Molecular Identification of Hyalomma Spp. Taken from Cattle in An-Najaf Province, Iraq.

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Abstract
The present study was carried out in the period May-August 2022 in An-Najaf Province, Iraq. The study aimed to classify hard ticks in cattle through molecular techniques. About 468 hard ticks were collected from naturally infested cattle which considered an important economic source of income. Phenotypic examination, then Polymerase Chain Reaction (PCR) were applied using a primer for cox1 gene for the diagnosis of Hyalomma. Sequencing of 18S rRNA gene was done to identified the Hyalomma species. Result of Hyalomma genus was proven in PCR, while sequence analysis and phylogenetic tree appeared one species Hyalomma truncatum that was recorded for the first time in Najaf.
Keywords: Hyalomma spp.; PCR, RNA, Iraq

1. Intoduction

The prevalence of cattle-infesting ticks is very high in Al-Najaf City, Iraq, especially during the beginning of summer (1). Ticks are from the phylum of blood-sucking arthropods and are of great importance in the medical and veterinary fields all over the world. They are second only to mosquitoes as vectors of disease-causing microorganisms. Ticks feed on host animals for several days and inject saliva with pathogens during feeding into the host's blood. (2). It is also found on all continents and is affected by climate changes (3).
These ectoparasites carry a wide range of infectious agents (viruses, bacteria, and parasites) therefore they considered most important vectors of diseases for mammals (4), tick-borne diseases cost the cattle industry billions of dollars each year causing significant financial losses to the cattle economy (5).
The research of tick species has become more crucial in Iraq (6), Determining the morphology of hard tick's genera using taxonomic keys is considered a traditional method of ticks identifying, an error in identification may occur during this process in terms of formality (7); hence, new techniques to be used to diagnose tick species. The present study aimed to use molecular techniques for cattle ticks' identification.

2. Materials & Methods

Sample collection: About 468 samples of hard ticks were collected from cattle's (43 sheep, 31 goats, 40 cows & 11 buffaloes) in the period from March-August 2022 in An-Najaf Province private fields.
Microscopic examination: Fine tip tweezer & medical cotton impregnated with alcohol were used for gathering and picking up ticks from the animal's body, considering caution to avoid ticks damage. Ticks were placed in clean sterile plastic tubes containing 70% ethanol. These tubes were labelled (date and place of sample collection, the host from which the sample was taken). Instructions for collecting hard ticks from livestock (8).
Then, samples were transferred to the Insects & Parasitology Laboratory at the Faculty of Education for Girls/ University of Kufa. Ticks samples were phenotypically examined via dissecting microscope with a magnification of 4X. To confirm the diagnosis, some samples were sent to the Iraq Natural History Research Center & Museum/ University of Baghdad, & diagnosis was documented by Professor Dr. Afkar Muslim Hadi/ Head of Vertebrate Department. Finally, these samples were transferred for the molecular diagnosis.

Molecular identification of hard ticks

Polymerase Chain Reaction (PCR)

Conventional PCR technique was used to diagnose 20 samples of hard ticks that collected from animals, after proven phenotypic diagnosis.

Extraction of DNA from ticks

Genomic DNA was extracted from hard tick samples (Tissue protocol, Geneaid, USA) gSYNC™, The purity and quality of tick DNA samples was evaluated by using a Nanodrop spectrophotometer and by running of samples on gel electrophoresis, these was done by preparing 0.5% of agarose gel (9).

Molecular identification of hard ticks

In this study, two type of primers were used: the 18S rRNA gene fragment of size (780bp) foreword 5'-ATTAAATCAGTTATGGTTCC-3' and reverse 5'-CGCCGCAATACGAATGC-3' (10).
& *Hyalomm spp. COX1gene* fragment of size (555bp), was able to catch different species of hard tick spp.,
foreword 5'- GCAAGCCCAGGGACATTAA-3' and
reverse 5'- CCACCGCCTGAAGGATCAAA-3' (11).

PCR Master Mix

The PCR master mix was prepared using (2 * Easy Taq R PCR Super Mix Kit) according the company's instructions as shown in the table below

Components PCR Master mix

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Table (1): Contents of PCR reaction mixture of this study.	
PCR master mix	Volume
DNA template	5 mL
Forward primer	3 mL
Reverse primer	3 mL
Nuclease free water	14 mL
Total	25 mL

The components of the PCR master mix are placed to a maximum of Stared PCR maxime PCR premix containing components such as (Taq DNA polymerase, cation buffer, SYBRB Green I, dNTPs, PCR enhancer & PCR stabilizer). All PCR tubes are transferred to a vortex centrifuge at 3000 rpm for 3 min. then placed in a PCR Thermal cycler.

PCR Thermal Cycler Condition

Table (2): The PCR program used in the present study.			
PCR Step	Temperature	Time	Repeat Cycles
Initial denaturation	95◦ C	5 min	1
Denaturation	95◦ C	30 sec	35
Annealing	52◦ C	30 sec	35
Extension	72◦ C	2 min	35
Final Extension	72◦ C	5 min	1
Hold	Forever	4◦ C	----

3. Results

PCR technique

PCR and Agarose Gel Electrophoresis results showed a positivity for COX 1 gene of *Hyalomma* at 555bp. as showed in Fig. (1):

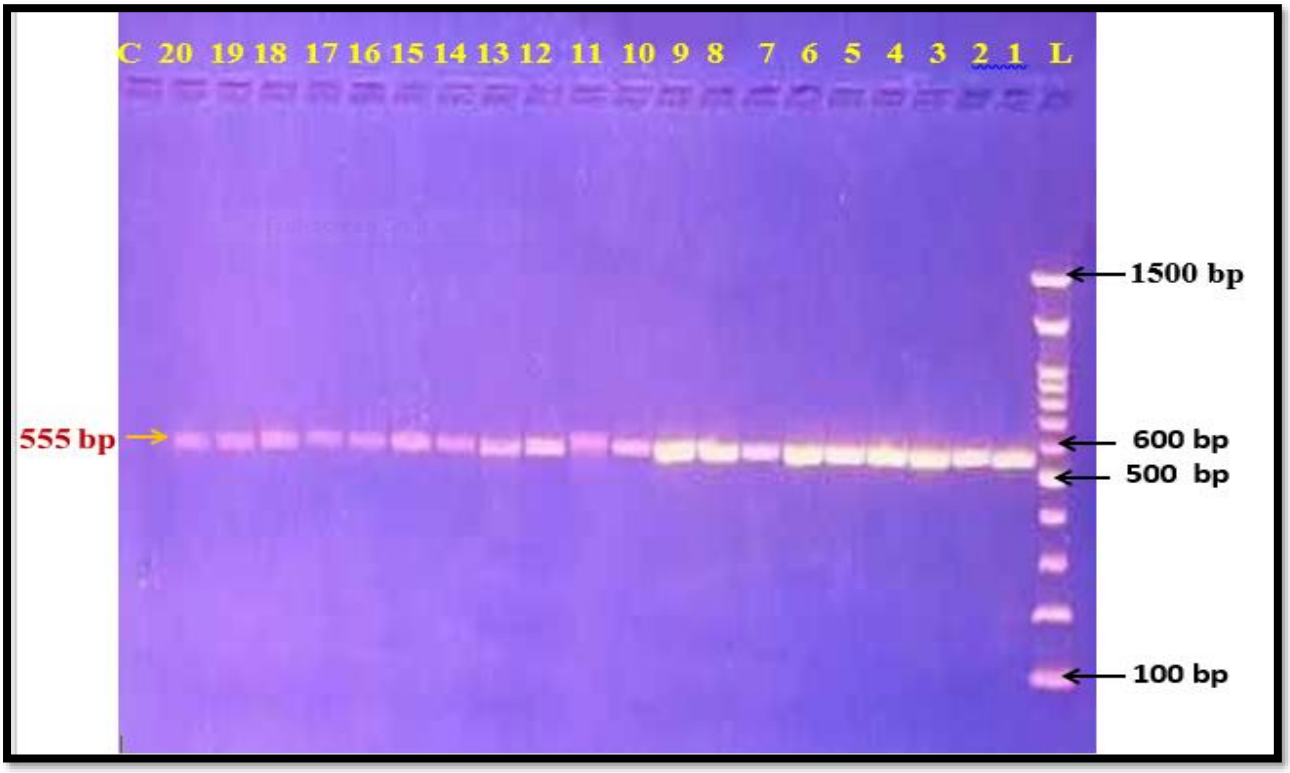

Fig. (1): Electrophoresis image of the agarose gel that showed the PCR product for the analysis of the cox1 gene for the diagnosis of Hyalomma (Marker ladder 100-1500 bp). Rows (1-20) are positive with a size of 555 bp under a voltage of 80 volts.

Sequencing of 18S rRNA gene fragment

Sequence analysis of the 18Sr RNA gene of the genus Hyalomma was performed by the basic local alignment search tool (Blast) accessed through the website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) Phylogenetic analysis and trees have become a key tool in a number of biological study domains because they enable the resolution of genetic connections between closely related species (12).

Dendritic analysis of gene 18sRNA for genera Hyalomma

The phylogenetic tree was analyzed based on the 18sRNA gene molecular sequencing of mitochondria in Hyalomma spp. local, and for the purpose of identifying the extracted isolates, a phylogenetic tree was established for seven isolates belonging to the genus Hyalomma using the evolutionary history inference using the method of joining nearby in the (MEGA X) version, and the ten genetic isolates had a genetic sequence in a bank, The NCBI global gene Pool-Blast Hyalomma truncatum. The sequence was submitted to GenBank (accession number from genBank as following:

Seq1 (OP787881), Seq2 (OP787882), eq3(OP787883), Seq4(OP787884),

Seq5(OP787885), Seq6 (OP787886), Seq7(OP787888).

One marker was used in this study: the first one was ribosomal ribonucleic acid 18SrRNA for the identification of tick species. This study used 18S for the identification and sequencing of tick species, which is as a good marker for the identification of hard tick species to solve morphological tick identification problems

Due to their quick development and maternal inheritance, several publications were used to establish the phylogenetic connections between various economic effects (13).

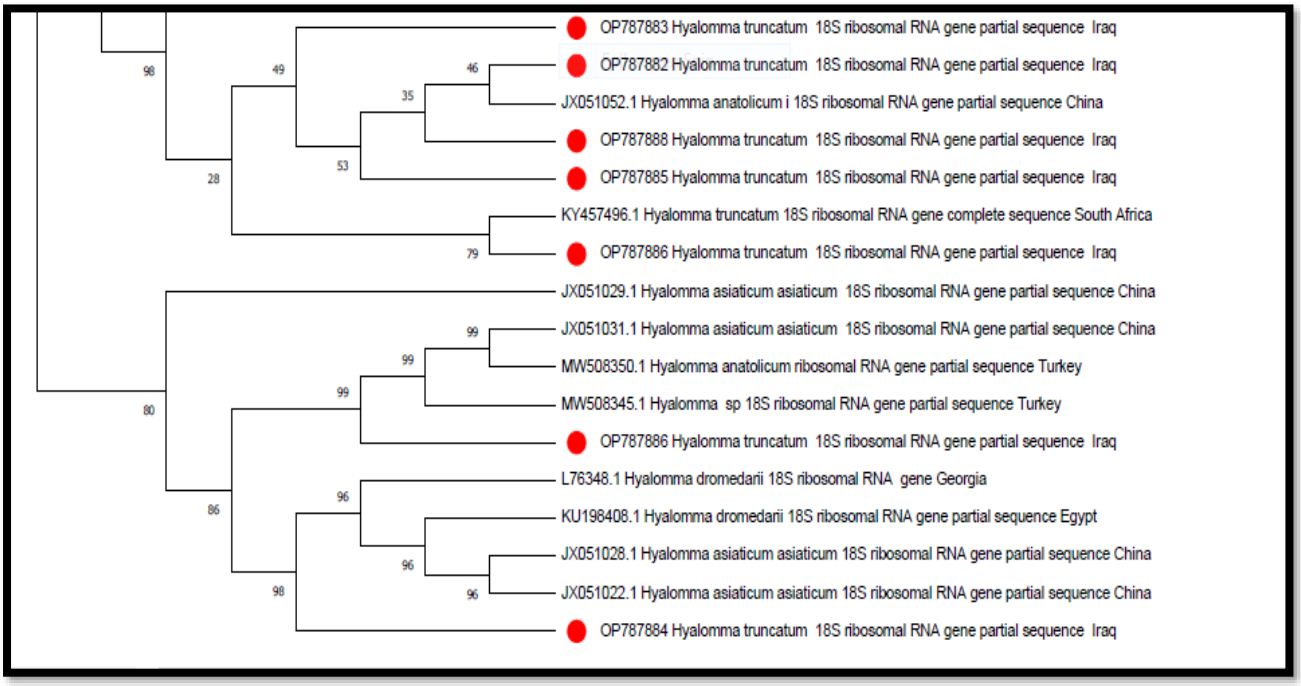

Fig. (2): Phylogenetic tree among tick species infested cattle's in An-Najaf province, Iraq.

4. Discussion

In this study the distribution of Hylomma spp., came first. There were several studies that support all these species in Najaf province by (14) through diagnosis of hard ticks in Al-Najaf, Iraq The result indicated that H. anatolicum is one of the wide-spread ticks that affect cattle.

Also, according to (15) several types of hard ticks were found in the north of Iraq. Rhipecephalus appendiculatus, H. atolicum anatolicum, & H. marginatum marginatum.

And that the result of this analysis was not close to what was found by(11) which recoded nine species of Hyalomma namely: H. aegyptium, H. anatolicum, H. asiaticum, H. scupense, H. dromedarii, H. excavatum, H. marginatum, H.rufipes and H. schulzei were identified.

In Thi Qar province, it was found that the tree analysis of the genetic isolates of the genus Hyalomma belonged to the species H. excavatium. H. anatolicum (17).

Another study in Duhok governorate six species under two genera of hard tick were identified by molecular study & sequencing including: three species were under the genus Hyalomma (18).

5. Conclusions

To our knowledge, this is the first study for the identification of *Hyalomma trunctum* from cattle in An-Najaf province/ Iraq by PCR and sequencing analysis.

6. Acknowledgements

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References

Sands, A.F., Apanaskevich, D.A., Matthee, S., Horak, I.G., Harrison, A., Karim, S., Mohammad, M.K., Mumcuoglu, K.Y., Rajakaruna, R.S. & Santos-Silva, M.M. (2017) Effects of tectonics and large scale climatic changes on the evolutionary history of Hyalomma ticks. Molecular Phylogenetics and Evolution, 114: 153–165.

Karasuyama, H., Miyake, K., and Yoshikawa, S. (2020). Immunobiology of acquired resistance to ticks. Frontiers in immunology, 11p.

Gilbert, L. (2021). The impacts of climate change on ticks and tick- borne disease risk. Annual review of entomology, Vet 66, 373-388.

Dabaja, M.; Tempesta, M.; Bayan, A.; Vesco, G.; Greco, G.; Torina, A.; Blanda, V.; La Russa, F.; Scimeca, S.; Lelli, R.; Ezzedine, M.; Mortada, H.; Raoult, D.; Fournier, P. & Mortada, M. (2017). Diversity and distribution of ticks from domestic ruminants in Lebanon. Veterinaria Italiana. 53(2): 147-155.

Lotfi, D. & Karima, K. (2021). Identification and incidence of hard tick species during summer season

2019 in Jijel Province (northeastern Algeria). J Parasite Dis.;45(1):211-217.
Fular, A.; Sharma, A. K.; Kumar, S.; Nagar, G.; Chigure, G.; Ray, D.D. & Ghosh, S. (2018). Establishment of a multi-acaridae resistant reference tick strain (IVRI-V) of Rhipicephalus microplus. Ticks & Tick-borne Diseases.
Hosseini-Chegeni, A., Hosseini, R., Abdigoudarzi, M., Telmadarraiy,Z. & Tavakoli, M. (2015). An additional record of Hyalomma marginatum rufipes Koch, 1844 (Acari: Ixodidae) in southwestern and southern Iran with a molecule evidence. Iranian Jou.of Anim.Bio.syst., 11(1): 79–80.
Darwin, S.K. (2010). Acaridae resistance in cattle tick. North. Territo. Gov. 845: 8236-8243p.
Green, M. & Sambrook, J. (2012). Molecular Cloning: A Laboratory Manual. 4th ed. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press.
Hadi, H.D, Hadi, A.M, Jassim, S.Y. (2020). Molecular diagnosis of ixodidae from canidae in Iraq. Iraq Natural History Research Center & Museum. plant Archives Vol.20, Supplement 2, pp.353-357.
Hosseini-Chegeni, Asadollah, Majid Tavakoli, and Zakkyeh Telmadarraiy. (2019) The updated list of ticks (Acari: Ixodidae & Argasidae) occurring in Iran with a key to the identification of species." Systematic and Applied Acarology 24.11: 2133-2166.
Yang, Z, Rannala, B (2012). Molecular phylogenetic: Principles and practice. Nat Rev Genet.; 13:303-314.
Brahma RK, Dixit V. (2013) Identification and characterization of Rhipicephalus (Boophilus) microplus and Haemaphysalis bispinosa ticks (Acari: Ixodidae) of North East India by ITS2 and 16S rDNA sequences and morphological analysis. Exp Appl Acarol. 62:253-265.
Al-Fatlawi MA, Ali MJ, Albayati HH (2019). Morphological and phylogenetic study of Hyalomma anatolicum in Al-Najaf, Iraq. Iraqi J Vet Sci. 2019;32(2):261-266.
Zangana, I. K.; Ali, B.& Naqid, I. A. (2013). Distribution of ectoparasite infested sheep and goats in Duhok province, north Iraq. Bas J Vet Res.;12(1).
Khorshid, N. H. (2021). Phenotypic and Molecular Characterization of Some Species of Hard Ticks (Acarina: Ixodidae) Isolated from livestock in Thi-Qar Province.
Ismael.S. S & Omer. L.T(2021). "Molecular identification of new circulating Hyalomma asiaticum asiaticum from sheep and goats in Duhok governorate, Iraq". Iraqi Journal of Veterinary Sciences, 35, 79-83.