



RESEARCH ARTICLE

Molecular Detection of *Hyalomma* Spp. Isolated from Sheep and Camel in Al-Samawah Desert of Al-Muthanna Province

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ARTICLE INFO	ABSTRACT
Received: May 5, 2024	Ticks, especially <i>Hyalomma</i> , are important vectors for the transmission of deadly microorganisms, such as Crimean-Congo Hemorrhagic Fever (CCHF) virus. The present study was conducted to identify <i>Hyalomma</i> spp. isolated from sheep and camel in Al-Samawah desert of Al-Muthanna Province. The study was performed with the collection of 60 tick samples. The samples were subjected to a PCR and partial gene sequencing of these ticks using the 18S rRNA gene. The results showed that 45/60 (75%) of the samples belonged to <i>Hyalomma</i> spp. The samples (17 PCR purified products) were sequenced, and the aligned data were placed in a phylogenetic tree. The phylogenetic analysis of these data revealed that the current study isolates of these ticks belonged to <i>Hyalomma</i> spp and stood close to different isolates deposited in the GeneBank. The present study reveal that these ticks are highly present in the sheep and camel in Al-Samawah desert of Al-Muthanna Province, and that could be an important indicator for the existence of certain pathogenic microorganisms, such as Crimean-Congo Hemorrhagic Fever (CCHF) virus, which needs more in-depth investigations for the purpose of disease control.
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INTRODUCTION

Ticks are important parasitic arthropods of domestic and wild animals and are considered important vectors of both livestock and human pathogens. It has been reported that ticks can transmit viral, bacterial, and protozoal diseases in ruminants, which negatively affect their health and significantly impact their productive and reproductive performance (1,36). Ticks require two or three blood meals to develop through their three host cycles. The number of hosts varies depending on their developmental stage. There are several tick species, but the genus *Hyalomma* attracts the attention of many researchers worldwide because it is considered one of the most harmful ticks to domestic and wild animals. The virus-infected ticks inside the venom reach the animal's blood, and when it bites the animal, the virus is transmitted during a blood meal (2,3).

Ticks (Acarina: Ixodida) are regarded as second to mosquitoes as vectors of human and animal diseases. They affect animals either mechanically or cause significant economic losses directly and through the transmission of tick-borne diseases (TBDs). In desert climates, particularly in Iraq, there are various problems associated with such ectoparasites (4, 5). In hot climates such as Iraq, ticks widely affect livestock, especially sheep and goat flocks. The distribution of ticks in Iraq is associated with changes in the environmental characteristics. A study concluded that the most frequent genera are *Hyalomma*, *Rhipicephalus* and in rural regions with abundant dense and dry grass. Sheep and goats, which are suitable hosts for ticks, are affected severely by these parasites. In Iraq, ticks which

affect ruminants such as sheep, goats and cattle the most are different species of the genus *Hyalomma* (6,7).

In areas with many hosts with pathogens of concern, it is advisable to treat both tick hosts (for example, livestock) and tick vectors. Control of tick vectors typically involves altering the habitat for the pathogen. For example, where tick vectors thrive in both wild animals and livestock, tick control and treatment to prevent disease in livestock through vegetation management, habitat alteration, and vaccination for wildlife are needed (8-11).

Ticks play crucial roles in pathogen transmission, inflicting extensive damage to animals. They use a range of hosts including farm animals, wildlife, and people. Tick-host-pathogen relationships are numerous and complex. Large mobile ticks such as *Hyalomma* spp. are able to travel long distances and readily exploit various hosts. These ticks are capable of supporting a high diversity of pathogenic agents related to the consistent feeding behavior on different hosts (12-15).

Hyalomma spp. are vectors of many livestock important diseases in Iraq, including bovine theileriosis, ovine theileriosis, and camel theileriosis. Bovine, ovine, and equine anaplasmosis occur in Iraq. Camels suffer heavily from dermatophilosis, and many of the protective techniques used to prevent other tick-borne diseases are ineffective in preventing dermatophilosis. Tick treatment after infection with the bacterium responsible for the disease has little or no effect on the disease state (16-18). *Hyalomma* spp. damage wool, hides, and skins of animals, which causes an increase in production costs and exploitation of the animals. Solvent residues in hides can cause skin irritation in humans. Using acaricide-impregnated collars reduces the damage to hides and skins. Sublethal doses of acaricides or resistance to acaricides are some of the problems that need to be addressed with this tick (19-35).

Hyalomma-tick transmitted diseases have a serious economic impact on animal production. The economic effects of *Hyalomma*-tick transmission occur following the bite of *Hyalomma* spp. associated with 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 on humans, and are vectors and reservoirs of viral, bacterial, and rickettsial diseases. In Iraq, *Hyalomma* spp. are the vectors of tick-borne piroplasmiasis, which has just been eradicated from a few areas outside the Kurdish region (20-22).

Ticks, especially *Hyalomma*, are important vectors for the transmission of deadly microorganisms, such as Crimean-Congo hemorrhagic fever (CCHF) virus. The present study was conducted to identify *Hyalomma* spp. isolated from sheep and camel in Al-Samawah desert of Al-Muthanna Province.

MATERIALS AND METHODS

Samples and DNA extraction

The present study was conducted to identify *Hyalomma* spp. isolated from sheep and camel in Al-Samawah desert of Al-Muthanna Province. The study was performed with the collection of 60 tick samples. The animals were identified with clinical abnormalities, such as abdominal pain, severe fever, and depression. The study was lasted during the period of November, 2023 to February, 2024. The tick samples were sterile-transported to a laboratory. Each tick was DNA- extracted using the Qiagene Kit, and the DNA was measured using a Nanodrop.

PCR

A gradient PCR technique was conducted on all isolates according to the instruction of the kit. In a total volume of 25 µl, the reaction contained each primer (23) (F; CATTAAATCAGTTATGGTTCC and R: CGCCGCAATACGAATGC, 780 bp) for the mitochondrial *18S rRNA* gene at 1.5 µl of 10 pmol, 5µg DNA, and 12.5 master mix. The thermocycler was set the annealing to (95 °C for 5 minutes, for 35

cycles of 94 °C for 30 seconds, 56 °C for 30 seconds, 72 °C for 45 seconds, and a final extension at 72 °C for 7 minutes. A 1.5% agarose gel electrophoresis was conducted and bands were displayed using a UV-set.

Partial sequencing of 18S rRNA gene

A 40 µl of each purified PCR product was sent out to Macrogen Company (Korea) for sequencing. NCBI BLASTn engine was used to analyze the data and find any close similarities with global isolates and presented in a phylogenetic tree using MEGA X software 11.0.11.

RESULTS

The results showed that 45/60 (75%) of the samples belonged to *Hyalomma* spp. The samples (17 PCR purified products) were sequenced (Figure 1).

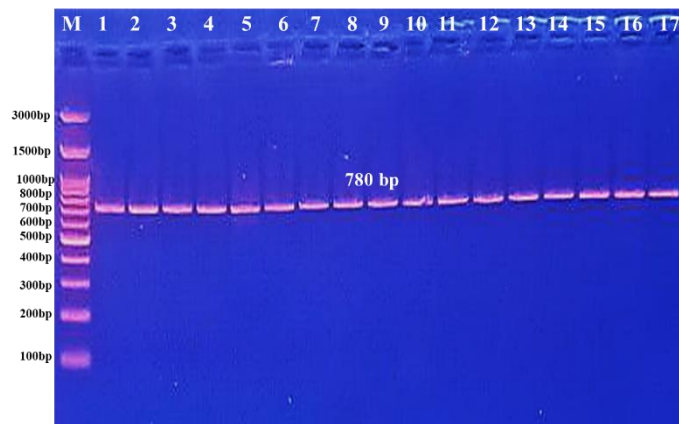


Figure 1: Image of agarose gel of 18S rRNA gene in *Hyalomma* spp.

M represent DNA marker, and 1- 17 lanes are positive PCR products. Lanes (1-9) refers to camel isolates while lanes (10-17) refer to sheep isolates.

The aligned data were placed in a phylogenetic tree. The phylogenetic analysis of these data revealed that the current study isolates of these ticks belonged to *Hyalomma* spp and stood close to different isolates deposited in the GeneBank (Figure 2).

DISCUSSION

The diagnosis of ticks of medical and veterinary significance is important with regard to disease pathogenesis, prevention, and treatment and has also been a useful and specific supplementary feature in the description of both new tick species and established species, and subsequent molecular studies of samples prepared from ticks on the Iraqi population and other host animals from around Iraq allow for confirmation of the pathogen status of tick collection hosts. Having comprehensive information on tick and pathogen life histories and associations will be important preventive measures for an organism, and our data will contribute important information on tick population dynamics (24, 25).

Other morphological and molecular-based techniques have sporadically shed light on the taxonomic status of different species and morphological cases within the *Hyalomma* genus. The classification and structural nature of these complex relatives are reflected in the literature, and standard keys are used to identify different biological stages. Research on the taxonomic status of the *Hyalomma* genus in some systematic fields is still in progress. The female *Hyalomma* bears two intermediate stages, which distinguish it from other ixodid species. The nymphs of *Hyalomma* are unique among ixodid ticks in that they resemble larval or adult stages of the same species on the same host. This altricial

development pattern, which is not seen in any other life-cycle tick that parasitizes a vertebrate host, is unique among the Ixodidae (26, 27). The larvae are monoxenously feeding on both larvae and nymphs, and they are not ready to repair for both sexes as adults. Adult females feed on the vertebrates during the respective stages from short periods to 1.5 to 2 weeks, and they appear with a remarkable sexual difference in body sizes, which generally measure 10–33 mm in length. While the hosts are still feeding, the larva and/or nymph remove their attachment, resulting in the completion of the secretion for the final molt and subsequent stage, and then they hide in various off-host micro habitats for 1 or 2 months. This life strategy enables the ticks to withstand high temperatures and low relative humidity. Adult ticks contribute to survival in areas not frequently visited by hosts (28, 29).

It is only in recent years that researchers have specifically investigated the distribution of individual *Hyalomma* species. In a study of Russia, 50 years of climate-driven geographic distribution changes in *Hyalomma* were assessed. Margos et al. (2021) mapped areas of central and eastern districts of Germany along with the center to southwestern Lithuania, displaying differences in biotopes, vegetation, and water bodies that were influenced by the climate. This may have facilitated the northern expansion of *Hyalomma* ticks, to 240 km north of its theoretically optimal latitudinal niche (30-32). Guglielmone et al. (2010) suggested that the expansion of the desert areas to the southeast of Kazakhstan, the dry steppes of the Kalmykia steppe, and central districts of Kobystan in Georgia would cause congestion on the local fauna. Initial estimates of 20–30 live ticks per animal resulted in an evaluation and subsequent study of all kinds of wild and domestic animals, discovering a total of about 1,500 *Hyalomma* ticks, including nymphs. The recordings cover 84% Atelidors cats, 9% foxes, 4.5% wolf ticks, and 2.5% omnivores. Bayisch and Hiechinger (2021) suggest that the introduced Asian meat-raccoon population is expanding and spreading, creating a potential danger for ticks and the pathogens they carry. In 2021, the European Food Safety Authority (EFSA) reported a review of the surveillance methods of *Hyalomma emarginatum* and *Hyalomma marginatum* hosts and transmission patterns, listing 32 pages of records of published literature on the topic. However, all the records on the list, except one European survey on host distribution, were related to the Middle East, Spanish North Africa, or the Mediterranean coastline. Information like that found in Srivastava et al. (2021) seems to be almost non-existent, i.e., the exact date of a potential European introduction from imported Arabian thoroughbred horses (33). While the interest in the geographical and species distributions of *Hyalomma* ticks has been clearly recognized, there appear to be no studies that have collected verifiable records of previous *Hyalomma* species distribution, prevalence rates (*Hyalomma persicum* = 215, *Hy. marginatum* = 154), and surveys post-1980 (*Hyalomma persicum* = 1343, *Hy. marginatum* = 2184) (34).

CONCLUSION

The present study reveal that these ticks are highly present in the sheep and camel in Al-Samawah desert of Al-Muthanna Province, and that could be an important indicator for the existence of certain pathogenic microorganisms, such as Crimean-Congo hemorrhagic fever (CCHF) virus, which needs more in-depth investigations for the purpose of disease control.

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