Infestation of Cattle with the Tick *Amblyomma mixtum* in the States with the Highest Cattle Inventory in Mexico

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ABSTRACT

Objective: The *Amblyomma mixtum* tick is one of the main parasites affecting cattle in Mexico. Epidemiological records of *A. mixtum* in leading states in the national cattle industry, such as Jalisco, Chiapas, Michoacán, Tabasco, and Veracruz, are outdated since this tick species was previously classified as *A. cajennense*. The objective was to update the records of the *A. mixtum* tick in the states of Jalisco, Chiapas, Michoacán, Tabasco, and Veracruz, as well as the main ixodicide molecules used for its control.

Methodology: From March 2022 to July 2023, ticks were collected from cattle in 46 bovine production units (BPUs) distributed in the states mentioned above. Identification was performed using standardized taxonomic keys. Scanning electron microscopy was performed on specimens corresponding to *A. mixtum*.

Results: Out of a total of 619 specimens of the genus *Amblyomma* from 22 PBUs, it was confirmed that 100% correspond to the species *A. mixtum*. Amitraz, an ixodicide molecule belonging to the chemical class of amidines, was used in 63.2% of the BPUs where the presence of *A. mixtum* was also reported.

Conclusions: This research confirms cattle infestation with *A. mixtum* in states with the highest cattle production in Mexico. Further studies with a focus on resistance and extension are required to prolong the usefulness of available tools, including ixodicides, for integrated control of *A. mixtum* infestations where this tick infests cattle in Mexico.

Keywords: Livestock, *Amblyomma mixtum*, distribution, acaricides, amitraz.

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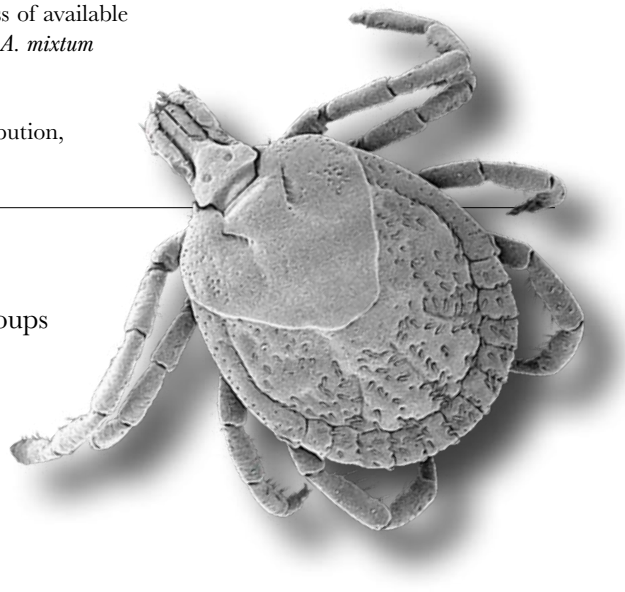

INTRODUCTION

Ticks are one of the most important groups of ectoparasites that affect cattle globally (Pérez de León *et al.*, 2020). In addition to feeding off the blood of their host, due to their hematophagous habit,

several species of ticks are vectors of pathogens that can cause high morbidity and mortality in cattle, as well as in other domestic animal species, wildlife and humans (de la Fuente *et al.*, 2023). Ticks belong to the Phylum Arthropoda, Class Arachnida, Order Acarina and within it, the suborder Ixodoidea; the family Ixodidea derives from this, which is divided in four: Ixodidae, Argasidae, Nuttalliellidae and Deinocrotonidae (Polanco-Echeverri and Ríos-Osorio, 2016; Peñalver *et al.*, 2017). Around 109 tick species have been reported in Mexico (Guglielmone *et al.*, 2010; Pérez *et al.*, 2014); from these, *Amblyomma mixtum* is considered in veterinary medicine as one of the species of highest economic impact for the livestock industry due to the decrease in cattle health and productivity in Mexico that it causes (Almazán *et al.*, 2018).

The genus *Amblyomma* is found in the subdivision Ixodidae and classified as the third largest (Rivera-Páez *et al.*, 2016), from which half of the species classified in it are located in the American continent (Guglielmone and Nava, 2006). Likewise, *A. mixtum* was originally described in 1844 (Koch, 1844), although the species was classified as *A. cajennense* (Beati *et al.*, 2013). Recent studies based on morphology, genetics and reproduction confirmed that *A. mixtum* is a species that is part of the *A. cajennense* complex (Beati *et al.*, 2013; Nava *et al.*, 2014). *A. mixtum* is a tick whose biological cycle has three hosts and in addition has the widest range of distribution, from the south of Texas to Ecuador (Aguilar-Domínguez *et al.*, 2021). Although cattle infestations highlight its importance in veterinary medicine, the larvae, nymphaea, and adults of *A. mixtum* can infest horses, dogs, several wildlife species such a white-tailed deer, and humans in the Neotropical region (Rodríguez-Vivas *et al.*, 2016; Aguilar-Domínguez *et al.*, 2019).

The cattle population in Mexico in 2022 was around 36.6 million heads including meat and dairy cattle (Consulta SIAP, 2022). Out of the 32 states in Mexico, the presence of *A. mixtum* has been reported in 16 states (Nava *et al.*, 2014; Castillo-Martínez *et al.*, 2020). However, information is necessary about cattle infestation of the main livestock producing states in the country, among them Veracruz, Jalisco, Michoacán, Tabasco, and Chiapas (Juárez and Salas-González, 2023).

It is also necessary to understand that ixodicides are used to treat cattle infested with ticks that can include *A. mixtum*. The use of these molecules is the most common practice to control ticks in cattle in Mexico. Chemical ixodicides are used primarily to control the *Rhipicephalus microplus* tick, which is considered as the most important tick for cattle that can be co-infested with *A. mixtum* in tropical and subtropical areas of the country where these two species of ticks coexist (Rodríguez-Vivas *et al.*, 2017; Almazán *et al.*, 2018).

The objective of this study was to update the records on cattle infestation with *A. mixtum*, as well as the main molecules of ixodicides used for their control in the states of Jalisco, Chiapas, Michoacán, Tabasco, and Veracruz.

MATERIALS AND METHODS

Sampling sites

Simple convenience sampling was carried out in a total of 46 Bovine Production Units (BPUs) distributed in the states of Chiapas, Jalisco, Michoacán, Tabasco and Veracruz,

in the period from March 2022 to July 2023. These states, in addition to having the largest populations of cattle, have geographic locations favorable for the development of the biological cycle of *A. mixtum*, because they are completely or partially within the Neotropical region of the country (Morrone, 2005). When sampling, the location of the BPUs was established based on the methodology described by Aguilar-Domínguez *et al.* (2021), where the potential distribution of this species of ticks in the states previously mentioned is indicated (Table 1).

Collection of specimens and data of the BPU/Host

In each BPU, cattle were inspected from head to tail against the hair to locate the specimens and with the help of entomological pliers, light tractions from the top down were carried out to remove them (CDC, NCEZID, 2018). Each tick was conserved individually in ethanol at 70% v/v. In addition to the collection, a survey was applied by BPU to gather data on the control of ectoparasites, including the use of acaricides to control infestation with ticks, and the presence of one or more domestic or wild species in contact with the cattle. The coordinates of the sampling points were taken through the implementation of GPSmap GARMIN® (Table 1). The ticks collected were transported to the Parasitology Laboratory, located in the Diagnostics Unit of the Torreón del Molino Ranch from the Veterinary Medicine and Zootechnics School of Universidad Veracruzana. The ticks were processed in the laboratory according to the methodology described by Aguilar-Domínguez *et al.* (2019).

Morphological identification and electron microscopy

The identification of specimens was conducted through morphological taxonomic keys established by Guzmán-Cornejo *et al.* (2011) for *Amblyomma cajennense* and by Nava *et al.* (2014) for *Amblyomma mixtum*. With the aim of describing the main morphological structures, ticks identified as *A. mixtum* and after determining through inspection that the specimen was complete, were selected for the processing of scanning electron microscopy, following the modified methodology from Corwin *et al.* (1979) and Dixon *et al.* (2000). For this purpose, cleaning with extra-fine Dumont® pliers and brushes was carried out and they were placed in ethanol at 70% v/v; and the sonic process was continued, where they are placed in a Cole-Parmer® 8848 ultrasonic cleaner for 5 min with 2 repetitions.

Dehydration was conducted through gradual ethanol in 80 and 90% for 90 minutes in each concentration and 3 changes in absolute ethanol for 30 minutes in each change. After this cycle ends, they are placed in xylene at a temperature of 40 °C for 24 hours. Then, they were transferred in absolute ethanol for 30 minutes with three repetitions. To dry the material, a critical point drier was used of the brand Quorum® model K850 using CO_2. After this phase is completed, the dry ticks were placed on aluminum slides adhered with double-face carbon conductive tape, to cover the specimens with gold using a Quorum® model Q150R S metal ionizer. To finish, the specimens were analyzed with the scanning electron microscope of field emission FEI Quanta 250 FEG.

Table 1. Location of sampling sites by state and municipality.

State	Municipality	Locality	Geolocalization	Altitude MASL
Chiapas	Tapachula	Oro Verde	14° 50' 31.2" N, 92° 20' 58.3" W	0
		Corlai	14° 52' 25.6" N, 92° 21' 46.4" W	0
		Tapachula	14° 54' 22.0" N, 92° 17' 42.1" W	0
	Mapastepec	Adolfo López Mateos	15° 26' 48.7" N, 92° 59' 56.5" W	0
		Dos Pasajes	15° 28' 57.1" N, 93° 03' 44.5" W	0
		La Trinidad	15° 32' 31.6" N, 92° 59' 56.6" W	0
	Pijijiapan	Puente Margaritas	15° 35' 37.0" N, 93° 02' 41.0" W	0
		La Herradura	15° 35' 48.1" N, 93° 04' 35.1" W	0
		Gabriel Toledo	15° 34' 57.2" N, 93° 10' 13.9" W	0
		Las Carmelitas	15° 36' 04.1" N, 93° 10' 41.0" W	0
		Caña Brava	15° 38' 34.1" N, 93° 10' 39.6" W	0
		Pijijiapan	15° 42' 16.0" N, 93° 13' 38.9" W	0
	Tonalá	Agua Prieta	16° 00' 59.5" N, 93° 37' 46.6" W	0
	Palenque	Palenque	17° 32' 17.9" N, 91° 58' 21.0" W	0
Jalisco	Cihuatlán	Emiliano Zapata	19° 17' 36.7" N, 104° 42' 50.4" W	0
	La Huerta	El Progreso	19° 19' 31.4" N, 104° 49' 11.8" W	0
	Concepción de Buenos Aires	Concepción de Buenos Aires	19° 59' 55.4" N, 103° 15' 50.3" W	0
	Encarnación de Díaz	Concepción de Buenos Aires	20° 00' 39.5" N, 103° 15' 18.1" W	0
		La Cuadra	21° 31' 39.7" N, 102° 11' 29.5" W	0
Michoacán	Lázaro Cárdenas	Lázaro Cárdenas	17° 59' 01.1" N, 102° 14' 23.2" W	0
		Buenos Aires	18° 02' 12.9" N, 102° 16' 46.2" W	0
		Playa Azul	17° 59' 20.7" N, 102° 22' 56.8" W	0
		El Habillal	18° 01' 22.0" N, 102° 21' 40.6" W	0
		El Habillal	17° 59' 34.7" N, 102° 22' 53.7" W	0
	Arteaga	Arteaga	18° 20' 31.1" N, 102° 17' 27.4" W	0
		Arteaga	18° 21' 08.2" N, 102° 17' 15.9" W	0
	Aquila	El Aguacate	18° 29' 54.4" N, 103° 17' 48.2" W	0
	Tuxpan	El Malacate	19° 32' 44.9" N, 100° 28' 40.4" W	0
	Morelia	Cañada de Buena Vista	19° 33' 15.6" N, 101° 15' 12.0" W	0
		Santiago Undameo	19° 35' 25.9" N, 101° 15' 28.0" W	0
		Santiago Undameo	19° 35' 34.4" N, 101° 15' 30.6" W	0
	Zamora	Romeo de Guzmán	20° 02' 29.7" N, 102° 14' 55.4" W	0
	Cuto de la Esperanza	Cuto de la Esperanza	19° 43' 43.0" N, 101° 20' 31.8" W	0
Tabasco	Balancán	El Tornillo	17° 50' 37.6" N, 91° 31' 00.6" W	25.5
	Tacotalpa	Puente de Piedra	17° 35' 33.2" N, 92° 37' 10.9" W	0
	Huimanguillo	Ocuapan	17° 49' 49.4" N, 93° 30' 08.2" W	0
Veracruz	Cosoleacaque	Calzadas	18° 07' 49.0" N, 94° 31' 36.0" W	0
	San Juan Evangelista	Rancho Azteca	17° 39' 30.2" N, 94° 58' 00.1" W	0
	Juan Rodríguez Clara	Perseveranza	17° 56' 19.1" N, 95° 11' 28.1" W	0
	Coatzacoalcos	Matilla de Conejo	18° 04' 11.3" N, 95° 15' 29.7" W	0
		Matilla de Conejo	18° 03' 49.3" N, 95° 16' 22.3" W	0
		Matilla de Conejo	18° 10' 24.4" N, 94° 15' 56.0" W	0
	Isla	El Tigre	17° 56' 09.2" N, 95° 21' 42.0" W	0
	Túxpam de Rodríguez Cano	Guillermo Prieto	18° 10' 24.4" N, 94° 15' 56.0" W	0
		San Isidro	18° 04' 52.1" N, 95° 32' 02.6" W	0
		Lindavista	20° 48' 58.6" N, 97° 14' 20.2" W	0
	Manlio Fabio Altamirano	Mata Loma	19° 08' 14.2" N, 96° 18' 00.7" W	0
	Tlapacoyan	San Francisco	20° 02' 08.0" N, 97° 06' 22.7" W	0

Statistical analysis

Descriptive statistics were conducted to obtain the frequency and percentage of *A. mixtum*, according to their taxonomic identification and to the molecule used. Likewise, the sampling sites were georeferenced to perform a detailed map with the information gathered through the Maptive GIS Mapping Software.

RESULTS AND DISCUSSION

In 22 BPUs of the states of Chiapas, Jalisco, Michoacán, Tabasco and Veracruz, a total of 619 *Amblyomma mixtum* ticks were collected from cattle (Figure 1).

Table 2 presents the distribution of *A. mixtum* ticks per state. Veracruz was the state where the largest number of *A. mixtum* ticks was collected (n=428), followed by Tabasco and Jalisco with 75 and 46, respectively.

Figures 2 and 3 present the main characteristics of *A. mixtum* through scanning electron microscopy. An ornamental shield1 is observed, the complete marginal furrow limiting all the festoons[2] as well as the presence of keratinous structures (chitin tubercles or mamelons) present dorsally to the festoons[3]. The complete spatulate hypostome can also be seen with dental formula 3/3[4], and the base of the sub-rectangular chapter with short cornu[5]. The

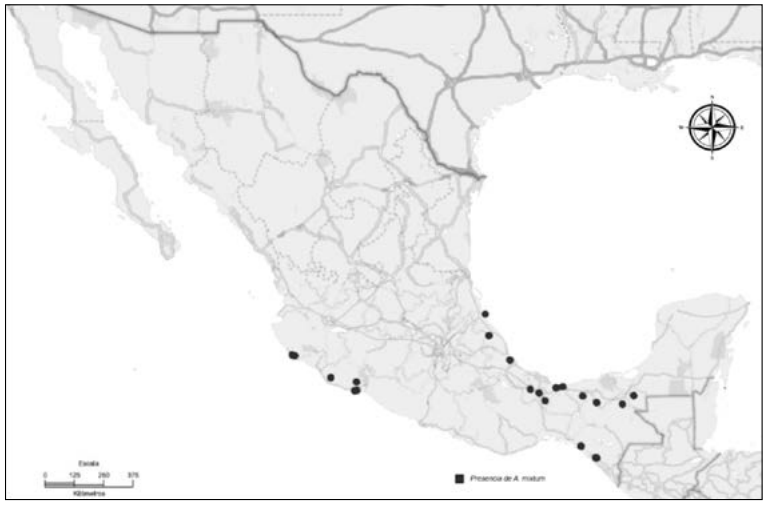

Figure 1. Sampling sites where the presence of *A. mixtum* was identified.

Table 2. Classification of *A. mixtum* ticks according to the genus and the state sampled.

State	Number of *A. mixtum*	(%)	Female (number)	(%)	Male (number)	(%)
Veracruz	428	69.1	240	70.8	188	69.6
Michoacán	45	7.3	29	8.6	16	5.9
Tabasco	75	12.1	30	8.8	45	16.7
Chiapas	25	4.1	16	4.7	9	3.3
Jalisco	46	7.4	24	7.1	12	4.5
Total	619	100	339	54.8	270	43.6

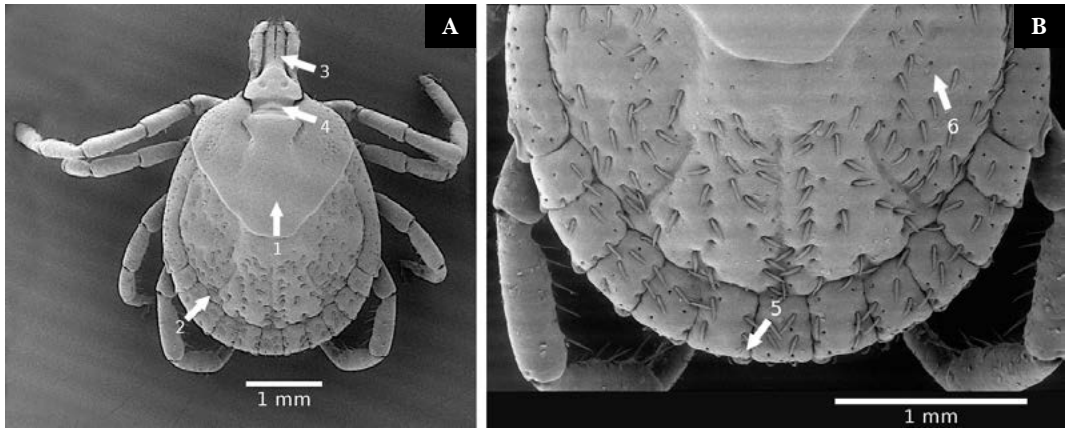

Figure 2. Dorsal view of an *Amblyomma mixtum* female.

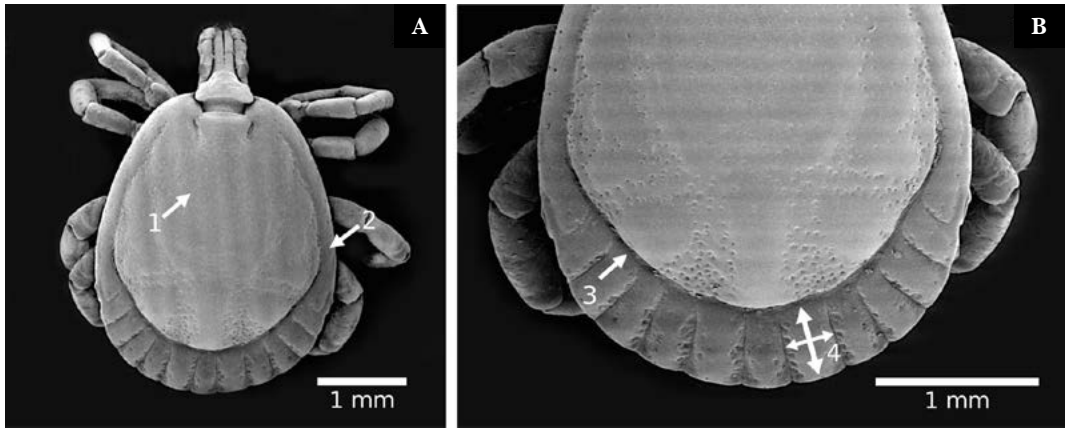

Figure 3. Dorsal view of an *Amblyomma mixtum* male.

oval idiosome with long gnatosome, as well as deep punctuations distributed throughout the body and with greater depth in the lateral fields[6].

The ornamental shield is observed with brown spots limited by whitish spots[1]. Complete furrow limited by festoons, deep to the IV coxa[2] followed by a line of pores or points that continue to the eyes[3]. Festoons that are wider rather than long[4]. Regarding the use of ixodicides in the BPUs, it was seen that tick control was carried out through the application of chemical products. The molecules used belong to the group of the Amidines, Organophosphates, Macrocyclic lactones (ML) and Pyrethroids (Table 3).

Amidines are the most frequently used acaricide in the BPUs (63.6%), followed by organophosphates (27.2%). Macrocyclic lactones and pyrethroids are the least used molecules in the BPUs, and the use of pyrethroids was found in the states of Michoacán and Chiapas, while macrocyclic lactones were only reported for the state of Chiapas. It is important to mention that this information is based on the frequency of the *A. mixtum* tick in the herds sampled.

Table 3. Molecules used in the BPUs where the presence *A. mixtum* was recorded.

Molecule	State	BPU where they are used	(%)
Amidines	Michoacán	14	63.6
	Jalisco		
	Veracruz		
	Tabasco		
	Chiapas		
Organophosphates	Jalisco	6	27.2
	Tabasco		
Macrocyclic lactones	Chiapas	2	9.1
Pyrethroids	Michoacán, Chiapas	2	9.1
Total		22	100

This study confirms the presence of *A. mixtum* in the states of Chiapas, Michoacán and Jalisco, which is consistent with what is reported for the state of Veracruz by Aguilar-Domínguez *et al.* (2019, 2021). This information updates and increases the knowledge of the distribution of *A. mixtum* in Mexico, in relation to the restructuring of the taxonomy of this species (Nava *et al.*, 2014), which generated a breach of outdating for more than 50% of the national territory. The information reported here about the infestation of cattle in the states with highest livestock production in the country suggests that the infestation of cattle with *A. mixtum* can be more prevalent than what is estimated. The frequency was 50.4% which agrees with what was reported by Ulloa-Ramones and Ulloa-Ramones (2021) and by Noda *et al.* (2015), who reported a frequency of 50% and 67%, respectively. It is likely that this increase of the mean corresponds to the displacement of the ecological niche of *R. microplus*, indicating that resistance problems are probably happening or it could be due to the need for management in extension work to improve the handling with ixodicide products that are commercially available for the use of livestock producers in Mexico (Juache-Villagrana *et al.*, 2023; Espinoza *et al.*, 2021; Alonso-Díaz *et al.*, 2013). These results highlight the need for more detailed epidemiological studies to determine the effectiveness in the use of acaricides to treat cattle co-infested with *A. mixtum* and *R. microplus* (Higa *et al.*, 2020), and to estimate the economic impact of *A. mixtum* in the livestock industry of Mexico.

Concerning the use of ixodicides in the BPUs, in this study it was seen that tick control is done through the application of chemical products. Several classes of acaricides are part of the veterinary products used as ectoparasiticides in 63.6% of the BPUs where the presence of *A. mixtum* is reported. Products based on amidines, including amitraz as representative of this class of acaricide, can be effective in the control of ticks and other ectoparasites in animals. However, its indiscriminate use has resulted in populations of *A. mixtum* that are resistant to amitraz (Alonso-Díaz *et al.* 2013). Resistance to amitraz of up to 100% has been reported in *A. mixtum* where a triggering factor of this extreme phenotype could be the more frequent infestation with larvae, nymphaea, and adults of

this same species in livestock where the signaling for treatment was the detection of *R. microplus* (Higa *et al.*, 2019).

Organophosphates, another class of ixodicides, have been used in Mexico since the 1980s, and they can still control ectoparasites where resistance is not prevalent (Sharma *et al.*, 2019). They act by interfering with their nervous system and have shown efficacy against various insects and mites (Bernal-González *et al.*, 2023). The frequency of use according to the BPUs where the presence of *A. mixtum* is reported was 27.2%, and it is only used in Jalisco and Tabasco. This indicates scarce use in the leading livestock production states, even when acaricide effectiveness is reported within the genus *Amblyomma* (Natala *et al.*, 2005).

Macrocyclic lactones, which include compounds such as ivermectin and other molecules (some in prolonged action products), are used as ectoparasiticides to treat livestock (Rodríguez-Vivas *et al.*, 2014). These veterinary products are effective against intestinal worms, mites, and other parasites. According to the frequency of *A. mixtum*, their use was reported only in the state of Chiapas. However, there is no literature inquiring about the resistance of these molecules in populations of *A. mixtum* in Mexico, in contrast with what was reported for *R. microplus*.

Pyrethroids, synthetic byproducts of natural pyretrins present in certain flowers (Lara Lafargue *et al.*, 2018), are widely used in insecticides and in products directed to pest control, ticks, and other insects (Díaz and Vallejo., 2013). Veterinary products in the market include flumethrin and cypermethrin as the most common pyrethroids in products used during the sampling period where the presence of *A. mixtum* was reported and it must be pointed out that its use was only reported in two BPUs. Resistance was reported in larvae of *A. mixtum* (Higa *et al.*, 2020). According to the data gathered, this group is the one that is least attributed the property of resistance and according to the information collected in the sampling, it is also the one that is least used. In some cases the problem of resistance to acaricides is so acute that there is a need to resort to the combination of many classes of acaricides, with the purpose of taking advantage of different modes of action and to broaden the range of action against the ticks that parasite cattle in Mexico (Rodríguez-Vivas *et al.* 2018). Additional research is required to develop and implement integrated management tools that mitigate the evolution of resistance to acaricides in populations of *A. mixtum* through their geographic range that affect the health and productivity of cattle herds in Mexico.

CONCLUSION

The presence of *Amblyomma mixtum* was confirmed in the leading livestock producing states in southeastern Mexico. The information obtained on control measures for *A. mixtum* in infested cattle, primarily in the use of various acaricide chemicals, generates alert for the risk of development of resistance to the classes of acaricides used by the producer. This situation highlights the need for additional studies with an approach on resistance and extension work to prolong the usefulness of the tools available, including acaricides, to control infestations with *A. mixtum* where this tick infests cattle in Mexico.

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COMPLIANCE WITH ETHICAL STANDARDS

This project was approved on April 12, 2023, by the Bioethics and Animal Welfare Commission of the School of Veterinary Medicine and Zootechnics in Universidad Veracruzana.

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