REVISTA DO INSTITUTO DE MEDICINA TROPICAL DE SÃO PAULO

JOURNAL OF THE SÃO PAULO INSTITUTE OF TROPICAL MEDICINE

CASE REPORT

http://doi.org/10.1590/S1678-9946202365037

A story of a lone star tick: an imported case of *Amblyomma americanum* (Linnaeus, 1758) infected with *Rickettsia amblyommatis* that parasitized a US traveler returning to Mexico

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ABSTRACT

In this study, we report the presence of a female *Amblyomma americanum* tick attached to a former resident of the East Coast of the United States who moved to Mexico city. The amplification and sequencing of gene fragments of the *16S-rDNA* and cytochrome c oxidase subunit 1 corroborated the identification of the species of the tick. Additionally, the presence of DNA of *Rickettsia amblyommatis* was confirmed. This work is the first report of an exotic tick of the genus *Amblyomma* in a traveler from the US to Mexico and represents the second record of an imported tick attached to humans in Mexico.

KEYWORDS: Lone star tick. Exotic tick. *Rickettsia amblyommatis*.

INTRODUCTION

Ticks are obligate hematophagous arthropods that are recognized as the main vectors of bacteria, protozoa and viruses to humans, right after mosquitoes[1-3]. The microorganisms carried by these hematophagous arthropods can cause diseases with a big impact on public health, such as Rocky Mountain Spotted Fever and Lyme Disease[1,2]. Humans are accidental hosts in the life cycles of different species of ticks. With the increasing globalization, the human population has also increased its mobility patterns, largely driven by international travel, migration and tourism[1]. Traveling to diverse geographical areas allows people to enter ecosystems with structured communities of ticks that visitors can accidentally carry to locations far from the native distribution of the ectoparasites. In recent decades, an increase in the number of reports of hard ticks from the Neotropical realm has been documented by visitors from various European countries, the USA and Canada[3,4], whereas the introduction of ticks from the Nearctic realm, such as *Dermacentor variabilis* and *Dermacentor andersoni*, has been reported by travelers that return to Panama and Brazil[5-8]. The introduction of imported ticks into new communities also leads to the carrying of bacterial pathogens. This phenomenon has been documented in the case of *Rickettsia africae* that infected a Brazilian traveler returning from South Africa[9]. This finding highlights the urgent need to train medical staff to be alert to deal with exotic pathogens outside their native distribution area. Medical personnel should have access to laboratory services and other trained personnel to help them identify ectoparasites and possible pathogens transmitted by imported tick species. This helps with early diagnosis and treatment, avoiding a delay in the treatment and

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Received: 16 December 2022

Accepted: 18 January 2023

thus complications due to a late diagnosis. In this article, we report the case of a female *Amblyomma americanum* that parasitized a former US resident who moved to Mexico a week prior to the study and we discuss the implications of monitoring exotic ticks for associated pathogens in Mexico.

Ethical statement

All procedures performed were in accordance with the ethical standards of the institution or practice in which the studies were conducted. The current report was approved by the Ethics and Research Committee of the Faculty of Medicine of UNAM (Universidad Nacional Autonoma de Mexico), FMED/CI/JMO/129/2017. The patient signed a written consent form that allows us to publish this case.

CASE REPORT

A 78-year-old woman, former resident of Cape Cod, Massachusetts, consulted an infectious disease physician upon her return to Mexico city due to the presence of a tick attached to her right thigh. The patient described that two days before traveling to Mexico city, she felt a mole in her thigh and, within a few days, perceived that it had grown. After bathing, she noticed that the site was tender, but felt no itching. A week after arriving in Mexico city, she consulted a dermatologist who detected an engorged tick causing a macular lesion of 0.5 cm in diameter. The tick was removed with tweezers and, while still alive, was placed in a small vial and sent for identification and detection of potentially transmittable pathogens.

Upon questioning, the patient reported feeling tired, experiencing dizziness, intermittent headaches, leg cramps, and pain in the limbs. She denied having a fever or arthralgia (joint pain). A 5-mL tube of whole blood with EDTA was taken from the patient for the identification of genetic material of multiple tick-borne pathogens. Prophylactically, antibiotic treatment with Doxycycline 100 mg every 12 h for 7 days was started, and the patient was scheduled for a follow-up evaluation.

The tick was studied at the Tropical Medicine Center of the Experimental Medicine Unit of the Faculty of Medicine of the Universidad Nacional Autonoma de Mexico. The identification of the tick species was performed using the specialized taxonomic keys of Keirans and Litwak, with the help of a Carl Zeiss Stemi 305 stereomicroscope[10]. Additionally, a photographic record of the most relevant characters was taken with the help of a Carl Zeiss Axiocam 208.

The genetic material of the specimen and 200 mL of blood from the patient were extracted using the Qiagen Blood and Tissue commercial kit, following the supplier's specifications, with an incubation period of 12 h for the tick as the only variation. To corroborate the identification of the specimen and as an endogenous control, a 400-bp fragment of the mitochondrial gene *16S-rDNA* and a 650-bp fragment of the cytochrome c oxidase subunit 1 (*CO1*) were amplified with the primers and thermal conditions previously reported[11,12].

Subsequently, a battery of oligonucleotides was implemented for the detection of bacterial (*Anaplasma*, *Borrelia*, *Coxiella*, *Ehrlichia* and *Rickettsia*) and protozoan (*Babesia*) pathogens[13], in tick and the patients' blood sample. The PCR was performed in a final volume of 25 µL, using 12.5 µL of GoTaq of Promega, 1 µL of each oligonucleotide (2 µM), 8.5 µL of DNA (300 ng) and 2 µL of nuclease-free water. The PCR products were run on 2% agarose gels, stained with Midori, at 100 V for 45 min.

Positive products were sent for purifying and sequencing to Macrogen, Korea. The recovered electropherograms were visually inspected and edited in Chromas. The consensus sequences were compared with those of species validated in GenBank by local alignment, using the BLASTn algorithm. From each sequence, the coverage percentage and the similarity with respect to the valid species were obtained. Additionally, the phylogenetic reconstruction was performed by concatenated analysis of our sequences and those available in GenBank, applying the Maximum Likelihood method with 10,000 bootstrap repetitions (gaps eliminated) in IQ-TREE.

The patient's blood sample tested negative for the presence of genetic material from the battery of tick-borne pathogens analyzed. In the follow-up visit, the patient confirmed that her initial signs had subsided, and she denied having fever in the days after starting antibiotic therapy, for which she was discharged.

The tick was identified as a female *Amblyomma americanum* based on the following morphological characters: dentition of the middle portion of the hypostome 3/3 and the presence of a whitish spot at the base of the scutum (Figure 1).

Both tick mitochondrial genes were successfully amplified: the *16S-rDNA* sequence exhibited a similarity of 99.53% (426/428 bp) with a sequence from *A. americanum* collected in Kansas (GenBank Accession Nº L34313.1), and the sequence from *CO1* exhibited 100% identity (635/635 bp) with the reference genome of *A. americanum* from Georgia (GenBank Accession Nº KP941755.1). Additionally, we detected the presence of *Rickettsia* DNA. Using BLAST analysis, the consensus sequences of the three genes were 100% (422/422 bp), 99.62% (778/781 bp) and 100% (468/468 bp) identical to the corresponding sequences of the *16S-rDNA* (CP015012.1), *ompB*

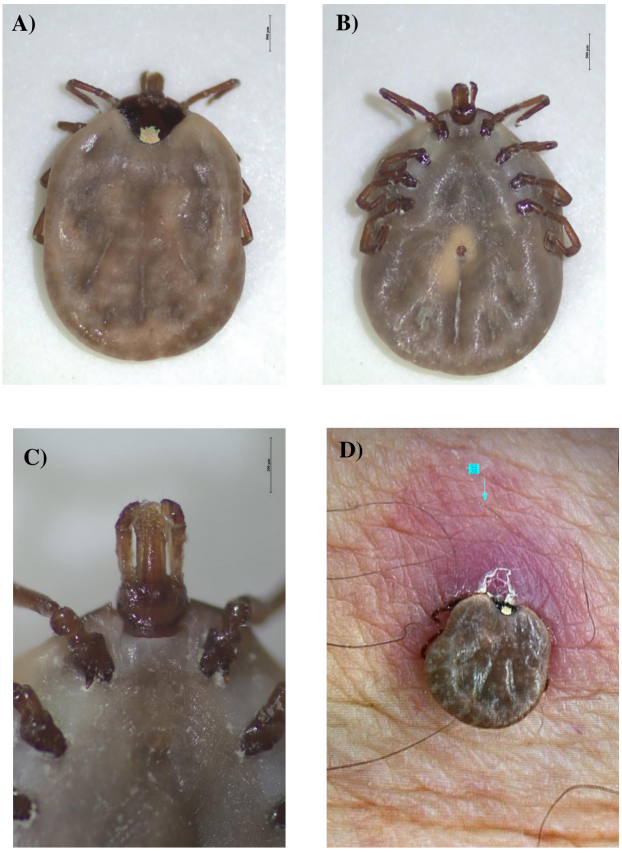

Figure 1 - Female of *Amblyomma americanum*: A) dorsal view; B) ventral view; C) hypostome and coxa 1; D) attached to the patient's right thigh.

(CP015012.1) and *ompA* (MN313363.1) genes of *Rickettsia amblyommatis* from US, respectively. Maximum likelihood phylogenetic analysis confirmed the identity of the bacteria as *R. amblyommatis* (Figure 2). None of the other bacterial or parasitic agents were detected. Sequences were deposited in GenBank under the following accession numbers: for *Rickettsia*, *ompA* (MT533590), *16S-rDNA* (MT535907), *ompB* (MW603199), for *A. americanum*, *16S-rDNA* (MT527093) and *CO1* (MW386401).

DISCUSSION

Although there are historical reports of *A. americanum* parasitizing cattle and horses in the states of Coahuila, Nuevo Leon, Tamaulipas, and Veracruz, there is no published record of the association of *A. americanum* with humans in Mexico[14]. This tick of Nearctic origin is one of the most studied in the USA due to its anthropophilic habits and its apparent aggressiveness towards humans passing through the woods[3].

The species *A. americanum* is important in terms of public and veterinary health because multiple disease-causing agents such as *Ehrlichia ewingii*, the etiological agent of human and canine granulocytic ehrlichiosis, as well as *Borrelia lonestari*, a relapsing fever species distinct from *Borrelia burgdorferi* s.l., the causative agent of Lyme Disease[15,16], and finally, *R. rickettsii*, which causes Rocky

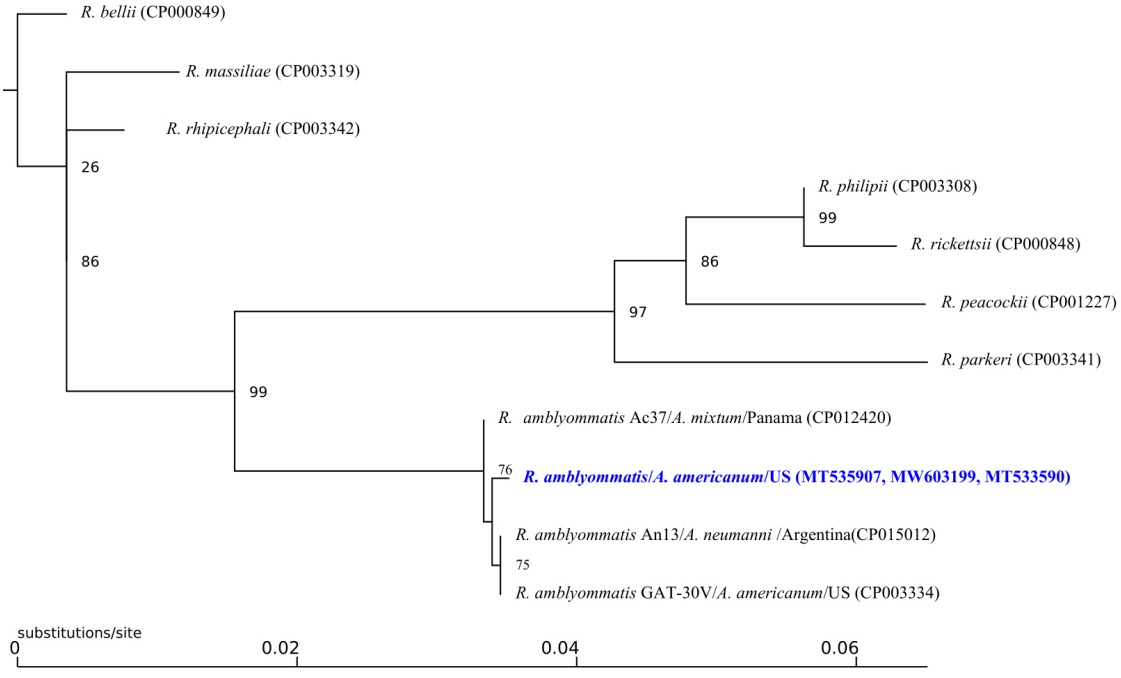

Figure 2 - Maximum-likelihood phylogenetic tree generated by using the Hasegawa–Kishino–Yano's model (HKY) with concatenated segments of the *ompB*, *ompA*, and *16S-rRNA* genes (1,676 bp total) of several members of the genus *Rickettsia* detected in ticks from America. Sequences generated in this work are highlighted in bold and blue. Bootstrap values > 50% are indicated at the nodes (–ln = –637.973). GenBank accession numbers are provided. The scale bar indicates nucleotide substitutions per site.

Mountain Spotted Fever[16], have been detected in this tick species. However, epidemiological studies have shown that populations of *A. americanum* exhibit a low prevalence of *R. rickettsii* (less than 1%)[16], which is why they are not considered to play an active role in maintaining the wild cycle of transmission of these bacteria[15,16]. This may be largely due to the fact that *A. americanum* populations are infected at a high prevalence (up to 85%) by another species of *Rickettsia* belonging to the spotted fever group-1, the *Rickettsia amblyommatis*, which is the most widely distributed species of tick-associated rickettsia in the Americas, reported in more than 14 species of ticks of the genus *Amblyomma* of one Nearctic and at least ten Neotropical countries[17]. This species has received special attention in recent years because experimental studies have shown a protective effect in the guinea pig model against highly virulent strains of the deadly *R. rickettsii*[18,19].

Rickettsia amblyommatis has been identified as the main bacterial agent detected in exotic hard ticks recovered from tourists traveling to the US and Latin American countries[4-8]. These findings are relevant because the potential role of this species as a human pathogen is still being discussed[18,19].

However, it is noteworthy that its presence can induce the production of antibodies in the mammalian host, which is why the serological immunofluorescence tests against *R. rickettsii* can show false positive results due to a previous Rocky Mountain Spotted Fever infection[18,19]. Thus, it is imperative to recover the ectoparasites of patients for accurate identification of potential circulating pathogens and to establish early treatment schemes before the first symptoms appear[2].

Previous reports in Latin America have identified the presence of two species of ticks (*D. andersoni* and *D. variabilis*) from US residents and/or visitors who had returned from or traveled to Panama and Brazil[4-8]. In Mexico, there is only one recorded case of an imported tick. *Ixodes ricinus* was recorded only in one study, where the tick was found on a traveler who had returned from Germany[20]. However, monitoring potential pathogens was not performed in that study, which is why our work provides the first published approach to tick-borne pathogens in exotic tick surveillance at the national level. For this reason, the need to reinforce the monitoring of imported ticks that enter Mexico is imperative.

AUTHORS' CONTRIBUTIONS

Patients' medical care: PV; conceived and designed the experiments: PV, EG, BS, IB and SSM; performed the experiments: EG and SSM; analyzed the data: PV, EG, BSS, HHJ, VAR, IB and SSM; contributed to reagents/materials/analysis tools: PV, BSS, HHJ, VAR, IB and SSM; wrote the article: PV, EG, BSS, HHJ, VAR, IB and SSM.

CONFLICT OF INTERESTS

The authors declare to have no conflict of interests.

FUNDING

This work was supported by the UNAM-PAPIIT IG201221 and CONACyT 6682 grants.

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