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Article

Body distribution of ticks (Acari: Ixodidae and Argasidae) associated with *Odocoileus virginianus* (Artiodactyla: Cervidae) and *Ovis canadensis* (Artiodactyla: Bovidae) in three northern Mexican states

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Abstract:

Ticks are important vectors of medical and veterinary importance pathogens in Mexico; however, the taxonomic studies of abundance, prevalence, intensity, and body distribution in white-tailed deer (*Odocoileus virginianus*) and bighorn sheep (*Ovis canadensis*) are limited. This study aimed to fill this knowledge gap in the Mexican states of Sonora, Nuevo León, and Tamaulipas. The area of study included authorized game farms where hunting is practiced. A total of 372 ticks [21 nymphs (5.65 %) and 351 adults (94.35 %); 41% female and 59 % male] were collected from 233 *O. virginianus* and four *O. canadensis*. The ticks collected from *O. virginianus* were identified as *Otobius megnini*, *Rhipicephalus* (*Boophilus*) *microplus*, and *Dermacentor (Anocentor) nitens*. *Dermacentor hunteri* was the only species collected from *O. canadensis*. Ears were the most infested region (83 females, 70 males, and 21 nymphs, 46.77 %), and the least infested body parts were the legs (10 males and nine females, 5.1 %). This study reports for the first time the abundance, intensity, and prevalence of ticks in *O. virginianus* in northern Mexico, particularly in the states of Tamaulipas and

Nuevo León, since the *O. canadensis* ticks had already been reported in Sonora. These results show that although ungulates are kept semi-captive, it is essential to control tick infestation by applying acaricide treatments on their preferred adherence sites to avoid the transmission of pathogens.

Key words: White-tailed deer, Bighorn sheep, Ticks, *Rhipicephalus microplus*, *Otobius megnini*, *Dermacentor nitens*, *Dermacentor hunteri*.

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Introduction

Ticks are hematophagous ectoparasites of amphibians, reptiles, birds, and mammals(1). As a result of their eating habits, ticks directly decrease their host weight gain and exert traumatic, toxic, infectious, or spoliation actions, in addition to the indirect effects that deteriorate the skin and cause death by dermal diseases(2,3). During their life cycle, ticks can horizontally or vertically acquire(4) and transmit a wide range of medical and veterinary important pathogens, such as *Babesia* spp., *Borrelia* spp., *Anaplasma phagocytophilum*, and *Rickettsia* spp.; hence, ticks are considered as vectors of global importance, surpassed only by mosquitoes(5).

Wildlife constitutes an important component in the transmission cycle of the vector-host-pathogen triangle, where humans are frequently included as accidental hosts, which makes it a zoonotic cycle(6). Therefore, the prevalence of new and reemerging tick-transmitted diseases represents a global public health problem(7). The spatial distribution of ticks is mainly influenced by climatic and geographic conditions, the type of vegetation, the agricultural landscape, the population dynamics of their wild hosts(3), and the illegal movement of cattle and *Odocoileus virginianus* for commercialization purposes without meeting the sanitary standards(8). These factors facilitate tick dispersion to places where it was not naturally found, increasing the human risk of acquiring diseases associated with these vectors(9,10). Previous studies have reported that *Rhipicephalus annulatus* has a specific preference for deer(11), and that *Dermacentor* spp. and *Ixodes* spp. specifically adhere to head and neck(12).

In Mexico, a total of 77 tick species has been identified; from these, those with national livestock importance due to their direct and indirect effects are *R. (Boophilus) microplus, B. anulatus, Amblyomma cajennense, A. imitador*. *A. maculatum, A. triste, A. americanum*, and

Anocentor nitens. However, the most important due to their economic impact are *R. microplus* and *A. cajennense*[13,14], with losses of US $ 573'608,076[15]. In Sonora, Nuevo León, and Tamaulipas, *O. virginianus* and *Ovis canadensis* have been used to increase income through legalized hunting practices in game farms. In 1996, a conservation program that consisted of constructing an enclosure for the reproduction of economically important species in semi-captivity was implemented in Rancho El Plomito, Sonora, in an area of 961 ha. By 2014, their reproductive population of *O. canadensis*, *O. v. couesi*, and *O. hemionus* was enough for the first repopulations performed by the Organización de Vida Silvestre (OVIS, AC)[16]. Mexican authorities reformed the operation of game farms by implementing the current system of Wildlife Management and Conservation Units (UMA, acronym in Spanish). This system allows the conservation and management of wildlife in their natural habitat, in addition to the rational use of wildlife or semi-captive populations and specimens[17,18]. In the studied Mexican states, regulated hunting of these ungulates is allowed, but population studies of ectoparasites, their body distribution, and the presence of species of game importance are scarce[3,12]. Thus, these results will allow to plan the body areas where proven successful acaricide treatments or devices will be applied for tick control[12]. This study aimed to i) taxonomically identify the tick species, ii) determine their prevalence, iii) estimate their abundance and intensity, and iv) describe their body distribution in *O. virginianus* and *O. canadensis* in game farms in Sonora, Nuevo León, and Tamaulipas. This information helps to understand the potential risk of ticks as vectors of diseases and implement preventive and corrective measures.

Material and methods

Areas of study

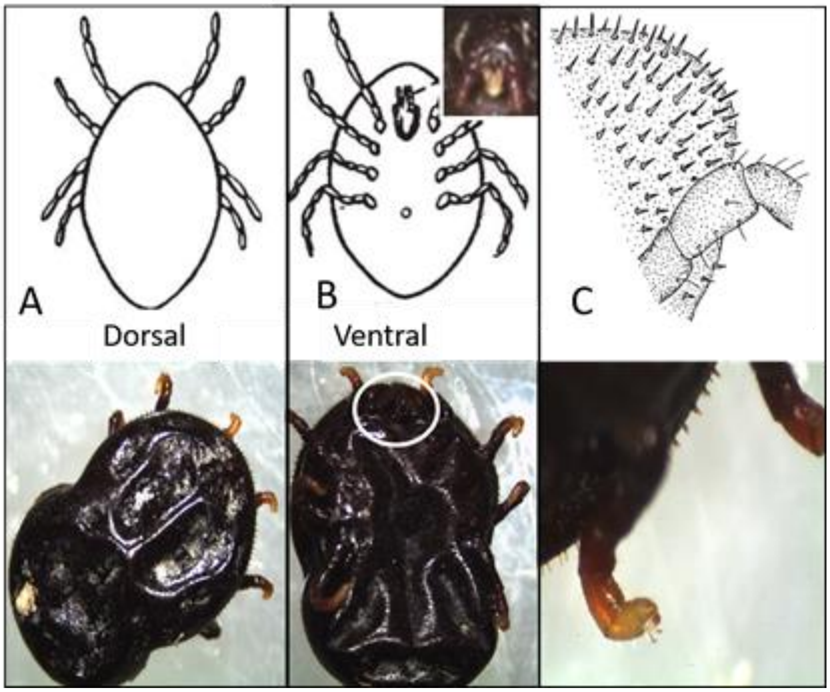
This study took place in different areas of Tamaulipas, Nuevo León, and Sonora from 2014 to 2018 in the months from October to February; these months correspond to the legal hunting period in UMAs *in situ* or *ex situ* of *O. virginianus* and *O. canadensis*[17,18]. Two areas of study were located in the Sierra Madre Occidental in Sonora: Rancho El Aigame (UMA registration: DGVS-CR-EX-1271-SON), La Colorada municipality (28º 43' 41" N, 110º 2'0.65" W) at 400 m asl and Rancho El Pitiquito (UMA registration: SEMARNAT-UMA-EX-250-SON) (30º 15' 0.0'' N, 112º 22'0.12'' W)[16-18], El Pitiquito municipality, with a predominantly arid and semiarid climate and mean annual precipitation of 450 mm[19]. In Nuevo León, ticks were collected in Rancho Mamulique (UMA registration: DFYFS-CR-EX-0333-NL), Salinas Victoria municipality (26º 7' 0.59'' N, 100º 19' 0.58''W), at 464 m asl, with warm arid steppe climate, annual mean temperature of 21-23 °C and annual mean precipitation of 380 mm[19]. In Tamaulipas, tick collection took place in two localities, Rancho Santa Clara (UMA registration: DGVS-CR-EX-1819-TAM), Nuevo Laredo (27° 33’0.11’’ N, 99° 47’ 59.9’’ W), which has the most arid and extreme climate in the State, ranging from -14 °C during winter and 40 °C during summer, and mean annual precipitation of 472.5 mm[19]. The second locality was Rancho Los Columpios (UMA registration: DGVS-CR-EX-2066-TAM), Guerrero municipality (26° 33’ 18’’ N, 99° 22’ 0.37’’ W), located on the Río Bravo basin, Tamaulipas. These localities have an arid climate with annual mean precipitation of 440 mm[18-20].

Tick collection and taxonomic identification

Ticks were collected from hunted specimens of *O. virginianus* and *O. canadensis* during the hunting season through the authorization issued by the Ministry of Environment and Natural Resources of Mexico to each UMA[16-18]. The utilization rate of these species is oriented to the hunting of male and adult specimens. After acquiring a hunting package, hunters were accompanied by OVIS technicians[16]. During collection, using sterile forceps, ticks were individually removed from the head (top part), ear, scapula, mid-dorsal neck, and inferior extremities of hunted animals[21]. Live ticks were transported to the Molecular and Experimental Pathology Laboratory (LPME, FCB, UANL) in 12 ml-vials containing a cotton pad moistened with sterile double-distilled water and labeled with the date, host, stage, locality, and body part from which the tick was collected; vials were stored at 4 °C. Ticks that died during transportation were placed in vials containing absolute ethyl alcohol as a preservative to avoid deterioration of the morphological traits needed for taxonomic identification[22].

The genus, species, sex, and stage of ticks were determined with a stereomicroscope at 10X - 40X (EZ4E, Leica Microsystem, Guadalajara, Jalisco, Mexico) and employing a specific tick taxonomic key[23-25]. In the taxonomic identification of ticks, the following distinctive structures of each of the species were considered:
Otobius megnini: Lack of dorsal shield, ventral gnathosoma, rectangular and straight basis capitulum, absent eyes, vestigial or atrophied hypostome, integument with spines, ambulacrum absent at the end of the legs (Figure 1).

Figure 1: Taxonomic characteristics of *Otobius megnini*

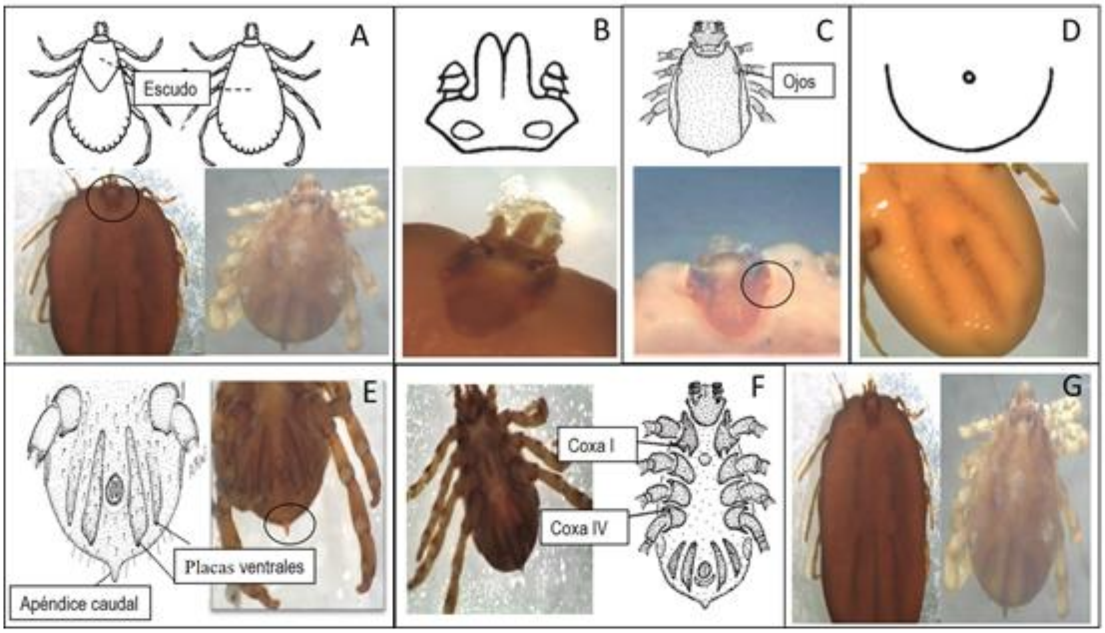


A) Dorsal view. Illustration and picture of a nymph. B) Ventral view with details of the anterior gnathosoma, rectangular basis capitulum in the box. C) Integument with spines (the integument is smooth between the spines).

Rhipicephalus (*Boophilus*) *microplus*: Males have ventral plates and a caudal appendage, a shield that covers the dorsal region of males and the anterior dorsal region of females, eyes, a hexagonal basis capitulum, a coxa I with double spines, a dorsally visible regular size and prominent coxa IV, and no festoons (Figure 2).

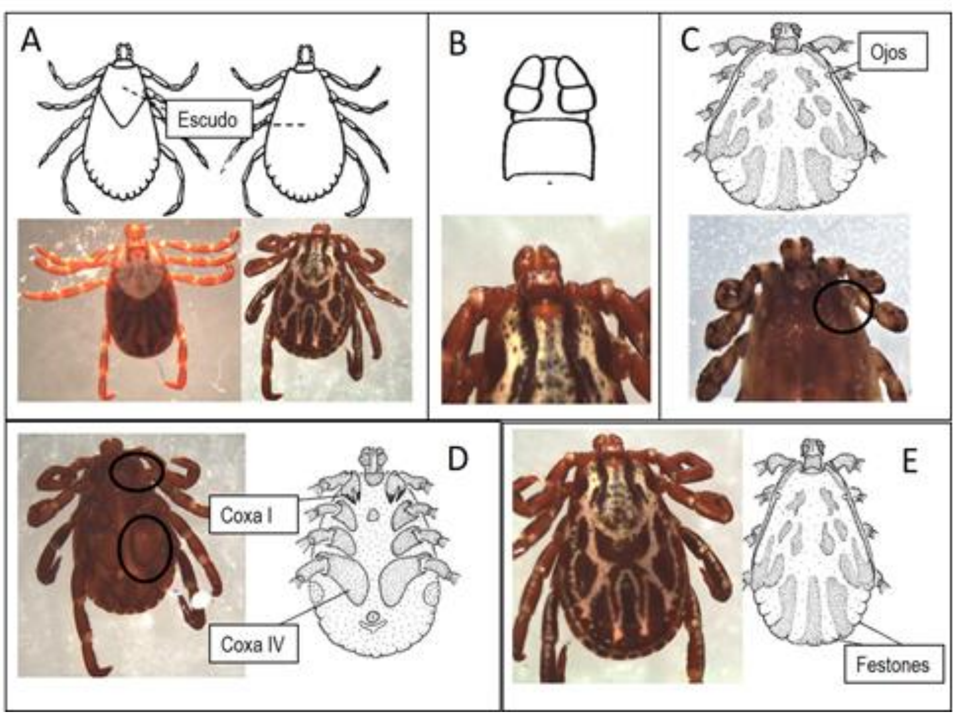
The *Dermacentor* species has an anterior gnathosoma with a rectangular and straight basis capitulum, festoons, a big coxa IV in males, and a coxa I with big and paired spurs (Figure 3). *D hunteri* and *D. nitens* differ in the number of festoons. *D. hunteri* has 11 festoons and an ornament with a characteristic pattern, with large spiracular plates forming a ring, rear to leg IV (Figure 4); *D. nitens* has seven festoons (Figure 5). All illustrations were obtained from tick identification keys[24,25].

Figure 2: Morphologic characteristics for the taxonomic identification of *Rhipicephalus* (*Boophilus*) *microplus*



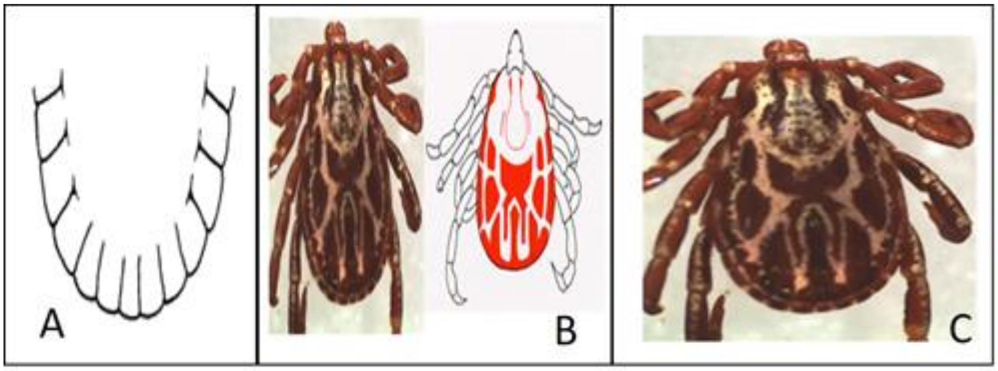
A) Dorsal shield. B) Anterior gnathosoma, basis capitulum with angular margins. C) Eyes. D) No festoons. E) Ventral plates and caudal appendage. F) Coxa I and IV. G) Female and male adult specimens.

Figure 3: Morphologic characteristics for the taxonomic identification of *Dermacentor* spp



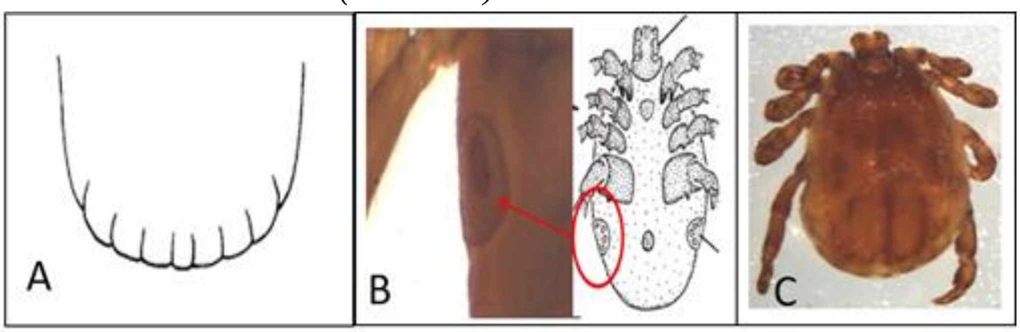
A) Dorsal shield. B) Anterior gnathosoma, rectangular and straight basis capitulum. C) Eyes. D) Morphology of coxa I and IV. E) Festoons in posterior end.

Figure 4: Morphologic characteristics for the taxonomic identification of *Dermacentor hunteri*



A) 11 festoons. B) Ornamentation (shown in the pattern). C) Complete male specimen.

Figure 5: Morphologic characteristics for the taxonomic identification of *Dermacentor (Anocentor) nitens*



A) Seven festoons. B) Big spiracular plates, posterior to leg IV (forms a ring). C) No ornamentation, complete male specimen.

Statistical analysis

The prevalence (percentage of infested hosts by tick species), intensity (number of ticks/infested hosts by each tick species), abundance (number of ticks by species/hosts), and sex proportion by tick species were calculated[26]. The significant association between the vector, host location, sex, and locality was determined with a Chi-squared (X^2) test at a significance level of 95% of the lower and upper confidence interval (CI). Additionally, the data were analyzed with the Z test to compare the population proportions between stages, species, localities, and hosts with the SPSS program, version 17[12].

Results

A total of 237 hosts were inspected, 233 *O. virginianus* specimens and four *O. canadensis* specimens; the body infestation percentages were 17.16 % and 100 %, respectively. Of the 372 ticks collected, 5.65 % were nymphs and 94.35 % adults. Four tick species were identified: *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini, 1887), *Dermacentor* (*Anocentor*) *nitens* (Neumann, 1897), and *Dermacentor hunteri* (Bishopp, 1912) from the Argasidae; and *Otobius megnini (*Dugès 1883) from the Argasidae family (Table 1).

Table 1: Tick identification by host (*Odocoileus virginianus* and *Ovis canadensis*), sex, and locality

Locality	Host (n/+)	Species	TS/ST (%)	Sex	
				F n/(%)	**M n/(%)**
Sonora	*O. virginianus* (16/6)	* *Otobius megnini*	19/204 (9.3)	NA	NA
	O. canadensis (4/4)	**O. megnini*	2/204 (0.98)	NA	NA
		Dermacentor hunteri	183/204 (89.7)	28/(13.7)	155/(76)
Nuevo León	*O. virginianus* (202/28)	*Rhipicephalus microplus*	98/151 (64.9)	84/(55.6)	14/(9.3)
		D. nitens	53/151 (35.1)	19/(12.6)	34/(22.5)
Tamaulipas	*O. virginianus* (15/6)	*R. microplus*	17/17 (100)	13/(76.5)	4/(23.5)
Total *O. virginianus*	(233/40)	NA	*19/372 (5.1)	NA	NA
			168/372 (45.2)	116/(41)	52/(59)
Total *O. canadensis*	(4/4)	NA	*2/372 (0.53)	NA	NA
			183/372 (49.19)	28/	155/

TS/ST= Ticks by species/ State total (%); n= number of specimens; *= nymphs; NA= non applicable.

Regarding sex, 41 % of ticks were female and 59 % male (Table 1). However, most ticks in *O. virginianus* were female (116/168, 69 %), only 31% were male (52/168). On the contrary, in *O. canadensis*, *D. hunteri* specimens were mainly male (155/183, 84.7 %), females represented 15.3 % (28/183). There was a significant association between the male and female proportion of ticks between *O. virginianus* and *O. canadensis* (X^2= 104.57, g.l.= 1, P<0.05). A significant association was also observed in the female and male proportion between the four tick species in *O. virginianus* (X^2= 39.92, g.l.= 1, P<0.05), and between males and females by locality (X^2= 105.01, g.l.= 2, P<0.05). These results were consistent with the population proportions between stages, species, localities, and hosts since it was confirmed with the Z test, which showed a significant association (Z> 1.2, IC 95%).

Table 2: Percentage of the body distribution of the identified tick species

Body region	**Sex**	***R. microplus* n/(%)**	***D. nitens* n/(%)**	***O. megnini* n/(%)**	***D. hunteri* n/(%)**	**Total n/(%)**
Head	♀	21 (5.7)	6 (1.6)	NF	7 (1.9)	34 (9.1)
	♂	5 (1.3)	5 (1.3)	NF	31 (8.3)	41 (11.1)
Ear	♀	58 (15.6)	17 (4.6)	NF	8 (2.2)	83 (22.3)
	♂	11 (2.9)	11 (2.9)	NF	48 (12.9)	70 (18.8)
	N	NF	NF	21 (5.7)	NF	21 (5.7)
Neck	♀	9 (2.4)	4 (1.1)	NF	5 (1.3)	18 (4.8)
	♂	1 (0.3)	5 (1.3)	NF	29 (7.8)	35 (9.4)
Back	♀	4 (1.1)	NF	NF	6 (1.6)	10 (2.7)
	♂	NF	NF	NF	31 (8.3)	31 (8.3)
Legs	♀	6 (1.6)	2 (0.5)	NF	2 (0.5)	10 (2.7)
	♂	NF	3 (0.8)	NF	16 (4.3)	19 (5.1)
Total by species (n/%)		115 (30.9)	53 (14.2)	21 (5.7)	183 (49.2)	372 (100)

N= nymphs; n= number of specimens; NF= not found.

In Sonora, 54.8 % (204/372) of the ticks from both hosts were collected. In *O. virginianus*, 9.3 % (19/204) were *O. megnini* nymphs. *O. canadensis* had 0.98 % (2/204) of *O. megnini* nymphs and 89.7 % of *D. hunteri* (Table 1). In Nuevo León, 40.6 % (151/372) of ticks were collected from *O. virginianus*; two different species were identified: *R. microplus* (98/151, 64.9 %) and *D. nitens* (34/151, 35.1 %). In Tamaulipas, only 17 specimens of *R. microplus* were collected from *O. virginianus*; this represents 4.56 % (17/372) of the total adult ticks collected in the three localities.

Ticks, from all four species, were more abundant on ears, with a total of 174 ticks (46.77 %) (22.3 % females, 18.8 % males, and 5.7 % nymphs), and the upper part of the head (9.1% females and 11.1% males), followed by the neck, scapula, and, lastly, inferior extremities. Without considering the host or locality, there was a significant association between the different tick species and their body distribution (X^2= 46.18, g.l.= 8, P<0.05); even *O. megnini* nymphs were located exclusively on the ears. Also, the females and males from different species were significantly associated with body location (X^2 = 13.25, g.l.= 4, P<0.05).

In *O. virginianus*, *R. microplus* was the most prevalent (30.9%), abundant (0.49), and intense (2.88) species; in *O. canadensis*, *D. hunteri* was the most outstanding species (Table 3).

Table 3: Prevalence, abundance, and intensity of infestation by tick species

Species	***O. virginianus* (40/233)**			***O. canadensis* (4/4)**		
	Prev (%)	**Abund $\bar{X}$**	**Intens**	**Prev (%)**	**Abund $\bar{X}$**	**Intens**
R. microplus	115 (30.9)	0.49	2.88	--	--	--
D. nitens	53 (14.2)	0.23	1.33	--	--	--
O. megnini	19(5.1)	0.082	0.48	2 (0.54)	0.5	0.5
D. hunteri	--	--	--	183 (49.2)	45.8	45.8

Prev= prevalence; Abund= abundance; Intens= intensity.

Discussion

Rhipicephalus (*Boophilus*) *microplus*, also known as cattle tick, was the most prevalent species in *O. virginianus* in Nuevo León and Tamaulipas because it inhabits the same hunting territory. This tick is considered of high incidence in livestock production systems due to the significant economic losses it causes worldwide(27); additionally, it is a vector of *Babesia bovis*, *B. bigemina*, and *Anaplasma marginale*. This study considers that *O. virginianus* plays an important role as a natural reservoir of these diseases in the areas of study, as it has been previously reported in Texas, USA, which shares borders with Nuevo León and Tamaulipas(27). During insecticide treatment, female ticks and larvae scape to favorable habitats to survive; this facilitates the upsurge of infestations in cattle and ungulates(28). However, the *O. virginianus* specimens analyzed in Sonora were free of *R. microplus*; SADER/SENASICA reported that the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) declared Sonora was free from this tick(17). In Nuevo León and Tamaulipas, efforts to eradicate *R. microplus* continue despite the program operating a permanent quarantine zone in south Texas, USA, along the Mexican border(28). In northern Mexico, *O. virginianus* is handled in game farms due to the income received from hunting permits, furs, and meat for human consumption. In these farms, semi-captive *O. virginianus* specimens share the same feeding, drinking, and transportation areas as cattle, representing a possible infestation risk factor and complicating the eradication of this tick(29,30).

The prevalence of *R. microplus* in northern Mexico was 31%, which is lower than in Yucatán, Mexico, where 97 % was reported in *Cervus elaphus*, an important host for this tick species; *R. microplus* not only feeds from its host, it also completes its nymph developing cycle(3). This species was also the most common in *O. v. yucatanensis* and *Mazama temama*, with a 28.4 % frequency and an intensity of 25.2 ticks per animal(31). In contrast, *R. annulatus* was exclusively reported in *C. elaphus* with a prevalence of 7.9 % in Cádiz, Spain(11).

In Nuevo León, *D. nitens* has not been previously reported in *O. virginianus*; however, it was able to collect specimens from this species. There are several reports of this tick in different Mexican states in hosts such as cattle, horses, dogs, mules, and rodents(32). The veterinary importance of this ectoparasite is that female specimens of *D. nitens* transmit *Babesia caballi* to their offspring transovarially, and all stages are competent for this disease, besides being the etiologic agent of equine piroplasmosis(33).

In Sonora, this study reported nymphs of the spinous ear tick (*O. megnini*) in *O. virginianus* (5.1 %) and *O. canadensis* (0.53 %); the adult stage is not an ectoparasite(34). Although this tick has been reported from the southwest of the USA to the south of Mexico and South America, it has not received the same importance as other ixodid ticks. *O. megnini* can have multiple blood ingestions and deposit batches of eggs that represent a danger in the veterinary and clinical fields, as it has a predilection for the ear canal, which can result in otoacariasis, with complications of external otitis, ear pain in 90 % of the cases, and other signs of internal otitis, such as facial and respiratory paralysis. This tick affects the people that have a close contact with livestock animals, be it cows, mules, goats, rabbits, and sheep(35). This ectoparasite can affect the host in several ways, such as severe irritations, weight loss, and offspring behavior(36). In ungulates and other hosts, when a tick or nymph feeds, it causes blood loss, which attracts other insects, causing stress to the hosts(37). Additionally, these ticks act as rickettsial vectors, responsible for spotted fever and *Coxiella burnetii* (Q fever)(38).

In Sonora, ticks were more abundant in bighorn sheep. From the total amount of collected ticks, 49.19% belonged to the *D. hunteri* species; males had a higher proportion with 41.6%. *D. hunteri* was almost exclusively collected from this host, similar to previous reports. This tick has also been reported in Baja California, in wild populations of *O. canadensis*(32,39). In California, USA, bighorn sheep populations were seropositive to *Anaplasma* spp(40); it is even considered as a primary vector of *A. ovis* (Lestoquard, 1924)(41,42) and *Rickettsia* spp., which suggests the importance of this ectoparasite in the epidemiology of these diseases(42).

Based on the body region, when considering the total amount of ticks, the four identified tick species preferred the ears, followed by the head. In *Capreolus capreolus*, 61% of ticks preferred the head area(12,21); in cattle, 32.02 % preferred ears and head; in sheep, 48.08 % also preferred the head, which included ears. These previous reports are similar to what was observed in this study. Ectoparasites prefer the head and ears because of the skin thickness in these areas, where the skin is relatively thin and vascularized[43,44].

In *O. canadensis*, males had similar proportions in each body region, with an increased number in ears and head. A study in Mexican tropical regions showed similar results; the Ixodidae tick distribution in sheep infestation was 26.50 % in head and neck[45]. Previous reports have stated that tick density in *O. virginianus* can vary depending on the collection season and the age of the ungulates[12,46]. In this study, ticks were collected during the fall-winter season, when hunting is allowed, and ticks are in their adult stage, except *O. megnini*, which parasitic stage is nymphal. *Ixodes* and *Dermacentor* ticks preferred younger ungulates due to their habits and thinner skin in *Capreolus capreolus*. Adult ticks had no preference for the sex of the host, but they did for their body mass[12,46]. Although in south Texas, where there is a quarantine area for eradicating ticks in cattle, ticks have not been eradicated due to unregulated movements of illegal cattle and the dispersal of wildlife animals such as *O. virginianus* in Mexico[27].

Conclusions and implications

This study reports three tick species in *O. virginianus* (*O. megnini*, *R. microplus*, and *D. nitens*) and two species in *O. canadensis* (*O. megnini* and *D. hunteri*) in game farms from northern Mexico. These species play an important role in pathogen epizootiology[47]; for this reason, it is essential to identify potential vectors of diseases and tick-associated pathogens in ungulates of game importance and implement control measures. The most and least infested body regions were ears and legs, respectively, due to skin thickness. One of the main strategies for tick eradication is knowing their host specificity (*O. virginianus* or other ungulates); this information helps recommend applying specific acaricides or ivermectins based on the type of soil or pasture[48]. It is also important to consider the life cycle of the tick since it could change its susceptibility to the insecticide[37]. Consequently, knowing the tick distribution in northern Mexico and their abundance and intensity in *O. virginianus* and *O. canadensis* will help implement preventive or control measures in game farms and livestock, as well as in the importation or hunting of these ungulates. This information will also prevent the development of new vectors of infectious diseases that could represent a public health or zoonotic problem.

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