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Original Research Article

Genetic Characterization of *Hyalomma Anatolicum* (Ixodoidea: Ixodidae) in Babylon Province Middle Iraq

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Abstract: This study was included molecular study of *Hyalomma anatolicum* (Ixodoidea: Ixodidae) based on *16S rRNA* gene collected from three regions' in Babylon province. The results of gel electrophoresis image (1.5 % agarose) shows the amplicons of a partial region within large subunit RNA gene (size= 450 bp). The sequencing data has reported species of *H. anatolicum 16S rRNA* gene (OQ162293) to OQ162298 these species revealed close matching on the phylogenetic tree to an isolate of *H. anatolicum* for different countries. This study represents first in Babylon Governorate to diagnose *H. anatolicum* by targeting *16S rRNA* gene.

Keywords: Sheep, *Hyaloma anatolicum*; PCR; *16S rRNA* gene, Phylogenetic tree.

INTRODUCTION

Ticks are blood-sucking arthropods that have a significant influence on both the veterinary and medical industries globally (Falih and Hamza, 2022; Makwarela *et al*., 2023). Ticks are the second line of harmful microbial vectors, after mosquitoes (Karasuyama *et al*., 2020). Ticks can have a significant effect on livestock and human health as blood feeders and carriers of several diseases, with great economic effects for the livestock industry (Perveen *et al.,* 2021). The normal dispersal species of *Hyalomma* is limited to African, European and Asian continents (Sands *et al.,* 2017). The hard tick of genus *Hyalomma* Koch, 1844 contains an estimated 30 recognized species and subspecies. The morphological identification of ticks is not adequate to identify the species (Dantas-Torres *et al.,* 2013). Additionally, molecular investigations were carried out to supply sequence data for Iraqi tick species that were not yet included in GenBank. Tick evolution and phylogeny have been successfully determined by the use of molecular markers, such as 16S rRNA and mitochondrial cox1 (Zhao *et al.,* 2020). For comprehending intraspecific and interspecific genetic variabilities among ticks, 16S rRNA and cox1 are helpful genetic markers (Ali *et al*., 2019, Zhao *et al*., 2020).

MATERIALS AND METHODS

Sample Collection

Samples of *Hyalomma anatolicum* were taken from various sheep body parts in an Babylon province – Iraq. A total of thirty *Hyalomma anatolicum* were removed from sheep between March and June of 2022. With extreme care and aseptic measures, the ticks were extracted from the sheep's skin using forceps and deposited in designated receptacles. Afterwards, the ticks and the containers were moved to the parasitology lab of the veterinary medicine college in Iraq—Al-Qasim Green University—Veterinary Medicine College. After that, it is submitted for molecular analysis.

DNA Extraction

Using the AddBio, Korea kit, DNA was extracted according to guidelines by the kit. The DNA that resulted was assessed with a NanoDrop.

PCR Primers

The Primers were designed by Han *et al.* (2019) targeting a specific region within *16S rRNA* gene to identify ticks. The universal primers Tick-F Forward (CCGGTCTGAACTCAGATCAAGT) and Tick-R (GCTCAATGATTTTTTAAATTGCTGT), synthesized by AddBio, Korea. The PCR strategy (table 1).

Table 1: PCR thermocycler conditions of 16S rRNA gene.

PCR step	Temperature	Time	Repeat
Initial denaturation	95 C	5 min	1
Denaturation	95 C	30 sec	39
Annealing	55 C	35 sec	
Extension	72 C	35 sec	
Final extension	72 C	5 min	1

DNA Sequencing

From the positive PCR samples, 6 samples were selected, and the Amplicons were shipped via DHL in an ice container to Korea Macrogen Company for DNA sequencing by sanger sequencing system. Next acquiring of the sequences, Genbank accession numbers were obtained by submitting the sequences to NCBI-GenBank.

Phylogenetic Analysis

Phylogenetic tree analysis were now conducted with the assistance of Molecular Evolutionary Genetics Analysis version 10 (Mega X) and multiple sequence alignment analysis based on Clustal W alignment analysis. This shows the similarity and sequences within this alignment region using MEGA7 software (Stecher *et al.*, 2020).

RESULTS

Present study based on PCR diagnosis was indicated that Hyaloma anatolicum were analyzed by using specific primers of DNA markers *16S rRNA* gene the products PCR amplified fragments size were 450 bp respectively as showed in Fig. (1).

Figure 1: Gel electrophoresis image (1.5 % agarose) shows the amplicons of a partial region within large subunit RNA gene (size= 450 bp). Some positive amplicons can be seen as in (1-6) while C is negative control in which similar PCR reactions components was used except PCR water was added instead of genomic DNA. M is Molecular marker from GeneDirex, Korea

After obtaining accession numbers, a comparison was made of the similarity rates with the global isolates, the similarity rates were between 99.74 and 100 % with the global isolates (Table 2).

Table 2: the NCBI-BLAST Homology Sequence identity (%) between local *Hyalomma anatolicum*. These sequences were deposited in gene bank under the following accession numbers (OQ162293, OQ162294, OQ162295, OQ162296, OQ162297 and OQ162298) and these were being compared with other global sequences

Sequence number	Obtained Accession number	Identical to	Accession number	Country	Identity %	Host
1	OQ162293	*Hyalomma anatolicum*	MK829042	Egypt	99.74	Camel
2	OQ162294	*Hyalomma anatolicum*	KC203340	China	100	Non
3	OQ162295	*Hyalomma anatolicum*	JX392003	India	99.21	Non
4	OQ162296	*Hyalomma anatolicum*	LC651063	India	100	cattle
5	OQ162297	*Hyalomma anatolicum*	LC651058	India	100	Buffalo
6	OQ162298	*Hyalomma anatolicum*	MZ976780	United Arab Emirates	99.74	goat

Phylogenetic Tree

Six isolates of *H. anatolicum*, have been checked in the GenBank database under accession no. OQ162293 to OQ162298. Phylogenetic tree shown there is an identity between the isolates of *H. anatolicum* (OQ162293, OQ162294, OQ162296 and OQ162297) of both sheep and goats, and it is similar to the *H. anatolicum* isolate in United Arab Emirates with

accession number MZ976780. Two isolates (OQ162295 and OQ162295) divergent form other. As well as through figure 2, we noticed the difference between the sequence of nucleotide of *H. anatolicum* due to of this difference, a divergent was found in the Phylogenetic tree.

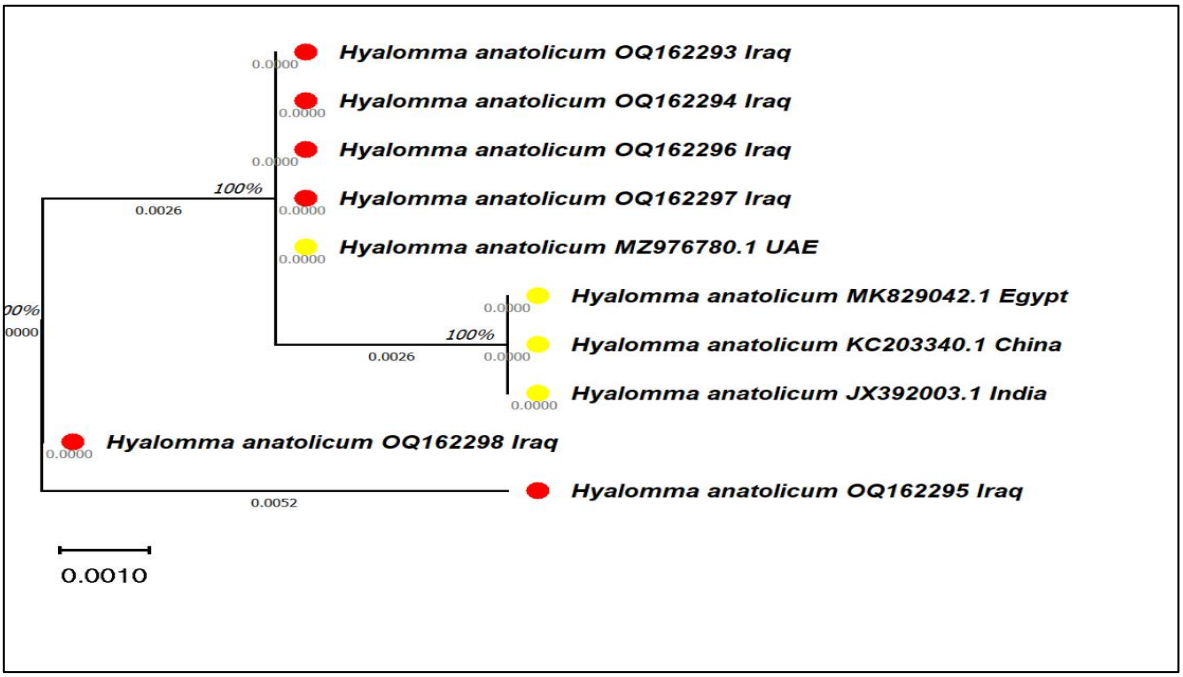

Figure 2: Phylogenetic tree analysis *of Hyalomma anatolicum* (Large subunit rRNA gene) constructed by Maximum Likelihood method (500 replicate). The percentage of tree in which the associated taxa grouped together is depicted following to the branches. The identified sequences are shown with red circles. These were compared with global sequences (referred as yellow circles). These were analysed by Mega X

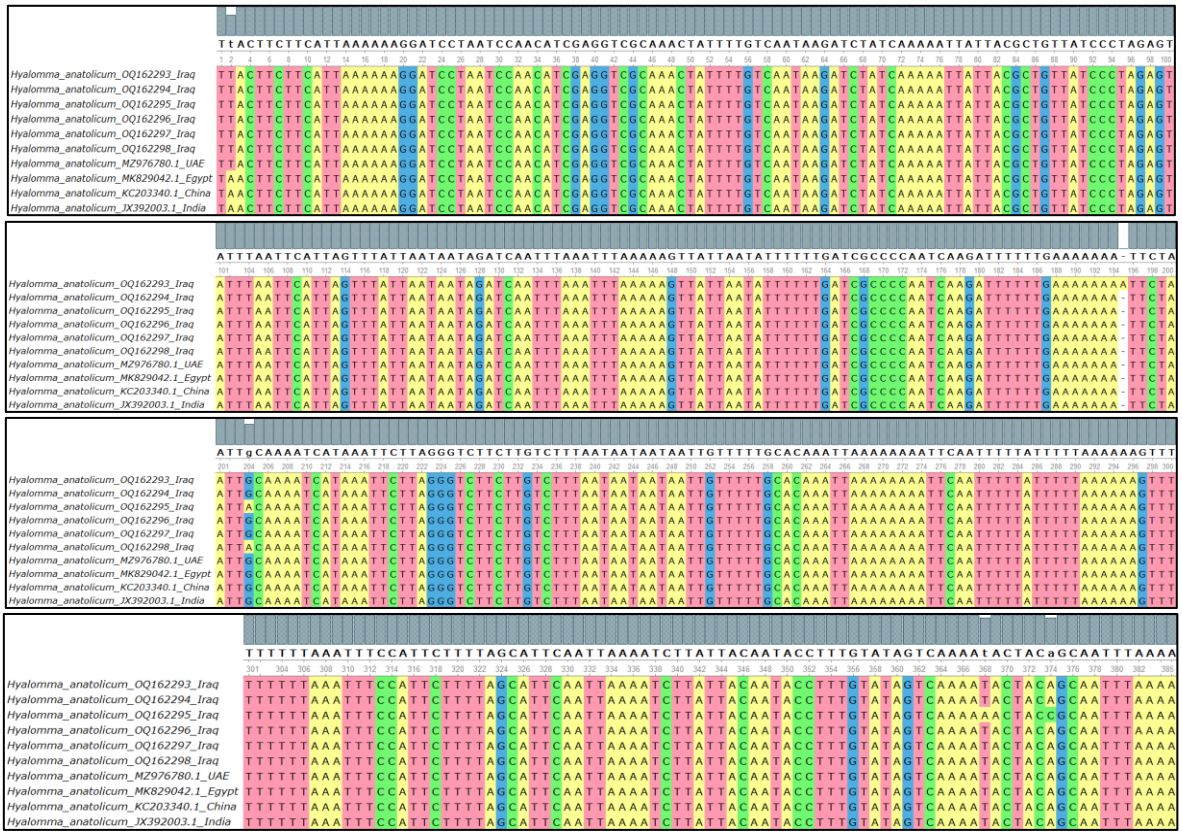

Figure 3: Multiple sequence alignment of the identified *Hyalomma anatolicum* (large subunit RNA gene). This highlights the similarity and differences between the identified sequences with four colours. This was analysed by Mega X

THE DISCUSSION

The results of the PCR and gel electrophoresis, as well as the sequence of nitrogenous bases, confirmed the sheep were infected with the *H. anatolicum*. This study is the first in Babil Governorate to study the genetic diversity of *H. anatolicum* using the *16S rRNA* gene.

The *H. anatolicum* parasite is widespread and recorded in all governorates of Iraq and recorded in a wide range of animals, including cattle, sheep, goats, camels, buffaloes, horses and donkeys (Shubber *et al.*, 2014). Additionally, the members of this genus can adapt to harsh weather conditions including heat, cold, and humidity, It can also parasitize a variety of hosts and are resistant to various medications (Sajid *et al.*, 2018), so all these reasons make it the most available genus. *H. anatolicum* play a significant role in the transmision of Theileria and Babesia, Modern methods of treatment and control of *H. anatolicum* depend on molecular identification of *H. anatolicum*. Because of morphology similarities, the existence of both engorged and immature stages, and damaged specimens, morphology alone is insufficient for the accurate identification of tick species (Ali *et al*., 2016; Estrada-Pena *et al.,* 2017; Zhao *et al*., 2020).

For the purpose of correct taxonomic classification of *Hyalomma* spp. have been identified using both morphological and molecular methods in a number of investigations (AL-Fatlawi *et al*., 2018; Ghosh *et al*., 2020; Al-Husseini, 2021; Ismael and Omer, 2021; Aziz, 2022).

Tick evolution and phylogeny have been successfully determined by the use of molecular markers, such as 16S rRNA (Zhao *et al.*, 2020). 16S rRNA was one genetic marker that helped to clarify the intraspecific and interspecific genetic variations among ticks (Ghosh *et al.*, 2020; Ismael and Omer, 2021; Aziz, 2022).

Nucleotide sequence databases show an vital role in providing meaningful genomic information on a variation of biological organisms (Moco *et al*., 2022). The DNA sequences of *H. anatolicum* have been deposited at NCBI for record in Iraq on a universal and comparison with other sequences of *H. anatolicum* in the world. The *H. anatolicum* found in the evolutionary tree (figure 2) is related and similar to isolates found in the UAE, Egypt, India and China, and this may be attributed to the animal imports from these countries. Also (figure 3) multiple sequence alignment of the identified *Hyalomma anatolicum* shows there is a difference between the bases of local isolates sent to the GenBank, this may be attributed to the mutations occurred within *H. anatolicum* for the purpose of adapting to environmental conditions.

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