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ORIGINAL ARTICLE

DISPERSAL OF HARD TICKS (ACARI, IXODIDAE) ON SHEEP *OVIS ARIES* LINNAEUS, 1758 IN DIFFERENT REGIONS OF IRAQ

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ABSTRACT

A total of 400 hard tick specimens (250 ♂♂, 150 ♀♀) were collected from 160 sheep *Ovis aries* Linnaeus, 1758 out of 200 samples examined in the different localities of Iraq, representing an infestation rate of 80% of the sheep. The results of the current research identified eight species of hard ticks belonging to two genera, *Hyalomma* C. L. Koch, 1844 and *Rhipicephalus* Koch,1844 and belonging to the Ixodidae family, as follows: *Hyalomma anatolicum* Koch, 1844, *H. excavatum* Koch, 1844, *H. impeltatum* Schulze & Schlottke, 1930, *H. scupense* Schulze, 1919, *Rhipicephalus bursa* Canastrini & Fanzago, 1878, *R. camicasi* Morel, Mouchet & Rodhain, 1976, *R. sanguineus* Latreille, 1806, and *R. turanicus* Pomerantsev, 1936. Molecular analysis and gene sequencing were conducted to confirm the species *Rhipicephalus camicasi*, using two genes, 12S ribosomal RNAs (PV155242.1, PV155243.1) and cox1 (PV139200.1, PV139201.1). The current study concluded that sheep are a new host for *R. camicasi* in Iraq.

Keywords: Cox1 gene, Hard ticks, *Hyalomma*, *Rhipicephalus*,12S gene.

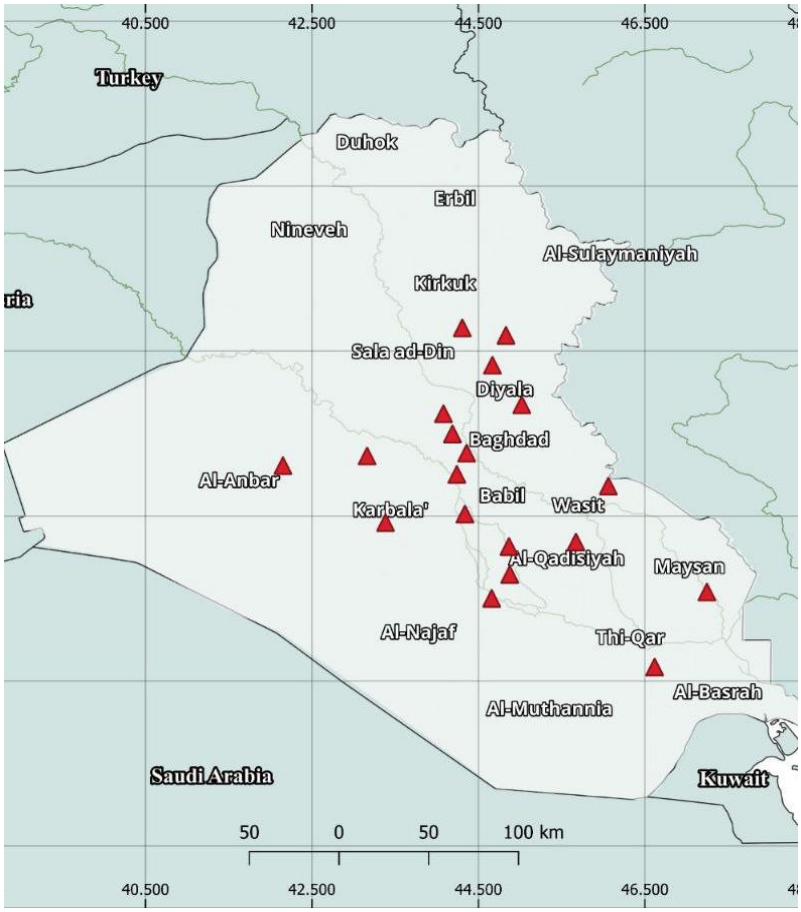
INTRODUCTION

Ticks are ectoparasites that feed on human and animal blood, endangering both human health and the environment (Amills *et al.*, 2017; Aldidge *et al.*, 2019). Many different kinds of animals, including birds, reptiles, and mammals, were infested by them (Jongejan, 2004). As stated by Dabaja *et al.* (2017), ticks are significant vectors that may spread several dangerous diseases to both people and animals. The two well-known families Ixodidae and Argasidae comprise the bulk of the 877-878 different species of ticks that now exist (Hamid and Al-Obaidi, 2023). However, hard ticks carry a number of infected diseases that can afflict both humans and animals (Brites-Neto *et al.*, 2015). Additionally, ticks have a high degree of environmental and host adaptation (Abdigoudarzi *et al*., 2009). Attempts at vaccination have been made; one such attempt used the salivary gland of *Hyalomma* Koch, 1844 to immunize sheep (Robson and Robb, 1967). Numerous investigations have been carried out on an array of subjects, including host inclination, infectivity, geographical dispersion, resistance to

pesticides, molecular identification of diverse species, biodiversity, seasonal fluctuations, categorization, fauna, and biology of hard and soft ticks in the country (Aghighi *et al.*, 2007). This study aimed to identify the hard ticks that infested sheep in Iraq. Additionally, the potential to add new information about tick hosts.

MATERIAL AND METHODS

Study areas: Tick specimens collected from sheep different area include the provinces and districts: Baghdad (Al- Yusufiyah), Al-Anbar (Fallujah, Al-Saqlawiya), Karbala (Ain al-Tamur), Wasit (Al Muwaffaqiyah, Badra, Shaihemiyh), Maysan (Qalaat Salih), Diyala (Baquba, Muqdadiya, Khanaqin), Salahaddin (Al-Dujail, Samarra, Tuz Khurmatu), Babil (Musayyib, Jiblah); Al- Diwaniyah (Al-Daghara, Afaq, Al-Sunniya) and Dhi Qar (Al-Rifai) (Map 1). Hard ticks were gathered and preserved in 70% ethanol; sheep were treated humanely.



Map (1): Dispersal of hard ticks in different regions of Iraq. Designed according to the program (Arc GIS On Iine, https://www.arcgis.com/index.html).

Microscopic examination and identification: Ticks were removed from the animal's head, thigh, ear, udder, and tail using tweezers and cotton soaked in ethyl alcohol. All of the specimens were brought to the lab at the Iraq Natural History Research Center and Museum-University of Baghdad, where they were examined under a dissecting microscope to determine the species of ticks after being cleaned of impurities using the guide to identification of species prepared by Walker *et al.* (2014).The specimens were identified using a dissecting microscope type ROMA and photographed using a Samsung SM-A225F Galaxy A22 mobile phone camera, while the terminal of ventral view of the *Rhipicephalus camicasi* was figured using the software Adobe Illustrator.

Molecular examination

DNA extraction**:** DNA was extracted from two *R. camicasi* specimens obtained and previously kept at -20ºC with a Korean DNA extraction kit.

Primers: The PCR amplification primers were created in accordance with Beati and Keirans (2001), based on 12S rDNA (12S ribosomal DNA) with Primer sequence F (5'-AAACTAGGATTAGATACCCT- 3'), R (5'-AATGAGAGCGACGGGCGATGT - 3') gene at 380 bp and Kushimo (2013) on the primer sequence of the cox1 gene (cytochrome oxidase subunit I) F (5' TACTCTACTAATCATAAAGACATTGG-3'), R (5' CCTCCTCCTGAAGGGTCAAAAAATGA- 3') at 656 bp.

PCR reaction preparation: The Master Mix (Promega/USA) was used to conduct the PCR reaction. The components included distill water (9µl), DNA (1.5µl), Forward primer (10 picomols/µl (1 µl)), Reverse primer (10 picomols/µl (1 µl)), and Master Mix or GoTaq® Green Master Mix (12.5µl).

PCR thermocycler conditions: A standard PCR thermocycler system for 12S ribosomal DNA was used to carry out the PCR conditions, which included Extension-1 at 72 °C for 1 minute with 35 cycles, Extension-2 at 72 °C for 5 minutes with 1 cycle, Initial Denaturation at 95 °C for 5 minutes with 1 cycle, Denaturation -2 at 95 °C for 45 seconds, and Annealing at 54 °C for 45 seconds. Meanwhile, the cox1 gene was processed using the same conventional PCR thermocycler system. Initial denaturation at 95 °C for five minutes with one cycle, Denaturation-2 at 95 °C for forty-five seconds, annealing at 58 °C for forty-five seconds, Extension-1 at 72 °C for one minute with thirty-five cycles, and Extension-2 at 72 °C for five minutes with one cycle were all included in this system.

Sequencing of DNA and phylogenetic tree: Two specimens were subjected to molecular identification of *Rhipicephalus camicasi* through genetic sequencing. The evolutionary lineage was deduced using the unweighted pair group method with arithmetic mean, commonly referred to as UPGMA.To secure accession numbers and verify identity, the sequencing outcomes were compared to GenBank utilizing BLAST. The Korean company Macrogen sent the cox1 gene and the 12S ribosomal DNA PCR product. Following their acquisition, the *Rhipicephalus camicasi* sequences were submitted to NCBI-GenBank in order to obtain GenBank accession numbers. Clustal W alignment analysis and molecular

evolutionary genetics analysis (MEGA6), were used for multiple sequence alignment in order to carry out the DNA sequencing study, particularly the phylogenetic tree analysis. The mentioned bootstrap value is installed on the tree. Comparing NCBI-Blast known sequences with phylogenetic tree analysis; was made to know *Rhipicephalus camicasi* in current study.

Statistical analysis: The chi-square (X2) analysis results for hard ticks were (1.812), showing that the infestation rate and gender did not significantly correlate (p=0.970).

RESULTS

Out of 200 samples analyzed, 160 sheep-infested samples contained 400 hard ticks (250♂♂, 150♀♀), representing an 80% infestation rate. Findings pointed to eight Hard tick species. The following two genera, *Hyalomma* and *Rhipicephalus*, the rate of infestation and gender did not significantly correlate (Pls. 1-8).

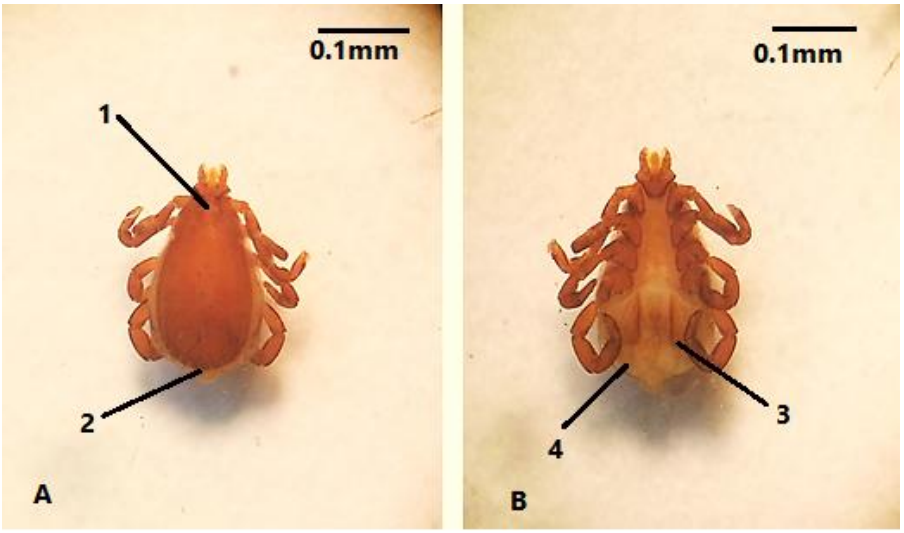


Plate (1): Male of *Rhipicephalus turanicus*; (A) Dorsal and (B) Ventral view. [1. There is a dip in the cervical fields, 2. The rear grooves are clearly visible, 3. The narrow shape of the adanal plates, 4. The accessory anal plates are big].

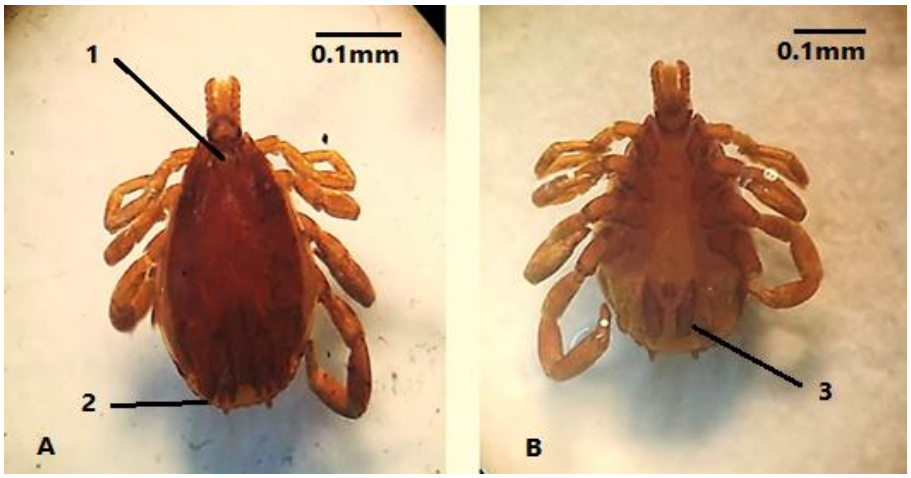


Plate (2): Male of *H. anatolicum*; (A) Dorsal and (B) Ventral view. [1. There is a depression in the cervical fields, 2. Paracentral festoons separate anteriorly, 3. Adanal plates have a circular termination].

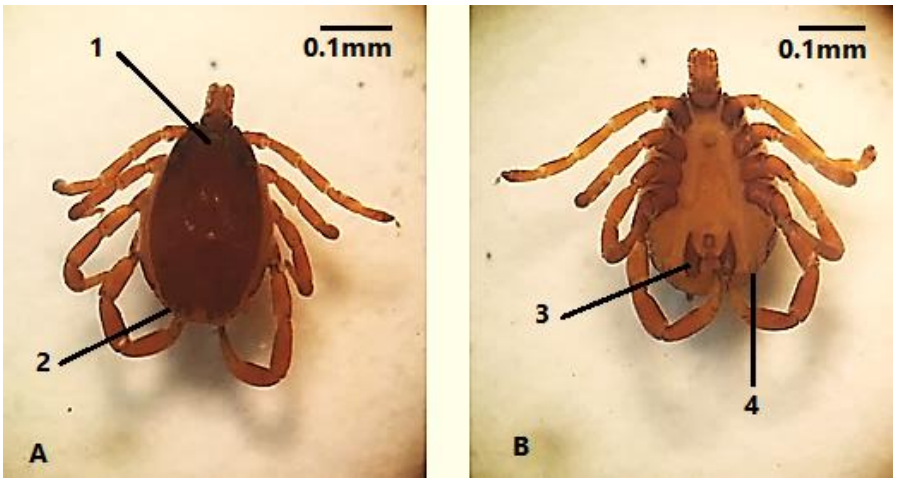


Plate (3): Male of *H. excavatum*; (A) Dorsal and (B) Ventral view. [1. Apparently depressed cervical fields, 2. Festoons paracentrally connected anteriorly, 3. The square-ended design of Adanal plate, 4. Distinct subanal plates].

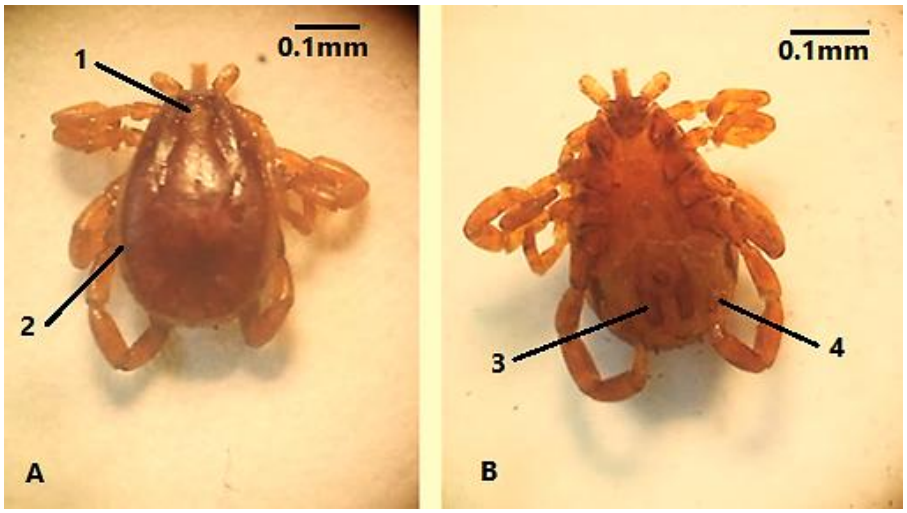


Plate (4): Male of *Hyalomma scupense*; (A) Dorsal and (B) Ventral view. [1. A slight yet noticeable dip in the cervical fields, 2. Long lateral grooves, 3. The ends of the adanal plates are square, 4. Subanal plates are clear].

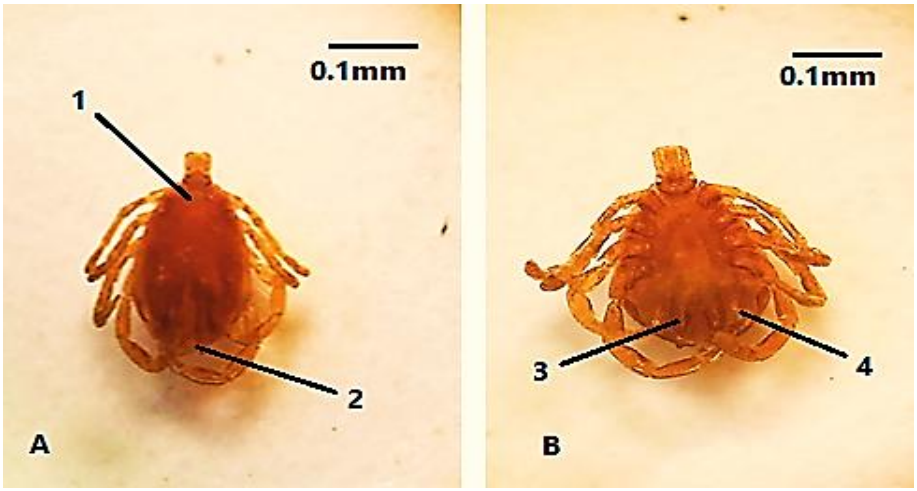


Plate (5): Male of *H. impeltatum*; (A) Dorsal and (B) Ventral view. [1. The cervical fields have a depression, 2. Pale central festoon, 3. Adanal plates have square ends, 4. Subanal plates are distinct].

Plate (6): Male of *Rhipicephalus bursa*; (A) Dorsal and (B) Ventral view. [1. There is no obvious depression in the cervical fields, 2. The eyes are clearly convex (as seen for the female), 3. The adanal accessory plates are little, 4. Adanal plates have a curved, wide form, 5. In fed males, the caudal appendage lacks information].

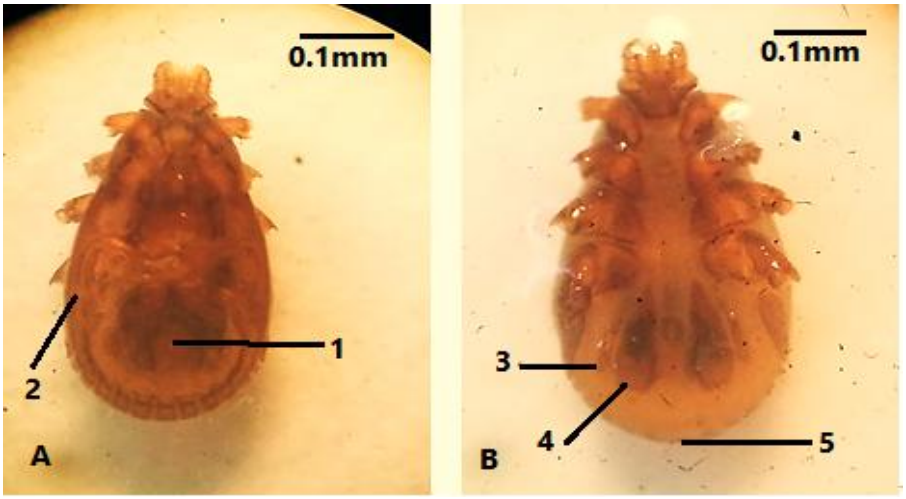


Plate (7): Male of *Rhipicephalus sanguineus*; (A) Dorsal and (B) Ventral view, [1. There are noticeable deep, broad, wrinkled grooves on the back, 2. The lateral grooves have a smooth texture and a specific kind, 3. There are large adanal auxiliary plates, 4. Although the adanal plates have a thin, trapezoid shape, they may look large and curved, 5. There is no information available on fed males' caudal appendage].

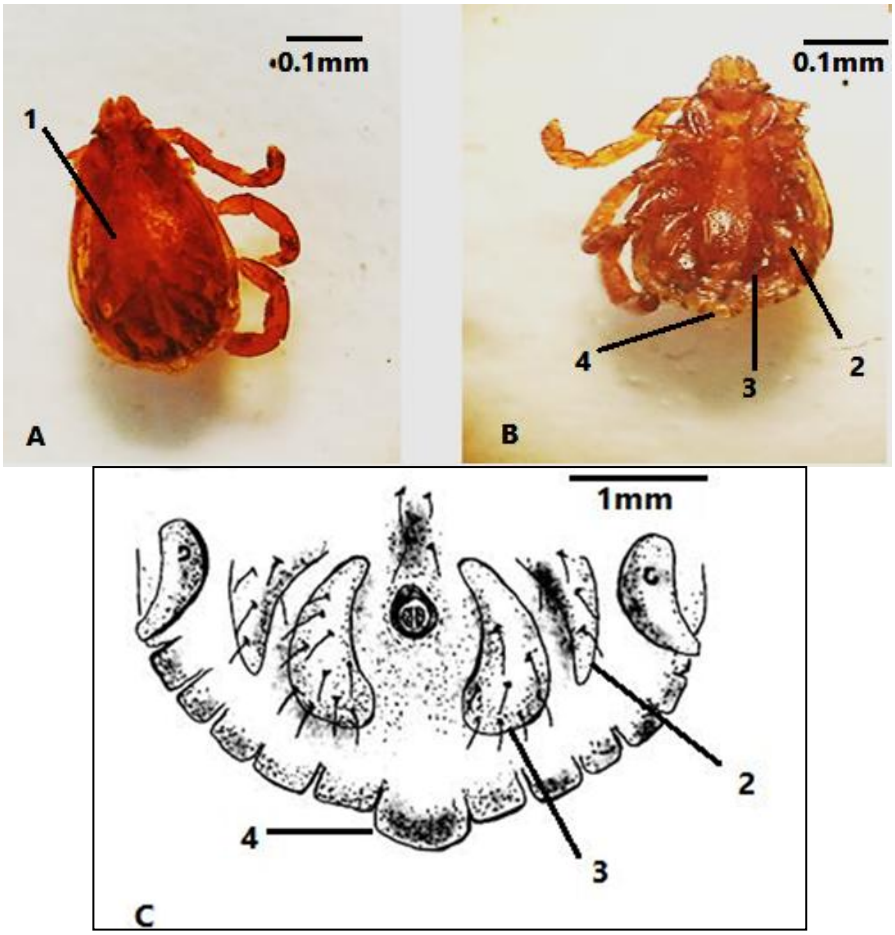


Plate (8): Male of *Rhipicephalus camicasi*; (A) Dorsal view, (B) Ventral view, (C) Terminal of ventral view. [1. Small to medium-sized interstitial punctures are seen in the distribution of interstitial punctations is sparse, 2. Adjacent adanal plates are big (but they may also be little), 3. The form of the Adanal plates is trapezoid and slender.4. In fed males, the caudal appendage is wide and protrudes as a small protrusion].

Table (1) indicated that the prevalence of male hard tick infestation is higher than that of female infestation, without a significant association between them. Identified eight Species of Hard ticks, with percentage ratios including: *R. turanicus* (31.75%), *H. anatolicum* (23.25%), *H. excavatum* (13.75%), *H. scupense* (10%), *H. impeltatum* (8.75%), *R. bursa* (6.25%), *R. sanguineus* (5.75%) and *R. camicasi* (0.5%).

Table (1): Hard tick species and their percentage ratios were identified on the sheep in Iraq.

No.	Species	No. of male	No. of female	Total	%	P-value
1	*R. turanicus*	80	47	127	31.75	
2	*H. anatolicum*	58	35	93	23.25	
3	*H. excavatum*	35	20	55	13.75	
4	*H. scupense*	25	15	40	10	
5	*H. impeltatum*	20	15	35	8.75	0.970
6	*R. bursa*	15	10	25	6.25	
7	*R. sanguineus*	15	8	23	5.75	
8	*R. camicasi*	2	0	2	0.5	
	Total	250	150	400	100	

Molecular findings derived from PCR analysis. Two hard ticks, suspected to be *R. camicasi,* were traditionally analyzed in the laboratory and subsequently subjected to molecular examination. The results indicated a positive identification of *R. camicasi* using 12Sr DNA and the cox1 gene (amplicon sizes of 380 bp and 656 bp, respectively) using the traditional PCR method (Pls. 9,10).

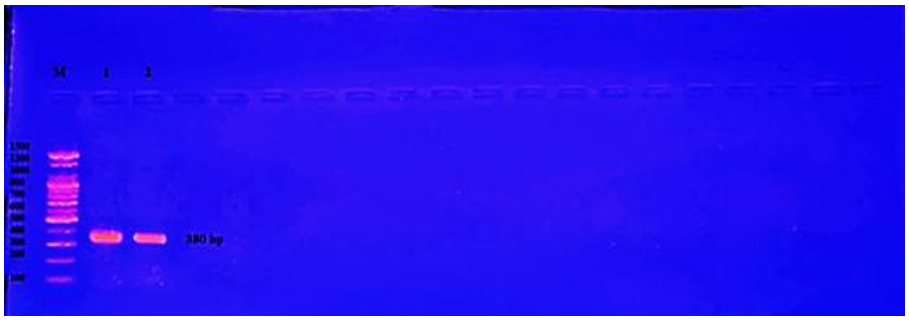


Plate (9): The gel electrophoresis image (1% agarose) demonstrates the *Rhipicephalus camicasi* positive. (amplicon size H – 380 bp) by targeting 12S ribosomal RNA gene.

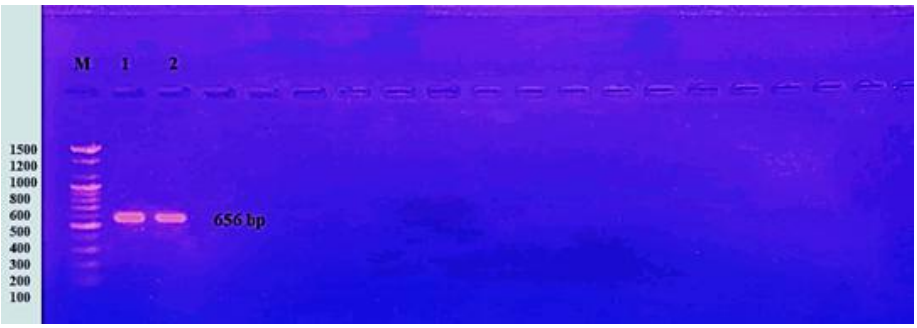


Plate (10): The gel electrophoresis image (1% agarose) demonstrates the *Rhipicephalus camicasi* positive. (amplicon size H – 656 bp) by targeting the cox1 gene.

Sequencing and phylogenetic analysis were conducted utilizing Mega X software, and multiple alignments were generated. Subsequently, these were sent to NCBI-GenBank in order to get sheep *R. camicasi* genetic codes, diagnosed by targeting12S rDNA with accession no (ID: PV155242.1, ID: PV155243.1), and *cox1*gene with accession no (ID: PV139200.1, ID: PV139201.1) respectively (Tabs. 2, 3).

Table (2): The percentage of homology sequence identity (%) as determined by NCBI-BLAST between local *R. camicasi* isolates from sheep, which were submitted to the 12S ribosomal RNA gene bank, and the strains deposited in NCBI-BLAST.

No.	Access No.	Affinity to	GenBank Access No.	Country	Affinity %	Bootstrap values
1	ID: PV155242.1	*Rhipicephalus camicasi*	ID: OQ565142.1	Kenya	99%	99.19%
2	ID: PV155243.1	*Rhipicephalus camicasi*	ID: OQ565142.1	Kenya	99%	99.19%

Table (3): The percentage of homology sequence identity (%) as determined by NCBI-BLAST between local *R. camicasi* isolates from sheep, which were submitted to the COX1 gene bank, and strains deposited in NCBI-BLAST.

No.	Access no.	Affinity to	GenBank Access no.	Country	Affinity %	Bootstrap values
1	ID: PV139200.1	*Rhipicephalus camicasi*	ID: OR573433.1	Kenya	99%	99.52 %
2	ID: PV139201.1	*Rhipicephalus camicasi*	ID: OR573433.1	Kenya	99%	99.76%

The Iraqi strains of *R. camicasi* are primarily closely related to one another, according to phylogenetic tree analysis. However, when targeting 12Sr DNA with bootstrap values (99.19%) and the cox1 with bootstrap values (99.52% and 99.76%) diagnosed *R. camicasi* similar to Kenya (Diags.1, 2).

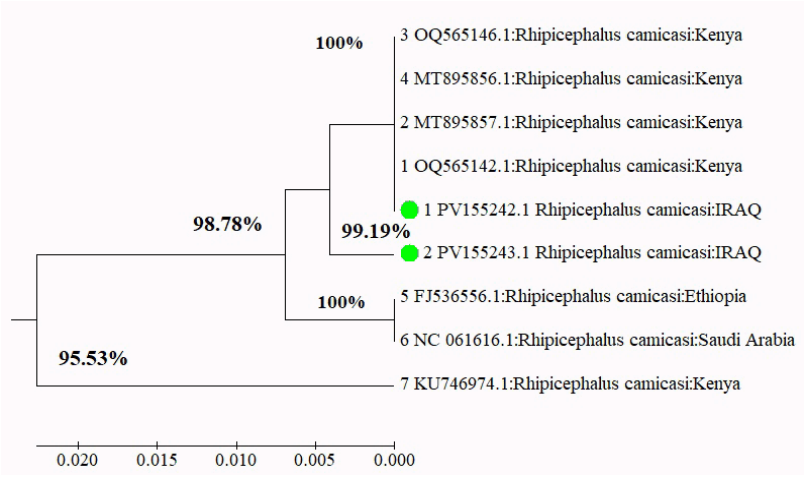


Diagram (1): Phylogenetic tree analysis for *Rhipicephalus camicasi* by targeting 12S ribosomal RNA gene.

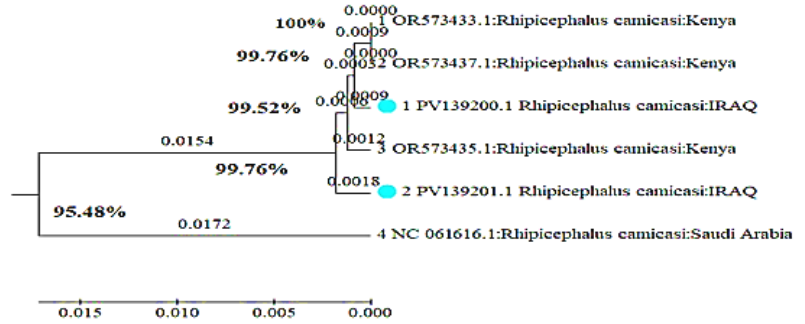


Diagram (2): Phylogenetic tree analysis for *Rhipicephalus camicasi* by targeting cox1 gene.

DISCUSSION

The current study recorded eight species of hard ticks on the basis of morphological examination of the hard ticks collected from sheep. These species included *Rhipicephlus turanicus*, *R. bursa*, *R. sanguineus*, *R. camicasi*, *Hyalomma anatolicum*, *H. excavatum*, *H. scupense* and *H. impeltatum*. These findings are consistent with previous studies conducted by Mohammad and Jassim (2011), which identified seven species of ixodid ticks, including *Hyalomma anatolicum, H. excavatum*, *H. detritum*, *Hyalomma* sp., *Rhipicephalus turanicus*, and *R. sanguineus.* Furthermore, in the extreme south of Iraq, in Basra Awad and Abdul-Hussein (2006), found that the only infestation found in the sheep were *R. turanicus.* Tahmaz, (2021) recorded *R. turanicus* and *R. sanguineus* on sheep in some regions from Erbil Province.

Also, eight species of *Hyalomma* have been identified as existing in Iraq, according to Al-Zubaidei *et al*. (2023). The study's main clinical findings were weight loss, mucous membrane

pallor, lymph node enlargement, and a gazing coat (Makawi and Hadi, 2023). Kadir *et al*. (2012), who revealed that *R. turanicus* was more prevalent in sheep, and these results were supported by our investigation (59.4%), According to El-seify *et al.* (2011), infestation rates in sheep were 18.22%, with *H. dromedarii* (16.67%), and *Rhipicephalus* spp. (45.14%). In contrast, Hasson's (2012); reported the identification of *H. anatolicum* which had the highest tick index (0.54 among all ticks). These variations might be the consequence of animal movement between various places, differences in the quantity of samples, or the presence of suitable climates (Makawi and Hadi, 2023).

According to this study, male hard ticks' infestation is more common than female. This agrees with the results of Ismael and Omer (2020), who found that there was a 2:1 gender distribution of ticks, with more ticks in male than in female. Distribution of tick species, including *Hyalomma,* and *Rhipocephalus,* was recorded during the present study. Relative rates are consistent with the findings of Abadi *et al.* (2010), who discovered that 57% of ticks were male and 34% were female. The demonstrated the co-infestation of two hard tick species in specific regions, this is in line with the results of Ahmad *et al.* (2021), who addressed this important problem. The decreased the number of ticks produced was due to irritability, anemia, and an increase in the morbidity ratio; this agreed with Makawi *et al*. (2023), who noted that a 100% infestation rate of one or two species of hard tick was present on the long-eared hedgehog. The current study is similar to Muhammad, (1996) who diagnosed *R. camicasi* in the jackal from Baghdad Province, the capital of Iraq. This is the same in Chandra *et al*. (2019), who found on dogs and dromedary camels from Saudi Arabia, and also in Chandra *et al.* (2022), who recorded *R. camicasi* from a camel in Riyadh, Saudi Arabia. Due to the opportunistic nature of *R. camicasi*, they prefer to be infested in cattle, sheep, goats, and camels (Walker *et al.*, 2005; Hekimoğlu *et al.*, 2016). That the parasite *R. camicasi* infested sheep as a new host in Iraq is attributed to the high proportion of imported sheep, as well as companion animals such as dogs, which are considered carriers of many external parasites.

CONCLUSIONS

The result of this study is the registration of *Rhipicephalus camicasi* as a new species on sheep in Iraq. The registration was confirmed using molecular and genetic sequencing, specifically the 12S ribosomal nucleotide sequence of the cox1 gene. This indicates a veterinary risk resulting from environmental changes in the country. Therefore, based on this study, we recommend conducting a comprehensive survey of hard ticks in the remaining Iraqi provinces to establish a database for researchers.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST STATEMENT

The author declares there are no contest interests.

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انتشار القراد الصلب (Acari, Ixodidae) على الضأن *Ovis aries* Linnaeus, 1758 في مناطق مختلفة من العراق

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الخلاصة

جُمِعتْ 400 نموذج للقراد الصلب (250 ♂♂، 150 ♀♀) من 160 رأسًا من الضأن *Ovis aries* Linnaeus, 1758 من أصل 200 رأس تم فحصها من مناطق مختلفة من العراق ، مما يُمثل نسبة إصابة بلغت 80% من الضأن. حددت نتائج البحث الحالي ثمانية أنواع من القراد الصلب تنتمي إلى جنسين، هما *Hyalomma* C.L. Koch 1844 و*Rhipicephalus* Koch, 1844 تعود إلى عائلة Ixodidae، وهي:

Hyalomma anatolicum Koch, 1844, *H.* excavatum Koch, 1844, *H. impeltatum* Schulze & Schlottke, 1930, *H. scupense* Schulze, 1919, *Rhipicephalus. bursa* Canastrini & Fanzago, 1878, *R. camicasi* Morel, Mouchet & Rodhain, 1976, *R. sanguineus* Latreille, 1806, and *R. turanicus* Pomerantsev, 1936.

أُجري التحليل الجزيئي والتسلسل الجيني لتأكيد تشخيص النوع *Rhipicephalus camicasi*، باستخدام جينين هما 12S ribosomal RNAs (PV155242.1، PV155243.1) و cox1 (PV139200.1، PV139201.1). اذ توصلت الدراسة الحالية إلى أن الأغنام تُعدّ مضيفاً جديدًا لـ *R. camicasi* في العراق.