



Occurrence of *Amblyomma mixtum* on the water buffalo (*Bubalus bubalis*) in Mexico

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ABSTRACT

The water buffalo (*Bubalus bubalis*) is an Asian species of bovine that was introduced in Mexico in 1992 as an alternative for milk and meat production. To date, no surveys have been conducted to identify ticks acquired by water buffaloes since their arrival in the country. Here we report, for the first time, the presence of *Amblyomma mixtum*, a neotropical tick in the *Amblyomma cajennense* complex, found on water buffaloes from Mexico and discuss its possible implications in veterinary public health for the region.

1. Introduction

The water buffalo (*Bubalus bubalis*) is an Asian species of bovine that was introduced to the Americas several times since 1895. This species was introduced to Mexico much later in 1992 as an alternative for milk and meat production. Due to their ability to readily adapt to neotropical conditions, water buffaloes were rapidly commercialized and disseminated across the country, particularly in the coastal states of Campeche, Tabasco, Oaxaca and Veracruz.

In Veracruz, water buffaloes are kept under the same conditions as dairy cattle and in some production units both groups of bovines coexist in close proximity to one another. Few studies have been conducted to identify the ectoparasites, particularly ticks, that may be present on water buffaloes in the Americas, some of which they may share with the cattle.

In Brazil, only a few species of ticks have been recorded on water buffaloes including *Amblyomma cajennense* sensu stricto, *Amblyomma sculptum*, *Amblyomma maculatum*, *Dermacentor nitens* and *Rhipicephalus (Boophilus) microplus* (do Nascimento Corrêa et al., 2012; Nava et al., 2014; do Nascimento Corrêa et al., 2012; Batista et al., 2018). Several of these species are vectors of microorganisms that can have an impact on animal health and production as well as human health, such as *Babesia bovis* (*R. (B.) microplus*) and *Rickettsia parkeri* (*A. maculatum*).

However, there are no additional reports of ticks associated with this species in other countries of the Americas, especially in Mexico, where the production of water buffaloes is around 50,000 head. In this work we report for the first time the presence of a tick species on water buffaloes from the states of Veracruz and Oaxaca, Mexico, and discuss the possible implications on veterinary public health.

2. Materials and methods

Tick surveys were carried out from July to August 2017 in two municipalities, Juan Rodríguez Clara in the state of Veracruz, and Matias Romero in the state Oaxaca, Mexico. The climate of this region is tropical, hot and humid, with a mean annual temperature of 23.4 ± 0.5 °C, an annual rainfall of 1991 ± 392 mm and a mean relative humidity of 85% (INAFED, 2017). The primary water buffalo production system is similar to that of beef and is based on extensive grazing system. There are no tick control programs in place for any of the farms utilized for the tick surveys.

Two water buffalo farms were visited to collect ticks. On each farm, at least 30 water buffaloes were inspected from head to tail, starting along the back and then to the ventral region, immobilizing animals by using a steering sleeve. Ticks were removed from hosts' body using forceps and each one was kept in a small flask with 70% ethanol,

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correctly coded with the natural region number, farm and buffalo number. Collected ticks were then transported to the Parasitology Laboratory FMVZ-UV, located in Veracruz, Mexico. All ticks were classified according to the morphologic characteristics described by Guzman-Cornejo et al. (2011) and Nava et al., 2014, using a Motic® stereoscope microscope (Motic, British Columbia) with a Moticom 1000 camera. In order to visualize additional morphological characters, a subsample of specimens was prepared for scanning electron microscopy using a conventional method previously described by Corwin et al. (1979). All the samples were placed in a FEI™ Quanta™ 250 FEG scanning electron microscope (FEI™, Hillsboro, OR) to take the micrographs.

To confirm the specific identification of the collected ticks, the 16S rRNA gene of two females and two males were sequenced according to Norris et al. (1996) using the DNA extraction protocol reported by Ballados-González et al. (2018). For PCR amplification, the reaction mixture consisted of 12.5 µL PCR Go Taq® solution (Promega, Madison, WI), 100 ng of each primer, 6.5 µL nuclease-free water, and 30 ng DNA in a final volume of 25 µL. PCR products were submitted for purification and sequencing to Laboratorio de Biología Molecular y de la Salud, Universidad Nacional Autónoma de México. Recovered sequences were assembled using Genious (Biomatters Ltd., Newark, NJ) and were compared with other tick species deposited in Genbank using the BLAST tool. Additionally, our sequences were deposited in Genbank under the following accession numbers: Submitted.

3. Results

A total of 291 ticks (237 ♀, 54 ♂) were collected from the two surveyed farms (Table 1). Most of the ticks were concentrated in the underarm, ventral and perianal region (Fig. 1). We detected a single tick species, *Amblyomma mixtum* due to the combination of the following morphological characters (Fig. 2): **Female**: scutum with low punctuations, presence of a very marked marginal groove and eyes. Notal setae densely distributed in the posterior half of notum (Fig. 2A); festoons with two lines of two or three stout setae in each festoon, and tubercles visible dorsally (Fig. 2C; porose areas of the female with marked punctuations. Scutum with punctuations concentrated on the margin of the scutum (Fig. 2E); coxa I with two spurs, the external larger than the internal (above half of the internal), genital aperture in form of a marked U (Fig. 2G); dental formula 3/3 (Fig. 2H). **Male**: dorsal view of the male with few punctuations, concentrated mainly in the marginal area (Fig. 2B); marginal groove which extends to the posterior region and reaches the festoons (Fig. 2D); presence of marked cornuas (Fig. 2F).

We recovered sequences from two male and two female *A. mixtum* which were 99–100% identical between them and exhibited a 100% similarity with two previous *A. mixtum* haplotypes detected in ticks parasitizing horses and cattle in the region (Genbank Accession numbers MG930055 and MG930056).

The prevalence recorded in both localities was over 40%, with an average abundance of three ticks per water buffalo surveyed and an average intensity of more than five ticks per water buffalo parasitized

Table 1

Prevalence, mean abundance, and median abundance of *Amblyomma mixtum* recovered from water buffaloes (*Bubalus bubalis*) from two farms in Veracruz and Oaxaca, Mexico. n, Host inspected; HP, host parasitized; TT, total of ticks collected; %, prevalence; A, mean abundance; MI, mean intensity.

Locality	Host inspected	Host Parasitized	Total of Ticks collected	%	MI
Juan Rodriguez Clara, Veracruz	40	18	111	45	6.16
Matías Romero, Oaxaca	35	21	166	60	7.90



Fig. 1. Occurrence of *Amblyomma mixtum* in the perianal region of a water buffalo (adult stage).

(Table 1).

4. Discussion

This is the first report of *Amblyomma mixtum* on water buffaloes in the Americas, particularly in Mexico. Although in Brazil the presence of a nearby species, *Amblyomma cajennense sensu stricto*., has been recorded in a few previous works (do Nascimento Corrêa et al., 2012; Batista et al., 2018), the record of *A. mixtum* is relevant because this species exhibits a greater plasticity and can be found in a large number of environments including semi-arid grasslands and subtropical secondary forests with preference of localities with sustained high temperatures (Estrada-Peña et al., 2004). Additionally, *A. mixtum* parasitizes a wide range of hosts and can take advantage of the nutritional resources present in the area (Guzmán-Cornejo et al., 2011; Nava et al., 2014; Rodríguez-Vivas et al., 2016). *A. mixtum* has adapted very well to utilizing cattle as a host and it is not surprising that it could be found on another bovine species such as the water buffalo.

The impact of *A. mixtum* on veterinary health is well documented (Labruna, 2009; Guzman-Cornejo et al., 2011). *A. mixtum* is a large tick that can cause anemia if infestations are large enough (Brites-Neto et al., 2015). Sites where *A. mixtum* attach and bite their hosts can become infected or can be colonized secondarily by larvae of flies that cause myiasis. Additionally, it is known that *A. mixtum* has been shown to transmit bacterial agents among cattle, including *Anaplasma marginale* (Rodríguez-Vivas and Domínguez-Alpizar, 1998), and *A. mixtum* is an important vector of *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever in human populations (Bustamante and Varela, 1946). Since the 16S haplotypes detected in this study are identical to those previously detected from ticks attached to cattle and horses in Veracruz (Aguilar-Domínguez et al., In Press), it could be possible that this tick species can exchange pathogens between domestic animals and water buffaloes. The characterization of the infestations found similar results as those from previous studies on cattle parasitized by hard ticks, in particular *Amblyomma variegatum* in Africa, where abundances of 8.42 and average intensity of 11.90 ticks per host parasitized were

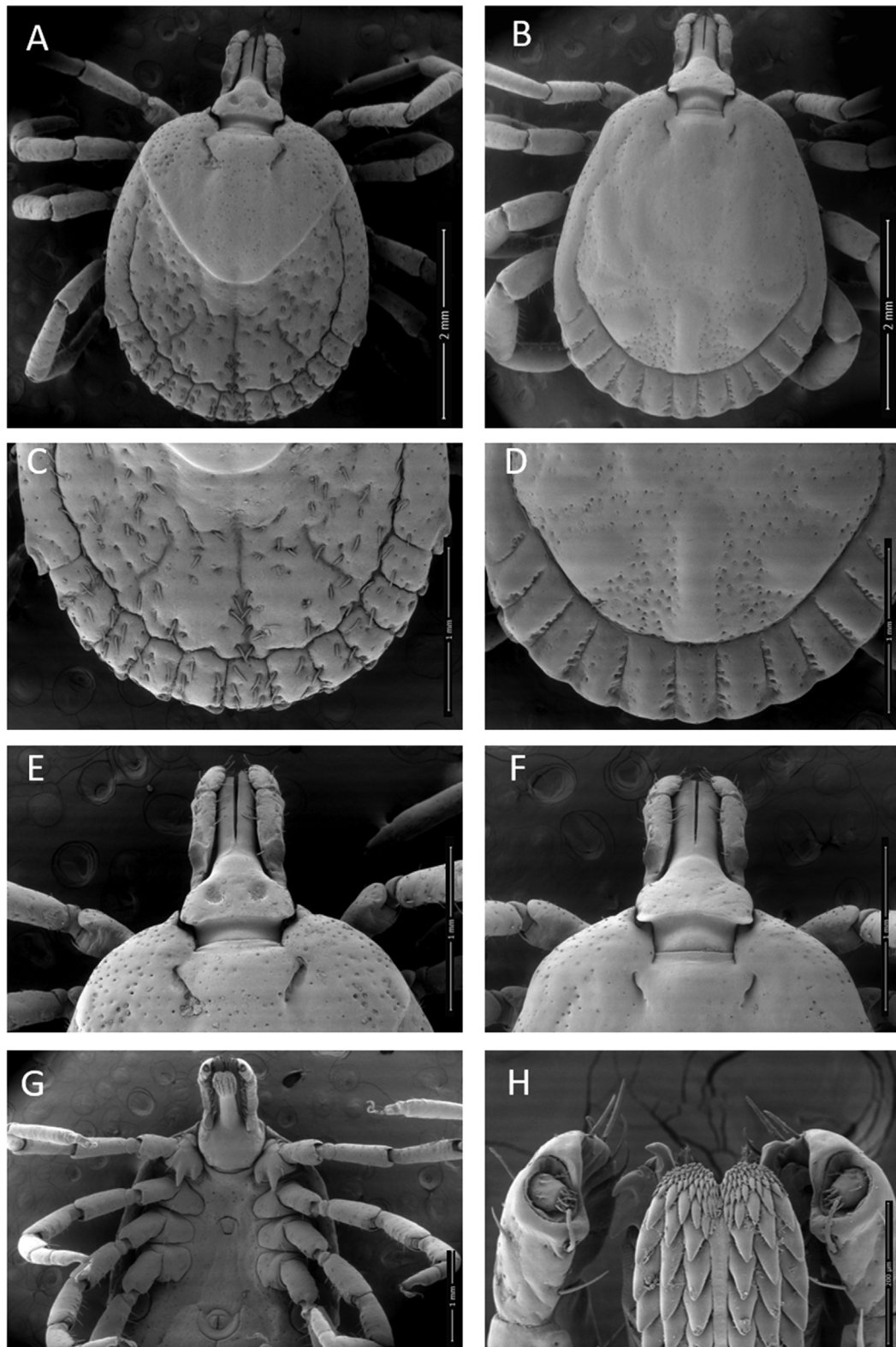


Fig. 2. Morphological characters used for *Amblyomma mixtum* identification: Dorsal view A) Female, B) Male; Zoom of the notum of the female C) and the scutum of the male D); Dorsal view of the capitulum of the female E) and male F); Ventral view of the female G); Dentition of the hypostome H).

recorded (Rodríguez-Vivas et al., 2016). The abundance of ticks recovered in both locations demonstrates the existence of ticks that have begun to establish close associations with water buffaloes. However, further studies are necessary to carry out systematized investigations to identify the impact of *A. mixtum* on water buffalo populations in order

to elucidate its relevance in the epidemiology of some tick-borne diseases, as well as determine the type and intensity of control measures that should be established.

Ethics approval and consent to participate

This project was approved by the Bioethics and Animal Welfare Commission of the Veterinary and Animal Science School (Facultad de Medicina Veterinaria y Zootecnia) of the State University of Veracruz (Universidad Veracruzana). Consent was obtained from the buffalo producers.

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