



Morphological and genotypical identification of *Hyalomma anatolicum* isolated from cattle in Al-Daghara city, Al-Qadisiyah province, Iraq

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Abstract

The present study looks at the morphological and molecular distinct features of the tick *Hyalomma anatolicum*, a livestock-important tick that transmits production-decreasing pathogens. A total of 17 ticks were collected from infested skin of cattle in Al-Daghara city, Al-Qadisiyah province, Iraq, from Oct 15, 2022, to Nov 15, 2022. All ticks were investigated microscopically to identify tick-external features and genotypical, using partial sequencing of the 16S rDNA gene. The microscopic results showed that the tick had mouthparts and genital pores, which were specific and identical to species *H. anatolicum*. The sequencing results confirmed the finding of the microscopic data. In conclusion, the ticks *H. anatolicum* identified in the present study could be similar to ticks previously identified in neighboring countries, such as Pakistan and Egypt, which encourages an explanation of why these similarities happened. Iraq had cattle imported from countries close to the Pakistan region, such as India, this could bring new *H. anatolicum* to Iraq.

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Introduction

Ticks are members of the Arachnida class, consist of spiders, and are distantly comparable to mites. Argasidae, namely soft ticks, and Ixodidae, namely hard ticks, are two tick groups that differ both via external and internal features (1-10). Worldwide, around 900 ticks inhabit a wide range of creatures, and a certain group can spread viral, bacterial, and/or parasitic agents (11). They are the primary carriers of both human and animal pathogenic microorganisms in the Northern Hemisphere, while in the Southern one, these vectors are the primary carriers of pathogenic organisms for animal health. After the start of the twentieth century, socioeconomic and climatic shifts have shaped the durability of disease foci by altering the geographical range and seasonality of several tick species, their hosts, and reservoirs (12). Therefore, the issue of the effects of the temporary or permanent development of novel tick species with their infectious agents in formerly tick-free regions becomes front

and center. The *Hyalomma* genus of ticks is the ideal illustration of this danger of emergence (13-18). Twenty-seven species belong to the *Hyalomma* genus, identified on all continents except both American continents (3,4). Their affinity for open areas with generally hot and dry weather (19-24). Genus *Hyalomma* has three lifecycle phases, including larvae, nymphs, and adults, which each requires just one blood meal. The vast bulk of *Hyalomma* species has a triphasic life cycle, featuring adults preying mostly on big large animals, particularly cattle, while larvae and nymphs prefer small vertebrate animals in hidden settings as hosts (25-30).

The present study was designed to investigate the morphological and molecular distinct features of the tick *Hyaloma anatolicum*, which were collected from affected cattle in Al-Daghara city, Al-Qadisiyah province, Iraq.

Materials and methods

Ethical approve

All the authors of the present work ensure that all procedures of our experiment were performed under the Ethical Norms approved by the scientific board of College of Veterinary Medicine, University of Al-Qadisiyah (committee approval number 1314 in 18/10/2022).

Samples collection

Seventeen ticks were collected from the infested skin of 17 cattle in Al-Daghara city, Al-Diwaniyah province, Iraq, from Oct 15, 2022, to Nov 15, 2022. Each tick collected from a single animal was preserved in a bottle containing 70% ethanol and labeled with the animal's sex, collection date, and region of the body where the tick was removed (31). The samples were transferred to the Parasitology Department, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah province, Iraq, for further investigation.

Microscopic investigation

The samples were investigated morphologically using microscopic methods according to Soulsby (32) and Vasilevich and Nikanorova (33).

DNA extraction

According to the manufacturer's instructions, the DNA was extracted using a kit (AddBio, Korea). In brief, the tick was placed in an Eppendorf tube, 1 µl liquid nitrogen was added, and the tick tissue was crushed utilizing a pestle. The

DNA was measured using a NanoDrop. The DNA was stored in a deep freezer (-20°C) for further investigation.

PCR amplification and sequencing

The forward 16S rRNA-F and reverse 16S rRNA-R primer used are described by Han *et al.* (34) (Table 1). The total PCR reaction was 20 µl, containing 10 µl master mix, 1 µl (0.5pmol/20µl) forward and reverse primers, 6 µl a. dest, and 2 µl DNA. The thermocycler condition was one cycle of 95°C initial denaturation for 5 mins, 39-cycles of (95°C denaturation for the 30s, 50°C annealing for 35s, and 72°C extension for 35s), and one cycle of 72°C final extension for 5mins. The PCR products were determined by electrophoresis of 10 µl of the reaction products in a 1.5% agarose gel with TAE buffer (pH 7.8) and then stained for 5 min with 5 µl/ml ethidium bromide solution. The DNA was then visualized under a UV trans-illuminator. An ethidium-bromide containing 1.5% agarose gel was electrophoresed at 100 volts of 80amp for the 60s. Then, the UV-dependent visualization of the gel was done, and pictures were taken. The PCR products of 17 samples were sequenced by MacroGen (Korea). The sequence data were compared with other reference sequences published in Genbank of NCBI using the Blast program of Genbank. All the sequences identified as *H. anatolicum* were submitted to NCBI to obtain the NCBI accession number. The target sequences were phylogenetically analyzed using molecular evolutionary genetics analysis, version 10 (Mega x) program. The Clustal W model was used for alignments, and NCBI's blast n server was used to compare the examined sequences.

Table 1: Primers of the 16S rRNA gene belongs to *Hyalomma anatolicum*

Primer name	Sequence '5-----3.'	Amplicon size (bp)
16SrRNA-F	CTGCTCAATGATTTTTTAAATTGCTGTGG	450
16SrRNA-R	CCGGTCTGAACTCAGATCAAGT	

Results

Microscopic identification

The results of the microscopic examination showed that the tick had mouthparts and genital pores, which were specific and identical to the species *H. anatolicum*. Figures 1 and 2 illustrate the unique features of the target tick.

Molecular identification

The PCR products revealed the amplification of the 16S rDNA gene at 450 bp (Figure 3).

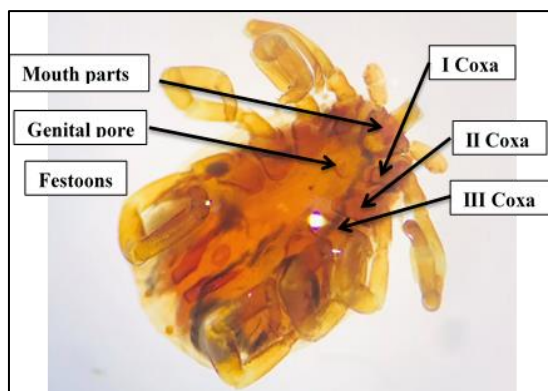


Figure 1: Microscopic features of *Hyalomma*. (Ventral view): Mouthparts and genital pore, I, II, and III Coxa and Festoons. Olympus (2.5X).



Figure 2: Microscopic features of *Hyalomma* (dorsal view). Olympus (2.5X).

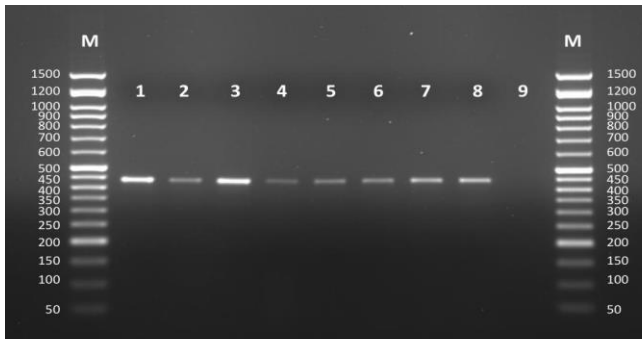


Figure 3: PCR products of 16S rDNA gene (450bp) of *H. anatolicum*. (Lanes: 1-8), negative control (lane 9), and M: marker GeneDirex.

The NCBI asseccion numbers of 17 sequences are acc. nr., OP740522; acc. nr., OP740526; acc. nr., OP740530; acc. nr., OP740522; acc. nr., OP740528; acc. nr., OP740537; acc. nr., OP740521; acc. nr., OP740529; acc. nr., OP740527; acc. nr., OP740536; acc. nr., OP740525; acc. nr., OP740534; acc. nr., OP740523; acc. nr., OP740531; acc. nr., OP740532; acc. nr., OP740524 and acc. nr., OP740533; The sequences of 17 ticks were analyzed by alignment with other sequences of Iraqi and neighboring countries (Figure 4).

Discussion

The present work identified the ticks collected from infested skin of cattle in Al-Daghara city, Al-Qadisiyah Province, to *Hyalomma*. Biglari *et al.* (3) identified *H. anatolicum* in cattle from the Central Area of Iran. *Hyalomma* ticks have extended mouthparts that can destroy tissue and set up the circumstances for myiasis and tick pyemia. *Hyalomma* may significantly influence animal productivity by its severe bite, swallowing up much blood that results in anemia, tick anxiety, and discomfort (35). Besides the direct damaging effects, various bacterial, viral, and protozoan diseases are transmitted by and/or maintained by *Hyalomma* ticks. The primary hemiparasitic infections

that cause babesiosis, theileriosis, and anaplasmosis in cattle are spread by these tick species, and the illnesses have a significant negative economic consequence on the cattle agricultural sector (36).

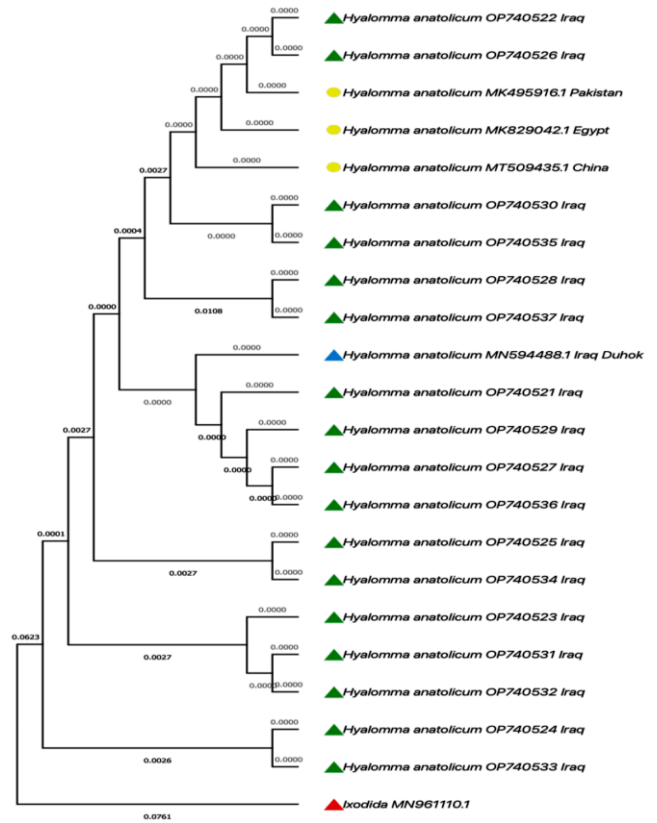


Figure 4: Phylogenetic tree analysis of *Hyalomma anatolicum* (16 rRNA gene) constructed by Maximum Likelihood method (500 replicates). The current study identified sequences shown with a green triangle.

Khan *et al.* (37) found that cattle made up most of the animals with tick infestations. This could be because of their delicate skin and Pakistan's tick-friendly ecosystem and climate. Their findings, which show lower tick infection rates in buffalo than in cattle, are consistent with earlier research, which also showed reduced tick infestation percentages in buffalo than in cattle (38). Dantas-Torres (39) reported that, generally, the most prevalent tick species found on cattle in the three ecological zones of Pakistan that we evaluated were *H. anatolicum*. There were discovered to be seven species of *Hyalomma*, constantly blood-feeding on animals. *Theileria annulata* is reported to be transmitted by the three-host tick *H. anatolicum*, while *Theileria* spp. is also known to be transmitted by the two-host ticks, such as *H. Dromedarii* (39). Also, Biglari *et al.* (3) identified *H. anatolicum* in cattle from the Central area of Iran.

The current study revealed that the Iraqi isolates were closely similar to those from different countries, such as Pakistan and Egypt, which encourages an explanation of why these similarities happened. A suggestion for this issue is that Iraq had cattle imported from countries close to the Pakistan region, such as India. This could bring new *H. anatolicum* to Iraq, and thus, genetic evolution could occur to a new but closely related species of this tick from these countries (40).

Conclusion

The current work indicates that the ticks (all 17 isolates) were from the *Hyalomma anatolicum* and that evolution strategies ensured their sequencing alignment to be similar to those from countries like Pakistan.

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Conflict of interests

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التحديد الشكلي والجيني لقراد الهيلوما اناتولكوم المعزولة من الماشية في قضاء الدغارة، محافظة القادسية، العراق

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الخلاصة

أجريت هذه الدراسة لدراسة الخصائص الشكلية والجزيئية المميزة لقراد الهيلوما اناتولكوم، وهو قراد هام للماشية حيث ينقل مسببات الأمراض التي تقلل الإنتاج. تم جمع ١٧ قرادة من جلد الماشية المصابة في قضاء الدغارة بمحافظة القادسية من ٢٠٢٢/١٠/١٥ إلى ٢٠٢٢/١١/١٥. تم فحص القراد مجهريا لتحديد الميزات الخارجية له كذلك استخدمت التقنيات الجزيئية، باستخدام التسلسل الجيني الجزئي إلى 16S rDNA. أظهرت نتائج الفحص المجهرى أن القراد يحتوي على أجزاء الفم والمسام التناسلية، والتي كانت خاصة ومتطابقة لقراد نوع لقراد الهيلوما اناتولكوم أكدت نتائج التسلسل الجيني لقراد الهيلوما اناتولكوم نتائج الفحص المجهرى. في الختام، يمكن أن يكون القراد الهيلوما اناتولكوم التي تم تحديده في الدراسة الحالية مشابهة للقراد الذي تم تحديده مسبقاً في البلدان المجاورة الأخرى، مثل مصر وباكستان والتي تفسر سبب التشابهات بين السلالات المختلفة. يستورد العراق الأبقار من بلدان قريبة من باكستان، مثل الهند، والتي تفسر نقل سلالات جديدة من القراد الى العراق.