

Morphological and Molecular Confirmation of Bovine Tick Species in Wasit Province, Iraq

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Abstract

Tick is the second largest vector of diseases, which infests many domestic and wild animals resulting in great economic losses due to morbidities and mortalities. This study aims for morphological characterization and molecular confirmation of bovine ticks using respectively the traditional microscopy and conventional PCR assay targeting the *18S rRNA* gene. Totally, 600 cattle of different ages and sexes were subjected for clinical investigation to identify the presence of ticks in various areas in Al-Kut city (Wasit province, Iraq) during June to July (2023). The findings revealed that 65.17% of study cattle were having the ticks on different bodily parts including udder (43.26%); while, significant lowering was seen in neck (4.93%), forelimb (7.4%) and hind-limb (10.82%) when compared to ear (14.04%) and perineal region (19.55%). Morphologically, the findings revealed that all samples were hard ticks and belong to the genus of *Hyalomma*; while molecularly, the PCR results confirmed that all collected ticks belonged to genus of *Hyalomma anatolicum*. In conclusion, this study demonstrated that the great naturally occurring the populations of the ticks in cattle, and that *Hyalomma anatolicum* remains the almost widespread species of ticks in Iraq. In addition, adult ticks are the easiest to identify and male and female ticks of the same species may look different, while nymphal and larval ticks are very small and may be hard to detect. This study recommended investigating all ticks species found in all Iraqi areas using the morphological and molecular techniques due to the definite role of this external parasite in transmission of various diseases to both animals and humans.

Keywords: Ectoparasite, Cattle, *Hyalomma anatolicum*, Polymeras chain reaction, *18S rRNA* gene

Introduction

Tick is an ectoparasite of marked distribution and considers the second largest vector of diseases in the world as infects many wild and domestic animals in several tropical and subtropical areas resulting in great economic losses due to morbidities and mortalities (Brites-Neto et al., 2015; Boulanger et al., 2019). Scientific classification of ticks involved three families are, *Argasidae* (soft), *Ixodidae* (hard) and *Nuttalliellidae* (Monotypic) which belongs to the *Ixodida* Order that belongs to the Arthropoda phylum (Kelava et al., 2021). Hard ticks, named due to presence of scutum on their bodies, represent the most widespread and important transmitters of pathogens as they fed on one, two or three hosts during its life cycle

that consists four stages; egg, larva, nymph and adult (Apanaskevich et al., 2014; Kahl, 2018; Okely et al., 2021). Worldwide, the spreading of infectious diseases is increasingly affecting the health of the world's population due to the ever-increasing number of infected people, therefore, the better understanding of interactions, vectors and pathogens, can aid in the development of prevention and control strategies (De la Fuente et al., 2017; Bouchard et al., 2019).

For diagnosis, the using of primary methods such as macroscopic and microscopic examination to identify tick may give unconfirmed results with the absence of the epidemiological history (Kemal et al., 2016). In a study on differentiation of some of *Hyalomma*, Apanaskevich and Horak (2006) showed that detection following the morphological characteristics like size and color of the scutum of various tick stages are greatly difficult and need to great expertise. Additionally, distinguishing of tick species through the morphology could cause a confusion particularly when the method of tick collection result in physical damaging of samples as a result of low expertise and engorging of collected ticks with blood (Estrada-Pena et al., 2017; Nava et al., 2017). Therefore, DNA-based methods such as polymerase chain reaction (PCR) assays are accurate diagnostic methods provide a valuable highly sensitive and specific data in particular in epidemiological studies (Lv et al., 2014 a, b; Amira et al., 2021). In Iraq, although researchers have provided information about characterization and distribution of ticks in different animal species (Algharban and Dhahir, 2015; Mohammad, 2015; Al-Fatlawi and Al-Fatlawi, 2019; Ali et al., 2021), these data remain under expected because ticks and their incriminated diseases remain existed widely. Hence, this study aims for morphological characterization and molecular confirmation of bovine ticks using the traditional microscopy and conventional PCR assay, respectively.

Materials and methods

Samples

Totally, 600 cattle of different sexes and ages were subjected for clinical investigation to identify the presence of ticks in various areas in Al-Kut city (Wasit province, Iraq) during June to July (2023). The tick samples of each infested animal were sprayed by ethanol 70% and collected by rotating manner using of forceps to avoid damaging of their mouthparts, and then, kept into labeled plastic containers that transported cooled using ice-box. In laboratory, ticks of each sample were divided into two parts, one for morphology which saved cooled and the other for molecular assay which saved frozen.

Morphological Identification

Tick samples were identified microscopically in the Iraqi Natural History Research Center and Museum (University of Baghdad, Baghdad, Iraq) based on the reference classification keys of other researchers (Walker et al., 2003; Estrada-Pena et al, 2004)..

Molecular analysis

Following the manufacturer instructions of the AddPrep Genomic DNA Extraction Kit (AddBio, Korea), DNAs were extracted from the ticks, and checked for its concentration and purity using the Nanodrop Sysytem (Thermo Fisher, USA). Targeting the *18S rRNA* gene, one set of primers [(F: 5'- GGC GAC GTT TCT TTC AAG TG -3') and (R: 5'- TCT CGA GGC ACA CAA TGA AG -3')] was designed based on sequence data of the NCBI global

Hyalomma anatolicum isolate (JX051051.1) and used to preparing the MasterMix (AddBio, Korea) tubes at a final volume of 20 µl. For PCR reaction, the thermocycler conditions were included 1 cycle (95°C, 5 min) for initial denaturation, 30 cycles for denaturation (95°C, 30 sec), annealing (58°C, 30 sec) and extension (72°C, 1 min), and 1 cycle for final extension (72°C, 5 min). Electrophoresis using the agarose-gel stained with Ethidium Bromide was done at 80Am, 100 volt for 1 hour. The PCR products were visualized using the UV-transilluminator and photographed using the digital camera. The product size of PCR positive samples were detected at 420 bp.

Statistical analysis

The *t-test* and One-Way ANOVA in the GraphPad Prism (version 6.0.1) Software were applied to detect significant variation between values of study groups at P<0.05 (*) and P<0.01 (**), , (Gharban et al., 2023, 2023).

Results and discussion

A total of 65.17% (391 / 600) cattle was positively having ticks that existed throughout various bodily parts including udder (43.26%), (Figure 1); while, significant lowering was seen in neck (4.93%), forelimb (7.4%) and hind-limb (10.82%) when compared to ear (14.04%) and perineal region (19.55%), (Figure 2).

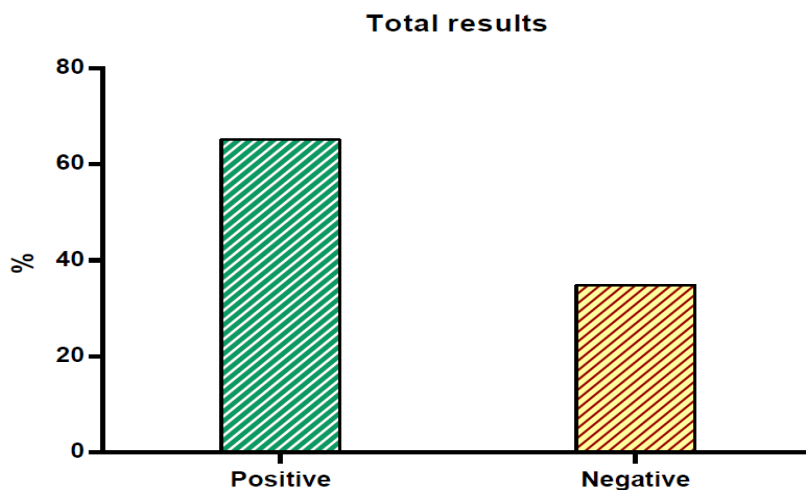


Figure (1): Total infested study cattle with ticks (Total No: 600)

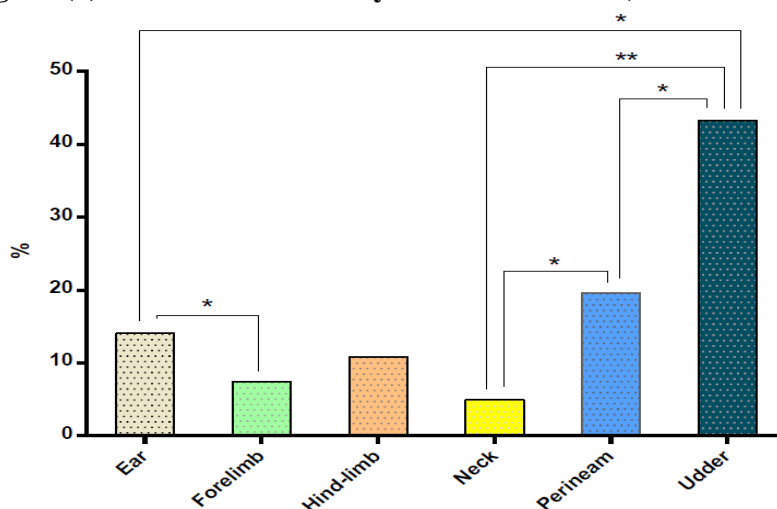


Figure (2): Distribution of ticks on different parts of infested cattle

The findings of this study were in agreement with other local studies such as Suleiman (2018) who recorded 36.77% infested cattle with hard ticks; but higher than those recorded in Sulaimanyia (11.8%) by Kadir et al. (2012); and in Baghdad (8.1%) by Hasson (2012) and 12.9% by Mallah and Rahif (2016); while were lowered than reported in Basrah (42.5%) by AL-Mayah and Abdul-Karim (2020). In comparison with other global studies, the overall prevalence of hard ticks in cattle was 85% in Pakistan (Ali et al., 2013), 67.5% in Iran (Ghashghaei et al., 2017), 40.26% in Ethiopia (Yalew et al., 2017) and 41.93% in India (Debbarma et al., 2018).

Morphologically, the findings revealed that all samples were hard ticks and belong to the genus of *Hyalomma* (Figure 3). Our results were similar with that reported by other researchers (Intirach et al., 2023; Luz et al., 2023).

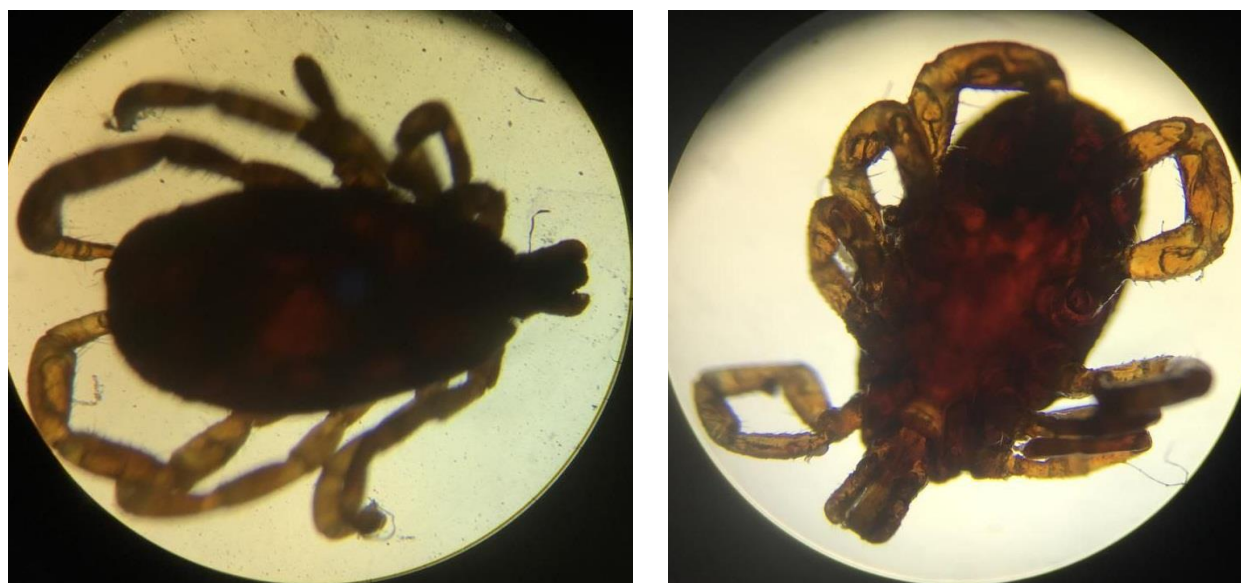


Figure (3): Morphological identification of study ticks based on their body characteristics

Targeting the *18S rRNA* gene, the PCR results confirmed that all samples belonged to genus of *Hyalomma anatolicum* (Figure 4). These results were in agreement with other locally studies carried out in Al-Najaf (Al-Fatlawi et al., 2018) and Babylon (Saadoon and Abid, 2021) provinces. Globally, the findings of this study were similar with that detected in Turkey (Aktas et al., 2006), Tunisia (M'ghirbi et al., 2008), China (Yu et al., 2018), Pakistan (Zeb et al., 2020), Egypt (Amira et al., 2021) and Saudi Arabia (Omer et al., 2021).



Figure (4): Electrophoresis of agarose-gel at 80AM, 100 volt for 1 hour; Lane M: Ladder marker (1500-100 bp); Lanes 1-9: representative positive samples at 452bp; Lane 9: Negative control

Many researchers in Iraq and neighboring countries have studied the epidemiology of persistent ticks and reported that dairy cattle grazing on pastures can become infected with ticks during grazing (Al-gharban and Dhahir, 2015; Desta et al., 2016; Kasaija et al., 2021). In Iraq, six species have been recorded including *Rhipicephalus annulatus*, *R. sanguineus*, *H. anatolicum*, *H. impeltatum*, *H. marginatum* and *H. excavatum* (Omar et al., 2007; Mohammed, 2015; Al-Abidi and Al-Ameri, 2021). An extensive study of some cattle herds in Basra province showed that *H. excavatum* can infect cows, calves and donkeys for the first time in Iraq. *Hyalomma asiaticum* was first recorded in animals in the south Iraq (Awad and Abdul-Hussein, 2006). In northern Iraq, Ismail and Omar (2021) recorded six species in Duhok city which belongs to two major tick genera, three species belonging to *Hyalomma* and three species of minor pollinators belonging to the genus *Rhipiciphalus*. A new species of the subgenus *Hyalomma* (*H. asiaticum asiaticum*) was reported for the first time in the Duhok region. In addition, a study was conducted to determine the prevalence of ticks of the family *Ixodidae* among horses and some domestic animals in Erbil province (Aziz and AL-Barwary, 2020). Another survey was conducted between September and February 2020 to determine the prevalence of the epidemic in the northern region of Basra province using 250 animal samples of different ages and sexes from the same region (Faraj et al., 2021). In other countries, *H. anatolicum* is well adapted to the dry climates of the Mediterranean and North Africa and other desert climates on both continents (Walker, 2014). In Saudi Arabia, a study on several dairy farms in the Al-Ahsa in the eastern region found *H. excavatum* (18.33%), *H. dromedarii* (17.63%), *H. anatolicum* (14.29%) and *R. turanicus* (14.04%), *H. impeltatum* (11.28%), *R. Praetextatus* (8.56%); *H. turanicum* (6.20%), *Haemaphysalis sulcata* (3.57%),

R. kohlsi (2.33%), *H. rufipes* (2.09%), *H. schulzei* (1.03%), *H. variegatum* (0.47%) and *A. gemma* (0.18%), (Abdally et al., 2020).

Conclusion

This study demonstrated that the great naturally occurring the populations of the ticks in cattle, and that *Hyalomma anatolicum* remains the almost widespread species of ticks in Iraq. In addition, adult ticks are the easiest to identify and male and female ticks of the same species may look different, while nymphal and larval ticks are very small and may be hard to detect. This study recommended investigating all ticks species found in all Iraqi areas using the morphological and molecular techniques due to the definite role of this external parasite in transmission of various diseases to both animals and humans.

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