

Genotypic analysis of ticks species infesting cattle in Al-Diwaniyah abattoir

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Abstract

Different vectors are considered critical for disease transmission between animals; however, ticks play a significant role in the dissemination of various infectious illnesses of animals and human importance. The current work was carried out to categorize ticks genetically of those isolated from cattle that entered Al-Diwaniyah abattoir. In the present study, 50 tick samples were collected and subjected to microscopic examination and genetic-based methods of polymerase chain reaction and partial gene sequencing, both utilized the mitochondrial cytochrome c oxidase subunit I (*COXI*) gene as a genotyping element. The findings of the microscopic examination showed that the ticks were from *Hyalomma* spp. Further analysis, the polymerase chain reaction revealed the genus of *Hyalomma* of the ticks, but when the PGS was performed, one sample of *H. detritum*, three samples of *H. excavatum*, and two samples of *H. marginatum* were identified. When the phylogenetic analyses were conducted, *H. detritum* showed close genetic similarity to an isolate from Spain EU827695.1. *H. excavatum* revealed similarity with isolates from India MK863382.1 and Turkey MT230050.1. In contrast, *H. marginatum* displayed close identity to an isolate from Iran (MG557555.1). In conclusion, these findings may indicate evolutionary links of the locally identified isolates to different world isolates, probably due to the trade-moving of animals.

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Introduction

Ticks are the most diversified arthropod vectors for human and livestock animals. Among such, ticks contain the most numerous collection of infectious agents. Human tick-borne pathogens, similar to the tick-borne associated diseases of farm animal species, are zoonotic diseases of livestock origin, in the epidemiology and ecology of tick-borne disease, complex interactions between the biotic and abiotic. Studies have established the theory that zoonotic organisms and their vectors are present in various environments, and landscape epidemiology has resulted in communicable diseases. Alterations in ticks density, in prevalence, as well as in animal interactions, transport, land use, and habitat modifications, can affect tick and tick-borne pathogenic demographic profiles (1-6).

There have been, are, and will continue to be modifications to the demographic makeup of tick communities. Changes of some of the known factors affecting tick populations will change the extent and abundance of these essential disease vectors. Synchronized changes are taking place among the complicated structures of micro- and macro-level factors requiring increased surveillance of these tick populations. In addition to many variables that may work together to create the observed changes, the geographical range and abundance of ticks are increasingly changing. A complex ecosystem of biotic and abiotic variables, including changes in animal and human demographics and behavior, influences both tick population densities and distributions. Aside from their immediate impact on ticks, a large number of tick-related micro-organisms and tick diseases, along with additional

danger to public health of tick-like paralysis and other toxicity is affected by biotic and abiotic elements (7-10). The dynamic, evolving complexity of the tick vectors and tick-borne diseases and dissemination needs persistent surveillance, including studying tick vectors, pathogens, and epidemiological characteristics. The collaboration of multidisciplinary teams involved in tick detection, identification, intervention approaches, awareness, study and testing, scientific and organizational preparation as well as public engagement promote community health responses to vector-borne diseases. As ticks and transmitted diseases do not acknowledge foreign borders, every tick and tick-borne infection network should become part of any comprehensive foreign disease tracking program offering accurate, evidence-based coordination for health agencies, healthcare professionals, other decision-makers, and stakeholders as well as the public (11,12).

According to the information mentioned above, the current work was carried out to categorize ticks genetically of those isolated from cattle that entered Al-Diwaniyah abattoir. The study might place a start point for epidemiological surveillance of ticks and tick-borne diseases in Al-Diwaniyah City, the southern provinces, and, eventually, the whole country.

Materials and methods

Samples and animals

The study was performed between February and May, 2019. The current work involved taking 50 tick samples from cattle that were prepared to slaughtering. The ticks were collected using ether-soaked pieces of cotton with forceps. Then, ticks obtained from each animal were placed in a separate 70%-ethanol-included container labeled with the animal sex, the collection date, and the part of the body from where the ticks were detached (13). After that, the ticks were transported to the Laboratory of Parasitology, Department of Microbiology, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq.

Microscopic examination

The ticks were intensively examined under the evaluation of Prof. Dr. Monyer Abdulameir Abd Alfatlawi (specialist in veterinary parasitology), Head of Department of Microbiology, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq. The ticks were visualized under a dissecting microscope and relying on standard information regarding methods and tick morphological features described by Soulsby (14) and Shubber *et al.* (15).
Genetic approaches

Polymerase chain reaction

Mitochondrial DNA was extracted using the gSYAN DNA Extraction Kit (Geneaid, USA) and following the kit

protocol. For the initiation of the process, 200 mg of each tick was used. A NanoDrop was utilized to evaluate the resulted DNA. The PCR was conducted using the primer set; F: AGGGTCCCCAGATATAGCATT and R: ACCGCCTGAAGGGTCAAAAA. The primer targeted a piece at 415 bp of the *COX1* gene (16). The AccuPower PCR PreMix Kit and relying on its instructions were employed for preparing the PCR reaction solution, which included using 5µl DNA, F and R primers 1.5µl 10 pmol/each, and 12 µl PCR water. Then, the mixture was placed in the kit tubes that had a ready-mixture of essential components such as DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, and MgCl₂. Later, the mixture was briefly vortexed and entered the thermocycler (MJ-Mini BioRad, USA) at 94°C, 1min, 1cycle of initial denaturation, 30 cycles of denaturation, annealing, and extension at 94°C, 0.5 min, 58°C, 0.5 min, and 72°C, 1 min, respectively, and the final extension at 72°C, 5min (16). After that, each well of a 1.5%-agarose gel pre-mixed with 3µl ethidium bromide was inserted with 10 µl PCR product except one well that received 5µl 100 bp ladder. The gel was run on 100 volts and 80 Amp for 1hr. Later, the gel and the bands were overviewed under a UV-light based visualizer.

Partial gene sequencing

The PGS was performed by the AB DNA sequencing system (Bioneer, Korea) using the purified PCR products of six samples and utilizing the same above primers. The sequencing data were deposited in the GeneBank database with an accession number for each isolate. These sequences were analyzed by recruiting the use of NCBI-based nucleotide websites and the MEGA 6.0 software for building the phylogenetic tree and identify comparable global isolates. The evolutionary distances were evaluated by the Maximum Composite Likelihood method.

Results

Microscopic examination

The findings of the ME showed that the ticks were from *Hyalomma* spp. The ME revealed characteristic morphological features of the mouth parts and genital pore (Figure 1A), Coxa (I, II, and III) (Figure 1B), and festoons (Figure 1C and D).

Polymerase chain reaction

Further analysis, the PCR revealed the genus of *Hyalomma* of the ticks. The amplification bands were at 415 bp (Figure 2).

Partial gene sequencing and phylogenetic analyses

The PGS uncovered that one sample of *H. detritum*, three samples of *H. excavatum*, and two samples of *H. marginatum* were identified. When the phylogenetic analyses were conducted, *H. detritum* showed close genetic similarity to an isolate from Spain EU827695.1 (Figure

3A). *H. excavatum* revealed similarity with isolates from India MK863382.1 and Turkey MT230050.1 (Figure 3B). At the same time, *H. marginatum* displayed close identity to an isolate from Iran MG557555.1 (Figure 3C).

due to the consumption of beef and lamb, such as Crimean Congo hemorrhagic fever (CCHF) that showed up in Iraq on several occasions, the last one in 2018 (17,18).

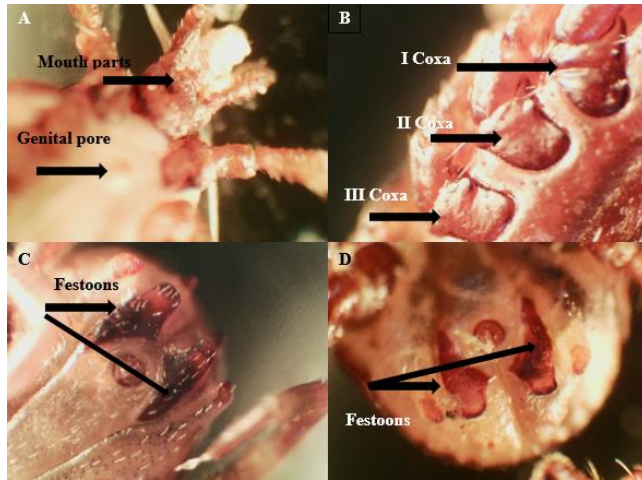


Figure 1: Microscopical examination of tick genus *Hyalomma*. A. Ventral surface: Mouth parts and genital pore. B. Ventral surface: I, II, and III Coxa. C&D. Ventral surface: Festoons. Olympus (2.5X).

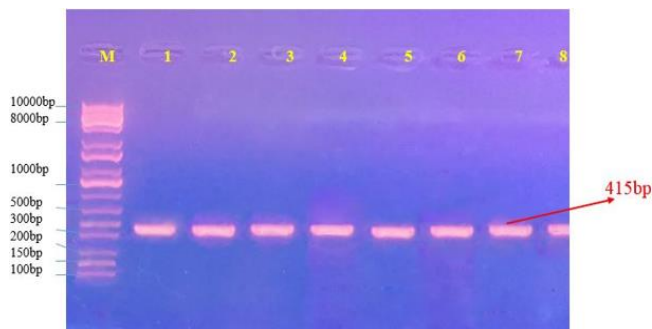


Figure 2: PCR analysis: Agarose gel electrophoresis 1% shows the DNA fragments of the *COX1* gene PCR products and the. Lane 1-8, 415bp Positive samples of genus *Hyalomma*, and M; a 10000 bp DNA ladder.

Discussion

Still, ticks and tick-borne-diseases represent a profound concern with a large global scale. These parasites exist with a broadly dense community of genera and species of human and animal involvement due to the direct effects of sucking blood from their victims and the infectious agents they transmit. For animals, infectious diseases from a long list can cause devastating effects on the health of wild and domestic animals such as protozoal illnesses; for example, babesiosis and theileriosis. On human health basis, many tick-borne diseases that represent a significant worrisome

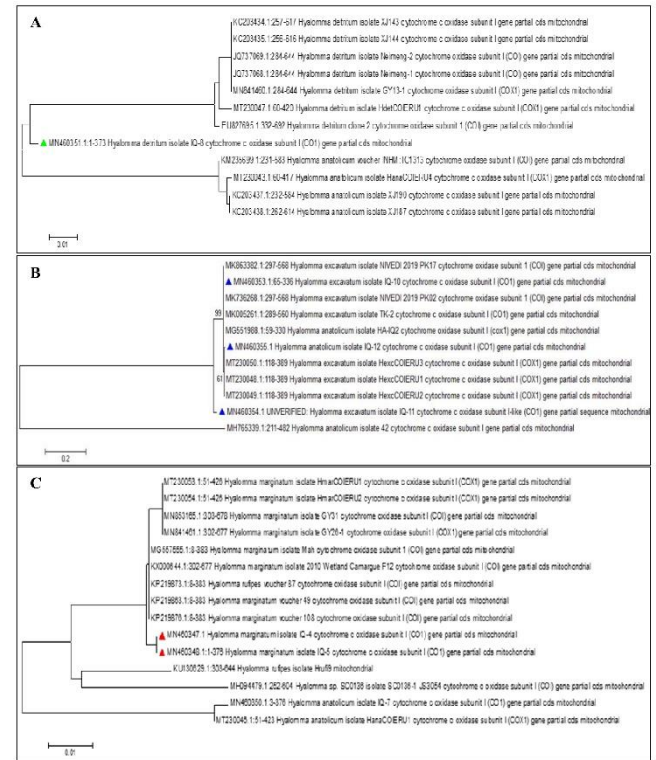


Figure 3: Phylogenetic tree based on the partial gene sequencing of *COX1* gene from *Hyalomma* ticks. A. *H. detritum*: Genetically similar to an isolate from Spain EU827695.1. B. *H. excavatum*: Similarity to isolates from India MK863382.1 and Turkey MT230050.1. C. *H. marginatum*: Similar to an isolate from Iran MG557555.1.

Many ixodid ticks are used as both the CCHF vector and reservoir. Nevertheless, for the ecology of this virus, ticks of the *Hyalomma* genus are especially important. CCHF outbreaks are strongly related to the world-famous *Hyalomma* spp. The *Hyalomma* genus is a small, robust species of ticks that are well suited to survive in arid biotopes. In Morphology (well-formed rounded head, strong legs), physiology (successful low moisture metamorphosis), actions (active host search), a significant degree of tolerance to hot, and stable in open habitations is evident. The primary host of most species are different animal types, and consequently, these ticks are generally broadened. The majority of this genus species have three hosts, but there are also species of one-host and two-hosts. Early phases feed primarily on mammals and birds, but some species non-adult ticks infect Reptiles of individual species. Together, 27 *Hyalomma* species invade domestic and wildlife animals, but a few species such as (*H. anatolicum*, *H. marginatum*, *H. nitidum*) species are

recognized as CCHF transmitters (19). The current results recorded the presence of *H. marginatum*, and according to the literature mentioned above, this species may assure the presence of the CCHF and probably its occurrence. Some of the *Hyalomma* species are considered rare, and if occurred, they go blood-feeding on cows/camels or cows, such as *H. detritum* and *H. schulzei*, respectively. Most *Hyalomma* species are active with high density during hot seasons rather than cold ones (20).

Several animal hosts, including pigs, camels, equines, horses, goats, and dogs, are accessible by *H. excavatum*, and it may target humans also. Based on the selection of hosts, it is served as a tick of two or three-host. The larvae and nymphs nourish on small vertebrates, birds, and humans with a low rate of presence in winters. This tick occurs in Egypt, Sudan, Eritrea, Iraq, Jordan, Italy, and Greece. CCHF is one of the major diseases that can be transmitted by *H. excavatum*. It can also transmit babesiosis, theileriosis, and rickettsiasis (21). The findings revealed evolutionary links of the locally identified isolates to different world isolates, probably due to the trade-moving of food animals (22).

Conclusion

The tick species recognized in the current study are among those that can transmit dangerous diseases to animals and humans, such as babesiosis and Crimean Congo hemorrhagic. The species identified represent significant similarity with global strains and may indicate evolutionary links of the locally identified isolates to the global isolates, probably due to the trade-moving of food animals.

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Conflict of interests

No conflict.

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مجزر الديوانية. تم جمع ٥٠ عينة من القراد التي خضعت للفحص المجهرى وفحص تفاعل سلسلة البلمرة ودراسة التتابع الجيني الجزئي، تم في الفحصين الأخيرين استخدام جين بيوت الطاقة المعروف بـ (COXI) لغرض التمييز الجيني. أظهرت النتائج التي توصلت إليها الفحوصات المجهرية إلى أن القراد كان من جنس قراد زجاجي العين. كشف فحص تفاعل السلسلة المتبلرة أيضا انتماء عينات القراد إلى جنس زجاجي العين، ولكن عندما تم إجراء فحص تتابع الجين الجزئي، اتضح أن إحدى العينات كانت تنتمي إلى نوع *H. detritum*، وثلاث عينات إلى نوع *H. excavatum*، وعينتين تعود إلى نوع *H. marginatum*. عندما أجريت تحليلات تطور الجينات، أظهر الفحص أن عزلة *H. detritum* تتشابه وراثيا مع عزلة من إسبانيا. كشف التحليل أيضا أن نوع *H. excavatum* تتشابه مع عزلات من الهند وتركيا. بينما أظهرت *H. marginatum* هوية قريبة لعزلات من إيران. يستنتج إلى احتمالية وجود روابط تطورية للعزلات المحلية مع العزلات العالمية بسبب النقل التجاري للحيوانات بين العراق وهذه الدول.

التحليل الجيني لأنواع القراد المتطفلة على الماشية المذبوحة في مجزرة الديوانية

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الخلاصة

يلعب القراد دورا مهما في نشر مختلف الأمراض المعدية ذات الأهمية الحيوانية والإنسانية. أجريت الدراسة الحالية لتصنيف القراد وراثيا في العينات المعزولة من الماشية التي كانت معدة للذباحة في