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Artícle

Ivermectin resistance in *Rhipicephalus microplus* (Acari: Ixodidae) in northeastern Mexico and associated risk factors

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Abstract:

Rhipicephalus microplus is the parasitic species that causes the most damage to Mexican and global livestock due to direct and indirect losses, such as the increase in multidrug resistance and cross-resistance. Currently, there are few studies on resistance to macrocyclic lactones in Mexico, most of them in the south. This study aimed to evaluate the status of ivermectin resistance in *R. microplus* in northeastern Mexico and its associated risk factors. A total of 20 populations of *Rhipicephalus microplus* were collected in the states of Veracruz, Nuevo León, Tamaulipas, and San Luis Potosí, and they were analyzed with the larval immersion test. Mortality data were subjected to a Probit analysis, estimating lethal concentrations (LC) of 50 % and 99 % and their respective 95 % confidence intervals (95 % CI), and to determine possible risk factors, a multivariate analysis and 2 x 2 contingency tables were performed for the exposure variables, with a 95 % confidence interval, and a binomial logistic regression model for those variables with a $P \leq 0.05$. Eighty (80) percent of the analyzed populations showed resistance with ranges of RR50= 2.07-11.14 and RR99= 3.03-47.93 ($P \leq 0.05$), and through the binomial logistic regression, it was observed that the variable of frequency of treatments obtained a $P \leq 0.0134$, a result that proved to be significant.

Keywords: Cattle tick, Veterinary epidemiology, Dose-response, Acaricides.

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Introduction

Ticks are hematophagous ectoparasites that are important in human and animal health due to the damage they cause by transmitting pathogens and feeding(1). *Rhipicephalus microplus* is the most important species in cattle farming because it is the main vector of hemoparasites such as *Babesia* spp. and *Anaplasma* spp., in addition to this, the economic losses it causes at the productive level in Mexico amount to more than 573.6 million dollars per year(2). This species is dispersed in the tropical, subtropical, and semiarid regions of all continents, except Europe(3). The geographical distribution of *R. microplus* in the country is recorded by SENASICA, which states that 30.60 % of the country is free of ticks, 3.44 % is under eradication, and 65.96 % are natural free zones and control zones(4).

Ixodicides have been used for years for the control of *R. microplus*, such as: organophosphates, amidines, synthetic pyrethroids, growth regulators, phenylpyrazolones, and macrocyclic lactones (MLs); the latter are a broad-spectrum family (endectocide) and act by binding to the transmembrane (TM) domains of Cys-loop receptors, such as the glutamate-gated chloride channel (GluCl), which are expressed in the motor and sensory systems of arthropods and nematodes, causing hyperpolarization and ultimately death(5,6,7).

What all these drugs have in common is that they have generated resistance due to operational factors such as inappropriate and continuous use(8). In Mexico, in 2010, resistance to ivermectin was reported for the first time in *R. microplus* populations(9), and it has been used since the beginning of the 21st century and currently there are few studies on resistance to MLs in Mexico, which are scarce in the northeast of the country. Therefore, the objective of this research was to determine the status of ivermectin resistance in *R. microplus* in cattle ranches in northeastern Mexico, as well as the possible risk factors associated with such resistance.

Material and methods

Area and place of study

The study was carried out in the Bacteriology Laboratory and the Multidisciplinary Research Laboratory (LMI, for its acronym in Spanish) of the Faculty of Veterinary Medicine and Zootechnics (FMVZ, for its acronym in Spanish) of the Universidad Autónoma de Nuevo León (UANL, for its acronym in Spanish).

From September 2021 to October 2022, 20 populations of ticks belonging to the *R. microplus* species were collected, which were located in 20 different cattle ranches belonging to the four states of the northeastern region of Mexico: Veracruz (Ver.), Nuevo León (N.L.), Tamaulipas (Tamps.), and San Luis Potosí (S.L.P.). To determine the sample size, a simple randomized model was used, which was based on data from SIAP-SADER(10).

Tick collection and identification

In the morning, 20 to 30 engorged (teleogynous) females belonging to the species *R. microplus*, which were located in the body areas of the bovine, were collected by hand following the recommendations of FAO(11). The identification of the specimens was carried out by means of an observational morphological analysis, with the use of dichotomous keys(12) and a Carl Zeiss™ Stemi™ DV4 stereoscopic microscope (Göttingen, Germany), in order to distinguish between other tick species that also parasitize cattle.

Production of infesting larvae

To carry out oviposition, the teleogynes were washed with distilled water and dried with paper towels; they were placed in groups of 10 in a Petri dish (100 x 15 mm) dorsoventrally and incubated in an ECOSHEL BOD-250 incubator at a temperature of 27 ± 2 °C and a relative humidity between 80 and 90 %. After oviposition (14 to 18 d), the eggs were collected and transferred to 10 mL glass tubes sealed with a cloth and a rubber band to await the hatching of the larvae; after another 14 d, we waited for the larvae to mature, and once the negative geotropism characteristic was observed, the larval immersion test modified for ivermectin was then carried out(8,9,13).

Larval immersion test modified for ivermectin (LIT)

A stock solution of 1 % IVM (Sigma-Aldrich, USA) was prepared in absolute ethanol and 2 % of Triton X-100 (Sigma-Aldrich, USA). From this solution, the maximum dose of IVM was prepared at 0.01 % (100 ppm). Subsequently, 11 serial dilutions were prepared at 30 %: 0.01 %, 0.007 %, 0.0049 %, 0.00343 %, 0.0024 %, 0.00168 %, 0.00117 %, 0.00082 %, 0.00057 %, 0.0004 %, and 0.00028 %. A solution of 1 % ethanol and 0.02 % Triton X-100 in distilled water was used as a diluent. In 2.0 mL Eppendorf tubes, 500 µL of each dilution was added in triplicate and a quantity of between 100 and 150 infesting larvae was placed; they were immersed for 10 min and then transferred to 8.5 x 7.5 cm Walkman papers closed with foldback clips. After 24 h, live larvae and the initial number of pack larvae were counted(8,13,14).

Statistical analysis

A PROBIT dose-response analysis was performed; lethal concentrations (LC) of 50 % and 99 %, with their respective 95 % confidence intervals (95 % CI) were calculated using the SPSS V.24 software. The hypothesis of normality and equality of variance was tested with a Chi-square test ($P \leq 0.05$).

The resistance ratio (RR) of each population was determined and compared with data previously obtained from the susceptible reference strain Deutch (USDA, Cattle Fever Tick Research Laboratory, Edinburg, TX, USA)(13). To determine susceptibility and resistance, the following classification was followed: RR50 ≤ 1: susceptible; RR50 > 1 < 2 incipient resistance, and RR50 ≥ 2 resistant(12). The formula for calculating the RR was:

$$\text{RR50} = \frac{\text{LC50 population}}{\text{LC50 reference strain}}$$

Epidemiological questionnaire

An epidemiological questionnaire was applied to each of the owners or managers of the cattle ranches studied in order to determine the practices in the use and management of MLs, as well as the control of *R. microplus*. Information related the following aspects was included: production, facilities, breeds, presence of ticks and other parasites, history of the use of macrocyclic lactones (MLs) and ixodicides, frequency of applications, rotation of ixodicides and pastures, among others.

The group with incipient resistance (RR50 > 1 < 2) was considered susceptible and a descriptive analysis was performed to calculate the frequencies of the variables found, as well as a multivariate analysis using 2 x 2 contingency tables to evaluate the interaction between the exposure variables, with a 95 % confidence interval using the Epi Info V.7.2 software. Fisher's exact test was used to determine the level of significance of each association and associations with a value of $P \leq 0.20$ were included in the binomial logistic regression model. A value of $P \leq 0.05$ was considered statistically significant in the binomial regression analysis[8,9,15].

Results

Place of collection of the populations

The data collected from the populations of *R. microplus* belonging to the northeastern region of Mexico are shown in Table 1, which were distributed as follows: four from Tamps., seven from Ver., five from N.L., and four from S.L.P.

Table 1: Geographic location of each *R. microplus* population collected in the northeastern region of Mexico

Population	Location	Geographic coordinates
ETHM	Tantoyuquita, Tamps.	22°31'05.5"N 98°31'26.5"W
JCG4	Ciudad del Maíz, S.L.P.	22°25'01.6"N 99°35'20.7"W
JAM5	Tantoyuca, Ver.	21°12'38.7"N 98°08'33.5"W
DALC	Cadereyta, N.L.	25°33'43.4"N 99°49'11.4"W
RAMT	Soto la Marina, Tamps.	23°48'30.3"N 98°08'24.9"W
JNSE	Santa Engracia, Tamps.	24°04'05.5"N 99°14'07.7"W
JVML	Los Ramones, N.L.	25°42'24.6"N 99°37'27.9"W
SNTM	General Bravo, N.L.	25°50'17.0"N 99°15'56.4"W
VMA1	General Terán, N.L.	25°10'06.4"N 99°32'55.3"W
PRVA	Aramberri, N.L.	24°06'19.8"N 99°55'20.1"W
MRNA	Hidalgo, Tamps.	24°04'41.0"N 99°14'28.8"W
ANGS	Tantoyuca, Ver.	21°23'42.1"N 98°08'32.3"W
LEX15	Tantoyuca, Ver.	21°18'06.0"N 98°15'42.4"W
ESHP	Tantoyuca, Ver.	21°19'42.7"N 98°20'44.0"W
JHE2	Tantoyuca, Ver.	21°24'05.1"N 98°11'15.5"W
JPN1	Tantoyuca, Ver.	21°17'15.3"N 98°15'57.3"W
VIHM	Tantoyuca, Ver.	21°27'41.4"N 98°18'30.5"W
KML1	Ciudad Valles, S.L.P.	22°01'19.9"N 99°04'23.5"W
EBEV	Casas Viejas, S.L.P.	22°11'22.2"N 99°05'53.2"W
ISALI	El Naranjo, S.L.P.	22°30'58.1"N 99°21'05.0"W

Cattle ranches with ivermectin-resistant *R. microplus* populations and the resistance ratio

Using the mortality rate and the PROBIT methodology, the lethal concentration in % (LC50 and LC99) and the resistance ratio (RR50 and RR99) were calculated (Table 2). The VMA1 population was susceptible to IVM (RR50= 0.73; RR99= 3.94) and the JCG4, JAM5 and JNSE populations showed incipient resistance (RR50 of 1.20, 1.55, and 1.61 respectively). On the other hand, the remaining 16 populations showed resistance to IVM (RR50= 2.07-11.14; RR99= 3.03-47.93) and of these, the JVML and LEX15 populations were highly resistant to ixodicide (RR50= 6.98; RR99= 11.11; RR50= 11.14; RR99= 47.93).

Table 2: Analysis of dose-response to IVM in *R. microplus* populations, lethal concentrations at 50 % and 99 % and resistance ratios 50 and 99 (RR_{50} and RR_{99})

Population	Slope	LC_{50}	95 % CI	RR_{50}	LC_{99}	95 % CI	RR_{99}
JCG4	4.77	0.00067	0.00028-0.00123	1.20	0.00203	0.00114-0.17975	1.20
ETHM	3.82	0.00154	0.00135-0.00174	2.75	0.00626	0.00491-0.00874	3.68
JAM5	3.10	0.00087	0.00074-0.00102	1.55	0.00490	0.00362-0.00758	2.88
DALC	3.46	0.00230	0.00200-0.00264	4.11	0.01083	0.00820-0.01616	6.37
RAMT	4.29	0.00148	0.00133-0.00164	2.64	0.00515	0.00418-0.00684	3.03
JNSE	2.82	0.00090	0.00072-0.00110	1.61	0.00602	0.00410-0.01090	3.54
JVML	3.40	0.00391	0.00313-0.00513	6.98	0.01889	0.01173-0.04563	11.11
SNTM	3.83	0.00226	0.00191-0.00269	4.03	0.00913	0.00695-0.01362	5.37
VMA1	1.91	0.00041	0.00027-0.00053	0.73	0.00669	0.00403-0.01585	3.94
PRVA	3.26	0.00206	0.00188-0.00225	3.68	0.01067	0.00883-0.01346	6.28
MRNA	3.62	0.00303	0.00265-0.00346	5.40	0.01326	0.01014-0.01927	7.80
ANGS	3.32	0.00213	0.00202-0.00224	3.80	0.01068	0.00948-0.01220	6.28
LEX15	2.09	0.00624	0.00547-0.00727	11.14	0.08148	0.05465-0.13760	47.93
ESHP	3.32	0.00177	0.00163-0.00192	3.16	0.00889	0.00741-0.01110	5.23
JHE2	2.70	0.00156	0.00138-0.00174	2.78	0.01136	0.0088-0.01572	6.68
JPN1	2.38	0.00225	0.00198-0.00256	4.02	0.02138	0.01526-0.03357	12.58
VIHM	3.10	0.00255	0.00222-0.00293	4.56	0.01435	0.01036-0.02122	8.44
KML1	2.53	0.00149	0.00134-0.00166	2.66	0.01242	0.00947-0.01750	7.31

ISALI	3.03	0.00116	0.00101-0.00132	2.07	0.00679	0.00513-0.00995	4.00
EBEV	2.87	0.00116	0.00095-0.00139	2.07	0.00750	0.00522-0.0129	4.41
DEUTCH[a]	4.72	0.00056	0.00052-0.00060	NA	0.0017	0.00150-0.00210	NA

a USDA susceptible reference strain, Cattle Fever Tick Research Laboratory, Edinburg, TX, USA.
LC= lethal concentration; CI= confidence interval; RR= resistance ratio; NA= not applicable.

Separating the populations by state, resistance to IVM was found to exceed 70 % in each of these. In the state of San Luis Potosí, there were three resistant populations (75 %) and one population showed incipient resistance (25 %); in Tamaulipas, values similar to those found in the state of San Luis Potosí were obtained: 75 % resistant, 25 % with incipient resistance. On the other hand, in Nuevo León it was found that 80 % of the population present resistance, while one population (20 %) showed susceptibility, it is highlighted that it was the only one in the present study. Finally, 86 % of the populations analyzed in Veracruz showed resistance, while 14 % showed incipient resistance.

Risk factors associated with resistant populations

A total of 14 independent variables were analyzed as possible risk factors associated with resistance to IVM (Table 3). On the one hand, the main farming system is the rangeland; just over half of the ranches have semi-technified facilities and landrace breeds between zebu and European. The density of animals per ranch is less than 50 head per herd, with a proximity of less than 5 km between ranches. Half of the ranches sampled have ticks year-round.

Regarding the management history of ixodicides and MLs, it was observed that all ranches implement ixodicide rotation by using various product families, such as organophosphates, amidines, synthetic pyrethroids, phenylpyrazolones, and developmental inhibitors. In addition, all ranches apply IVM and other MLs, such as doramectin, half of which are used for the treatment of ectoparasites. More than 50 % of the farmers surveyed mentioned using IVM formulations with concentrations greater than 1 %, applying them more than five times a year and adjusting the dose according to the weight of the bovine. In addition, most ranches have veterinary assistance and carry out pasture rotation.

The exposure variables "frequency of treatments" (P=0.026) and "formulation administered" (P=0.1531) showed statistical significance according to Fisher's exact test (Table 4). Therefore, both variables were included in the binomial logistic regression model (Table 3), where regression estimates, 95 % confidence intervals (95 % CI), odds ratios (OR), *P*-values, and standard error of the regression coefficient were obtained. A value of $P \leq 0.05$ was considered significant, indicating a positive statistical association between the variables.

Table 3: Frequency analysis of exposure-independent variables as possible risk factors associated with *R. microplus* resistance to IVM

Variable	**Analysis**	**Frequency (%)**	***P* (Fisher's exact test)**
Farming system	Housed	6/20= 30	
	Rangeland	14/20= 70	0.6573[a]
Type of facilities	Semi-technified	11/20= 55	
	Familiar	9/20= 45	0.6253[a]
Breeds	Pure	2/20= 10	
	Landrace	18/20= 90	0.3684[a]
Animal density (number of heads)	> 50	8/20= 40	
	< 50	12/20= 60	0.5345[a]
Proximity to another ranch	> 10 km	5/20= 25	
	< 10 km	15/20= 75	0.2487[a]
Season with ticks	Seasonality	10/20= 50	
	All year	10/20= 50	0.7089[a]
Target parasite (s)	Ectoparasites	10/20= 50	
	Endo- and ectoparasites	10/20= 50	0.7089[a]
Frequency of treatments (year)	1- 3	9/20= 45	
	4- > 5	11/20= 55	0.026[b]*
Application of treatments	Prevention	7/20= 35	
	Presence	13/20= 65	0.5607[a]
Formulation administered	1	8/20= 40	
	3.15 - 4 %	12/20= 60	0.1531[b]*
Application according to the weight	Yes	17/20= 85	0.5087[a]
	No	3/20= 15	
Veterinary assistance	Yes	15/20= 75	0.2817[a]
	No	5/20= 25	
Ixodicide rotation	Yes	20/20= 100	0.4738[a]
	No	0/20	
Pasture rotation	Yes	14/20= 70	
	No	6/20= 30	0.3426[a]

a= not significant; b*= significant ($P \leq 0.20$).

Table 4: Binomial logistic regression analysis in significant variables as possible risk factors associated with *R. microplus* resistance to IVM

Variable	OR	95 % CI	SE (β)	*P*≤0.05
Frequency of treatments	Not defined	0.0	291.26	0.0134
Formulation administered	6.59	0.5428	1.27	0.1101

OR= odds ratio; CI= confidence interval; SE (β) = standard error.

Discussion

Chemical control of ticks in Mexico and the world has become ineffective, given the emergence of populations resistant and multi-resistant to ixodicides(16,17,18). Since its introduction in the 1980s, IVM has been the most important animal health product worldwide(19). There have been few studies on the status of resistance to IVM in *R. microplus* in Mexico(8,9,15). This highlights the importance of conducting studies on the evaluation and diagnosis of resistance to this drug in the northeast of the country.

Applying the LIT and following the Probit methodology, the LC50 and LC99 of the study populations were determined. In the results obtained, a significant difference was found with the reference strain Deutch, with a susceptible population (5 %) (RR50= 0.73), three populations with incipient resistance (15 %) (RR50= 1.20-1.61), and the rest (80 %) with resistance (RR50= 2.07-11.14). These results coincide with those reported for the first time in Mexico(9), where 100 % of the populations analyzed showed resistance to IVM, with RR50= 2.04-8.59 and RR99= 2.67-87.86, in addition to exponential growth in different sampling periods. The importance of using a susceptible reference strain lies in the fact that it is a reference parameter for biochemical and molecular resistance studies(20). In addition, they are regulated by international organizations. In the study carried out in 2006(9), a comparison was made between the results obtained in their research using the Deutch strain and another study(15), which used the Porto Alegre strain. This study(9) highlights that the result obtained by this team is superior to those of the second, even so, slightly higher or equal RR50 values were obtained. In the present research, similar results were found when analyzing the Porto alegre, Mozo and Deutch strains(9,13,21) as possible candidates for the reference strain, so it was decided to select the Deutch strain because, when analyzing the results of the three, there was no significance at the time of determining the already stipulated classification, and it was more in line with what was desired. On the other hand, the Mexican strain Media Joya is only susceptible to organophosphates, synthetic pyrethroids and amidines, and there is no toxicological characterization of susceptibility to ivermectin[(22)].

Authors[(23)] mention that resistance is given by biochemical/genetic factors, operational factors, and ecological factors; the latter include intrinsic traits and interactions of populations with their surroundings and environment. In addition, the development of resistant individuals is dependent on the frequency of occurrence and the selection pressure[(9,24,25)]. In addition, in different studies of Latin American countries, resistant populations of between 40 and 100 % of the populations analyzed were obtained[(26,27,28)].

The response of populations to dose increase (slope) is an important indicator of resistance. A low slope ≤ 2 and a high LC (higher than the reference strain) are common in resistant populations, while a high slope ≥ 2 and low LC are common in susceptible populations with heterogeneous response[(13,29)]. In the present study, populations that respect this statement were found: JCG4 (S.L.P.), JAM5 (Ver), JNSE (Tamps), VMA1 (N.L.), and the JPN1 population (Ver), while, surprisingly, three populations from Tamaulipas (ETHM, RAMT, and MRNA), four from Nuevo León (JVML, SNTM, PRVA, and DALC), five from Veracruz (ANGS, LEX15, ESHP, VIHM, and JHE2) and three from San Luis Potosí (KML1, EBEV, and ISALI) showed high LCs and slopes. To date, there are no reports that determine a strain of *R. microplus* that is highly resistant to IVM[(28)]; according to these statements, the populations described have suffered a loss of heterogeneity and susceptible genes, demonstrating for the first time in the present research that resistant alleles are fixed in the population and they present a homogeneous resistance response. Other studies mention that the heterogeneity of resistant alleles would lead to the loss of susceptible populations and the emergence of resistant populations with homogeneous alleles[(9,30,31)].

Of the resistant populations obtained in this study, two were classified as highly resistant (RR50= 6.98 and RR50= 11.14), results that are similar to those that showed the highest values of resistance (RR50= 6.84, 7.37 and 10.23) and RR50= 5.89, 6.25 and 8.21[(8,9,15)]. Even so, molecular studies are needed to analyze all frequencies of resistant alleles in populations.

On the other hand, frequencies were analyzed based on the responses obtained in the epidemiological questionnaire (Table 3). The municipalities included in this study are located between parallels 26° N to 21° N, relative humidity between 65 and 79 %, average temperatures of 21° C, and an average water evaporation between 1,200-1,400 mm, optimal conditions for the development, distribution, and survival of the tick, as well as for the increase of generations per year[(32,33,34)]. Some authors mention that geographic location and abiotic niche are factors that promote the greater development of ticks[(3,35)].

Of the 14 variables studied, two showed significances of $P \leq 0.20$: frequency of treatments (P=0.026) and formulation administered (P=0.1531), which were included in the binomial logistic regression model.

Animal management systems, as well as the number of annual treatments, are considered factors that influence the efficacy of drugs, playing an important role in the development of resistance(4). In 55 % of the ranches, IVM treatment is applied 4 to more than 5 times per year, similar to that obtained by Fernández-Salas *et al*(36), where cattle ranches that apply MLs 4 or more than 5 times a year are up to 13 times more likely to develop resistance(8). IVM has a period of decrease in concentration after application, but due to its high affinity to fat and its persistence in tissues, it is not completely eliminated, so prolonged exposure to therapeutic doses favors the emergence of resistant organisms(9,15,36). This assumption is known as the "tail effect"; if organisms are present during this period, the selection of IVM-resistant organisms is possible(37,38). *R. microplus* reacts quickly to selection pressure and higher concentrations of ixodicides(39), therefore, the application of the chemical should be carried out less frequently at 30-d intervals with the intention of reducing this pressure, not only for the tick, but also for non-target organisms such as helminths(40,41).

By applying the binomial logistic regression, it was observed that a $P \leq 0.0134$ was obtained for the variable of frequency of treatments, a result that proved to be significant, but with an undefined OR due to the fact that in one of the groups of the 2 x 2 contingency table, there was a box in which there was no susceptible population and that the IVM was applied 4 or more than 5 times a year, which had to be computed as a zero; since the OR is the quotient of two ratios(42). Including a zero in the division generates an incalculable result. It was determined that the administration of the treatment 4 or more than 5 times a year may be a risk factor since, on the one hand, the calculated frequency measures resulted in values greater than 1; the relative risk obtained was 1.8 and the ORs are in a range from 1.27 to infinity. Therefore, the increase in frequency in the exposed group can be considered to be due to the effect of the independent variable. One way to solve the fact that the OR is incalculable is to proportionally increase the values of each box(43), so when doing so, a value of OR= 11.14 and P=0.032 was obtained; although this result cannot be taken as reliable, it leaves open the possibility that, in future studies, including a larger number of farms studied, the increase in ORs for farms that apply treatments 4 or more times a year can be verified.

Regarding the independent variable of formulation administered, it was observed that more than half of the farmers use IVM-LA formulations of 3.15 % to 4 % due to the lack of efficacy of the 1 % formulation. IVM-LA formulations have a higher risk of generating resistant populations when applied with high frequency compared to 1 % short-acting formulations(1). This is due to several factors, such as a higher concentration of the active ingredient in IVM-LA formulations, an applied dose that is three times higher (630 μg/kg), a prolonged withdrawal period, a decrease in natural immunity and a faster selection of resistant parasites(41,44,45). The binomial logistic regression analysis showed that for the variable of formulation administered, a $P \leq 0.1101$ (OR= 6.59, 95 % CI= 0.5428 and SE= 1.27) was obtained, which was not significant as a possible risk factor, but with a positive association. With these data, the only susceptible population (VMA1) was related to the possible associated risk factors due to the fact that, in this population, a lower frequency of treatments was found: 1-3 per year and a lower formulation administered: IVM at 1 %.

Conclusions and implications

Based on the results obtained, it was shown that, in the states of Veracruz, San Luis Potosí and Tamaulipas, there are no populations susceptible to IVM and 14 to 25 % of these have incipient resistance. On the other hand, in the state of Nuevo León, only one susceptible population was found. *R. microplus* is resistant to IVM in northeastern Mexico (80 %). Currently, the frequency of applications of 4 or more than 5 times a year is the only risk factor that could be associated with the presence of resistant populations. Therefore, it is necessary to migrate to new control methods, such as including several families of ixodicides, carrying out integrated control, responsible management, and a culture of diagnosis in order to reduce the selection pressure to which populations are exposed.

Acknowledgments and conflicts of interest

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