Molecular Identification of *Rhipicephalus 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 *Rhipicephalus.* Sequencing of 18S rRNA gene was done to identified the *Rhipicephalus* species. Result of *Rhipicephalus* genus was proven in PCR, while sequence analysis and phylogenetic tree appeared two species *Rhipicephalus turanicus* and *Rhipicephalus glabroscutatum* that was recorded for the first time in Najaf.

Keywords: *Rhipicephalus* , molecular identification, tick- infested cattle, Najaf.

INTODUCTION

The prevalence of cattle-infesting ticks is extremely high in Al-Najaf City, Iraq, particularly during the start of summer (1). Ticks are blood-sucking arthropods that are very important in the medical and veterinary professions all around the world. As vectors of disease-causing bacteria, they are only second to mosquitos. Ticks feed on host animals for several days and inject pathogens into the host's blood during feeding. (2). It can also be found on all continents and is influenced by climate change (3). These ectoparasites transport a diverse spectrum of infectious agents (viruses, bacteria, and parasites), making them the most important vectors of illness for mammals (4). Tick-borne diseases cost the cattle industry billions of dollars each year, inflicting considerable economic losses (5). Determining the morphology of hard tick genera using taxonomic keys is considered a traditional method of tick identification (6); however, an error in identification may occur during this process due to formality (7); thus, new techniques to diagnose tick species are required. The current study sought to identify cattle ticks using molecular approaches.

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).

& ***Rhipicephalus*** *spp. COX1gene* fragment of size (500bp), 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:

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	----

RESULTS

PCR technique

Rhipicephalus Genus

PCR and agarose gel Electrophoresis results showed a positivity for COX 1 gene of *Rhipicephalus* at 500bp. as appeared in Fig. (1).

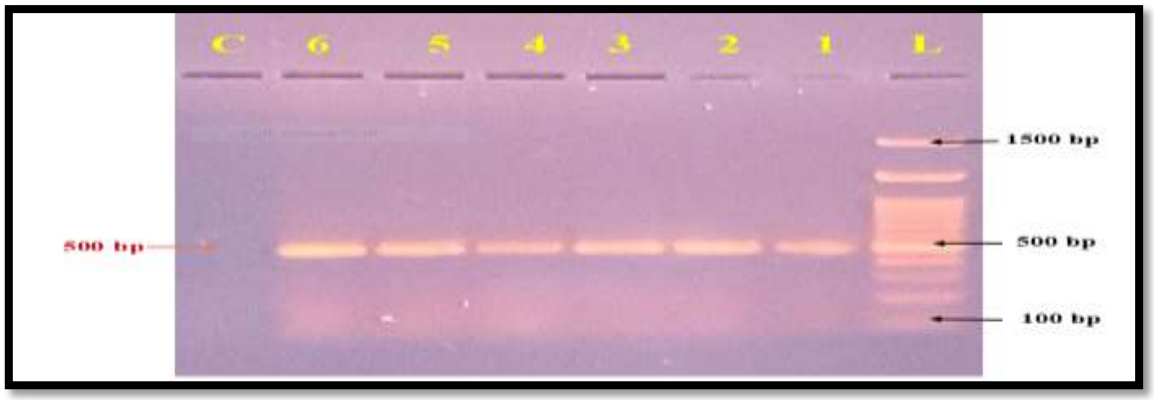

Fig. (1): Electrophoresis Image of the Agarose Gel that Showed the PCR Product for the Analysis of the cox1 Gene for the Diagnosis of *Rhipicephalus*. L= Ladder 100-1500 bp. C= Control. Rows (1-6) are Positive with a size of 500 bp under a voltage of 80 volts.

Sequencing of 18S rRNA gene fragment

Sequence analysis of the 18Sr RNA gene of the genus *Rhipicephalus* 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 *Rhipicephalus* genera

The phylogenetic tree was analyzed based on the 18sRNA gene molecular sequencing of mitochondria in *Rhipicephalus spp*. local, and for the purpose of identifying the extracted isolates, a phylogenetic tree was established for seven isolates belonging to the genus *Rhipicephalus* 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 *Rhipicephalus turanicu*s. The sequence was submitted to GenBank (accession number from genBank as following: Seq8(OP787887), Seq9(OP787889) & Seq10 (OP787890)

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).

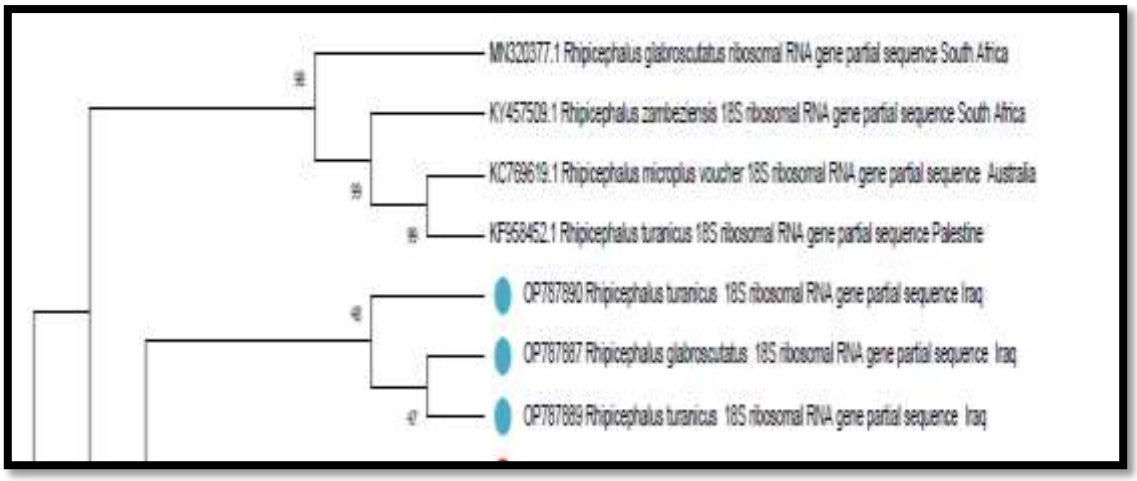

Fig. (2): Phylogenetic tree among tick species infested cattle's in An-Najaf province, Iraq.

DISCUSSION

In this study the distribution of *Rhipicephalus spp*., came second. There were several studies that support all these species in Najaf province by (14) Also, according to (15) several types of hard ticks were found in the north of Iraq. *Rhipecephalus appendiculatus*, *H. atolicum anatolicum*, & *H. marginatum marginatum*.(16) in Duhok governorate found six species under two genera of hard tick were identified by molecular study & sequencing including: three species were under the genus *Hyalomma* & three species were under the genus *Rhipicephalus.*

CONCLUSIONS

To our knowledge, this is the first study for the identification of *Rhipicephalus turanicus* from cattle in An-Najaf province/ Iraq by PCR and sequencing analysis and *Rhipicephalus glabroscutatum* that was recorded for the first time in Najaf.

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