ASIAN JOURNAL OF AGRICULTURE
Volume 10, Number 1, June 2026
Pages: 8
E-ISSN: 2580-4537
https://doi.org/10.13057/asianjagric/g100180

Cypermethrin resistance in *Rhipicephalus microplus* from cattle farms in the Ecuadorian Amazon

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Manuscript received: 20 February 2026. Revision accepted: 26 June 2026.

Abstract. *Zapata J, Cabezas L, Vaca M, Llerena J, Masaquiza D. 2026. Cypermethrin resistance in* Rhipicephalus microplus *from cattle farms in the Ecuadorian Amazon. Asian J Agric 10 (1): g100180. https://doi.org/10.13057/asianjagric/g100180.* Acaricide resistance in the cattle tick *Rhipicephalus microplus* represents an important sanitary challenge associated with herd management practices in tropical livestock systems. In the Ecuadorian Amazon, control of *R. microplus* frequently relies on the non-standardized use of chemical acaricides, with cypermethrin widely applied and often used with limited product rotation and technical guidance. The continued use of the same active ingredient contributes to the resistance of ticks to this product. In this study, the in vitro sensitivity of *R. microplus* to cypermethrin was evaluated using the Larval Packet Test (LPT) at four livestock farms in La Joya de los Sachas, and the relationship with management practices was determined. One hundred adult female ticks were collected and incubated under laboratory conditions to obtain larvae for the bioassays, which were exposed to different concentrations of cypermethrin; lethal concentrations were determined using Probit models. Additionally, a survey was administered to producers regarding the use of acaricides, treatment frequency, treatment rotation, herd size, and access to veterinary assistance. With regard to mortality, a dose-response pattern was observed across the different concentrations evaluated. LC_{50} values ranging from 0.009 to 0.037 mg/mL were found, indicating variability in susceptibility to cypermethrin across different farms. When classified based on the thresholds, one population was susceptible (RR_{50}=1.00), and the others were classified as tolerant (RR_{50}=3.11-4.11). Farms reporting acaricide rotation, lower treatment frequency, and technical assistance tended to exhibit lower RR_{50} values, while larger herd sizes and more frequent treatments were associated with higher resistance ratios. These findings suggest that integrated approaches combining resistance monitoring with improved management practices may contribute to sustaining tick control in cattle production systems of the Ecuadorian Amazon.

Keywords: Acaricide resistance, cattle farms, farm management, larval packet test, *Rhipicephalus microplus*

INTRODUCTION

The constant growth of the world's population has increased global demand for food, constituting a crucial challenge for agri-food systems. This challenge is directly related to human development indicators, given that food security is a pillar of social and economic stability (Sánchez-Hernández et al. 2020). Agriculture and livestock farming have responded with technological innovations aimed at increasing production efficiency; however, both sectors encounter structural and environmental limitations, particularly in rural areas of developing countries (Álvarez 2020).

Cattle production is one of the main economic activities in the Ecuadorian Amazon. The region's climatic conditions characterized by high temperature, humidity, and rainfall favor the proliferation of ectoparasites as the cattle tick *Rhipicephalus microplus* (Makwarela et al. 2025).

Rhipicephalus microplus is the principal ectoparasite responsible for direct economic losses in livestock production through blood loss, skin damage, decreased weight gain, reduced milk yield, and increased veterinary costs (Rodríguez-Vivas et al. 2018). It is also the primary vector for the transmission of diseases, such as bovine babesiosis and anaplasmosis, leading to high morbidity and mortality rates in cattle. The combined effect of ticks and the pathogens affects productive performance, reproduction, commercial value of the animals, and consequently the producer's profits (Abbas et al. 2014; Rodríguez-Vivas et al. 2018). Infestation by this ectoparasite is one of the main challenges for livestock farming in tropical areas.

The economic losses caused by ticks are significant. They are estimated at more than USD 7 billion annually worldwide (Rodríguez et al. 2023). In Latin America, studies report losses exceeding USD 3 billion annually due to *R. microplus* alone (Galván et al. 2023).

The use of chemical acaricides is the primary method of tick control in tropical livestock farming such as: cypermethrin, being the most widely used product due to its low cost. However, its use and without an adequate rotation promotes the development of resistance in tick populations (Obaid et al. 2022). In Latin America, specifically in tropical regions, this phenomenon is widely documented, affecting the viability of using chemicals as a long-term strategy (Rojas-Cabeza et al. 2025). Resistance to acaricides more than doubles control costs on farms with limited access to technology, a situation that increases the vulnerability of small-scale farmers and justifies the need to adopt integrated management approaches (Paucar-Quishpe et al. 2024).

The resistance of *R. microplus* to various chemical as pyrethroids can be attributed to various physiological and genetic mechanisms, for example: mutations at target sites and increased metabolic detoxification via enzymes: esterases, cytochrome P450 monooxygenases, and glutathione S-transferases (Abbas et al. 2014; Cossio-Bayugar et al. 2024). Furthermore, management practices on each farm, the treatment frequency, product rotation, herd size, and access to technical assistance, also influence resistance (Guerrero et al. 2012; Cossio-Bayugar et al. 2024). The design of sustainable strategies is a crucial element that must be adapted to the production realities in each region (Lagunes-Quintanilla et al. 2024).

Currently, there is limited information on studies determining the susceptibility status of tick populations in the Amazon region, as well as their relationship to management practices on farms. However, recent studies in Ecuador have demonstrated increasing resistance to pyrethroids and macrocyclic lactones, as well as the importance of environmental factors in acaricide resistance (Paucar-Quishpe et al. 2024; Pérez-Otáñez et al. 2024; Masaquiza et al. 2025).

Assessing tick susceptibility to acaricides is a strategy that has been used in integrated pest management programs for this ectoparasite. Its importance lies in the early detection of resistance to various active ingredients. Likewise, one of the main tools used in the laboratory to diagnose resistance to acaricides is the Larval Packet Test (LPT), which allows the establishment of a dose-response relationship in larvae exposed to different concentrations of acaricides; thereby yielding Resistance Indices (RI). These facilitate the comparison of sensitivity levels among populations making it possible to compare populations from different regions and production systems (Jongejan et al. 2024; Pérez-Otáñez et al. 2024).

This study was focused on the hypothesis that *R. microplus* populations from cattle farms in the Ecuadorian Amazon exhibit heterogeneous susceptibility to cypermethrin, due to differences in farm-management practices and acaricide selection pressure. Specifically, farms characterized by larger herd sizes, higher treatment frequency, limited product rotation, and reduced technical assistance were expected to show higher resistance ratios (RR_{50}) and reduced cypermethrin susceptibility compared with farms by implementing more integrated management practices. Therefore, this study aimed to evaluate the in vitro susceptibility of *R. microplus* to cypermethrin using the larval packet test and to explore descriptive patterns between resistance indicators and tick management practices in cattle farms of the Ecuadorian Amazon.

MATERIALS AND METHODS

Study area

The study was conducted in La Joya de los Sachas, Orellana Province, Ecuador (00°17´S, 76°52´W; 270-320 m a.s.l.), an area characterized by humid tropical climate conditions typical of the Ecuadorian Amazon. Mean annual temperatures range between 24 and 27°C, with relative humidity above 80% and annual precipitation exceeding 3000 mm (Instituto Nacional de Meteorología e Hidrología (INAMHI) 2023). These environmental conditions favor the proliferation and persistence of *R. microplus* ticks in bovine production systems. Sampling was conducted between May and July 2025, corresponding to the period of higher tick infestation reported by producers in the study area.

Procedures

Selection and collection of tick samples

A total of 100 adult *R. microplus* ticks were collected from naturally infested cattle on four livestock farms within the study area (Figure 1), with 25 engorged adult females collected per farm. On each farm, approximately 10 to 15 animals were inspected during routine sanitary evaluation to identify animals with sufficient tick infestation for sampling.

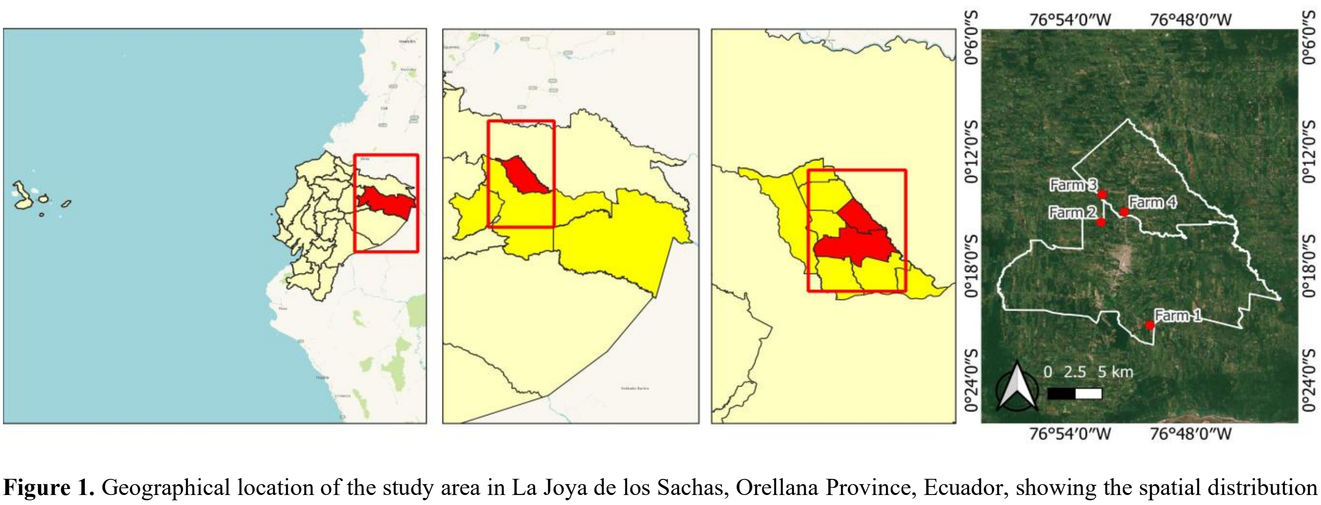

Figure 1. Geographical location of the study area in La Joya de los Sachas, Orellana Province, Ecuador, showing the spatial distribution of the cattle farms sampled for *Rhipicephalus microplus* collection and cypermethrin susceptibility evaluation during 2025

Between 1 and 3 engorged ticks were collected from each animal, up to a total of 25 per farm. Animals were selected based on visible infestation, defined as the presence of at least 10 adult ticks per animal, without restriction on breed or age, reflecting typical field conditions. Farms were selected using purposive sampling to represent contrasting tick management conditions typical of the study area. Selection criteria included differences in acaricide use patterns, treatment frequency (≤6 to >6 applications per year), herd size (<25 to >60 cattle), and access to veterinary technical assistance, classified as presence or absence of professional guidance.

The influence of these variables on selective pressure, as well as on the development of resistance to acaricides in *R. microplus*, is widely documented. Similarly, although the number of farms was small (n=4), they are representative of production systems in the Amazon region, allowing for comparisons of field conditions.

Fully engorged adult females were selected as the inclusion criteria for larval production, following general recommendations for tick bioassays and larval production procedures (Food and Agriculture Organization (FAO) 2004; Jongejan et al. 2024). Ticks were manually removed from body regions with visible infestation, prioritizing specimens in adequate physiological condition, including complete engorgement, intact body structure, and absence of visible damage. Removal was performed carefully to avoid damage to the hypostome and reproductive structures (Rivera et al. 2009). This procedure was carried out during routine sanitary inspections with the consent of farm owners.

Specimens were taxonomically identified using dichotomous keys and stereoscopic observation according to Dantas et al. (2019). Collected ticks were transported to the laboratory under controlled conditions, cleaned by gently removing external debris with distilled water and absorbent paper, visually inspected, incubated and processed for larval production for subsequent bioassays.

Larval production

The engorged ticks were placed in labeled Petri dishes and incubated at 27-28°C and 85-95% relative humidity until oviposition. Egg masses were maintained in the incubator under the same conditions until hatching. Larvae between 14 and 21 days old were used for the larval packet test.

Farm management component

A structured epidemiological survey was conducted among farm owners to determine the control practices used to combat ticks. The survey included questions about farm management and the use of acaricides. Data was obtained about: acaricides used, rotation of active ingredients, frequency and method of application, number of animals, production goals, livestock replacement, acaricide dosage, and access to veterinary care. This information was cross-checked with the records available at each farm.

The variables collected were previously classified to facilitate comparison among the farms (Table 1). Farm size was classified based on the total number of animals, and the total amount of acaricide applications made during the year was considered the treatment frequency; similarly, the change in active principle during the year was considered product rotation. Given the small number of farms in the study (n=4), the comparison was descriptive for data related to management practices.

Preparation of dilutions

Technical grade cypermethrin (40% active ingredient) was used. Serial dilutions were prepared in a vehicle mixture of olive oil and trichloroethylene (7:14 v/v) following the standardized larval packet test protocol described by Jongejan et al. (2024). Eight concentrations were prepared: 0.00312%; 0.00625%; 0.0125%; 0.025%; 0.05%; 0.1%; 0.2%, and 0.4%, plus a control treatment containing only the solvent (Table 2).

Larval packet test

The Larval Packet Test (LPT) was conducted following a sequential protocol, including filter paper impregnation with the cypermethrin solution, solvent evaporating, package preparing, larval placement, incubating, and mortality assessment. Bioassays were performed 14-21 days after egg hatching, when larvae were fully developed according to Anderson and Magnarelli (2008), following standardized procedures (Pérez-Otáñez et al. 2024).

Table 1. Management variables considered in the analysis of cypermethrin resistance in *Rhipicephalus microplus*

Variable	Description	Category
Farm size	Number of animals per farm	<25 - 25-60 - >60
Production purpose	Primary production objective	Milk - Meat - Mixed
Treatment frequency	Acaricide treatments per year	≤ 6 times/year - >6 times/year
Treatment type	Application method	Spraying - Pour-on - immersion bath
Product rotation	Alternation of different acaricide classes within one year	Yes - No
Tick seasonality	Season reported by farmers as having the highest tick infestation	Rainy season - Dry season - Year-round
Recommended dose	Uses of the recommended dosage	Yes - No
Veterinary assistance	Receives veterinary assistance	Yes - No

Table 2. Experimental design of the larval bioassay with cypermethrin

Treatment	Description	Concentration (%)	No. of replicates	Larvae per replicate	Total larvae
T0	Control	0.00000	3	100	300
T1	Cypermethrin	0.00312	3	100	300
T2	Cypermethrin	0.00625	3	100	300
T3	Cypermethrin	0.0125	3	100	300
T4	Cypermethrin	0.025	3	100	300
T5	Cypermethrin	0.05	3	100	300
T6	Cypermethrin	0.1	3	100	300
T7	Cypermethrin	0.2	3	100	300
T8	Cypermethrin	0.4	3	100	300

Impregnated filter papers (Whatman No. 1, 7.5 × 8.5 cm) were treated with 0.67 mL of each solution and allowed to dry for 30 minutes in a fume hood to ensure solvent evaporation prior to larval exposure. Paper strips were folded into packets and sealed with metal clips. A total of 100 viable larvae were counted and introduced into each packet, with three replicates per treatment. Packets were incubated at 27-28°C and 85% relative humidity for 24 hours (Klafke et al. 2024), and mortality was assessed by stereomicroscopic observation.

Data analysis

Larval mortality data from each bioassay were analyzed using Probit analysis with POLO PLUS software (LeOra Software 2004). Although cypermethrin concentrations were prepared and applied as percentages following standard larval packet test protocol, all Lethal Concentration (LC_{50} and LC_{99}) values were expressed in mg/mL for statistical analysis. Percentage concentrations were converted to mg/mL based on the 40% active ingredient concentration of the technical grade cypermethrin, and these converted values were used as input for Probit analysis. This conversion was necessary to standardize input values for Probit analysis based on the active ingredient concentration. For each tick population, lethal concentrations required to cause 50% (LC_{50}) and 99% (LC_{99}) mortality were estimated along with their respective 95% confidence intervals.

In the absence of a cypermethrin-susceptible reference strain, the tick population exhibiting the lowest LC_{50} and LC_{99} values was used as the susceptible reference population to calculate the Resistance Ratio (RR), following the approach proposed by Daniele et al. (2021). Resistance status was categorized using resistance ratios derived from LC_{50} values (RR_{50}), according to previously proposed thresholds for cypermethrin susceptibility in *R. microplus,* where $RR_{50}<3$ indicates susceptibility, RR_{50} between 3 and 5 indicates tolerance, and $RR_{50}>5$ indicates resistance (Cabrera-Jiménez et al. 2008).

Differences among populations were interpreted descriptively based on the overlap of 95% confidence intervals. Given the limited number of farms included in the study (n=4), the relationship between management practices and resistance indicators was assessed descriptively, without formal statistical inference. The absence of a standard susceptible reference strain represents a limitation, and comparisons should be interpreted relative to the most susceptible population identified in this study.

RESULTS AND DISCUSSION

Dose-response analysis and lethal concentrations

The Probit analysis provided the dose-response relationship between cypermethrin and mortality, determining the 50% Lethal Concentration (LC_{50}) and the 99% Lethal Concentration (LC_{99}). Variations in LC_{50} were observed among farms with Farm 1 registering the lowest value (0.009 mg/mL), which was used as a susceptibility reference (Table 3). The other populations showed higher LC_{50} values, reflecting reduced sensitivity to the evaluated product.

With respect to the LC_{99} values, these were higher than the LC_{50}, reaching 3.03 mg/mL, resulting in the near-total mortality of the tick populations. Differences were observed across all the farms evaluated, where the dose-dependent response varied in relation to the dose administered. This demonstrated variation in susceptibility among the farms; confirming that the toxic effect of cypermethrin in La Joya de los Sachas does not have the same intensity.

These differences are reflected, both in the separation of the mortality curves and in the proportion of response observed at intermediate and high concentrations (Figure 2). At the highest concentrations, larval mortality was near-total in all populations, suggesting that, despite the variability in susceptibility observed at lower doses; cypermethrin remains effective when applied at high concentrations.

Resistance ratio and population classification

Based on LC_{50} values, Resistance Ratios (RR_{50}) were calculated for each population using the farm with the lowest LC_{50} value (F1) as the susceptible reference population. RR_{50} values were used to classify the evaluated populations according to previously established thresholds for cypermethrin susceptibility (Table 3).

This classification indicates that, within La Joya de los Sachas, tick populations with different susceptibility levels coexist, suggesting variability in selection pressure exerted by acaricide use across the evaluated livestock systems. The identification of tolerant populations suggests an intermediate stage in resistance development, where control may still be achieved at higher concentrations, but reduced susceptibility is already evident.

Larval packet mortality by farm

Analysis of larval mortality by farm revealed differences in the response of *R. microplus* populations to the evaluated cypermethrin concentrations. In F1 and F2, high mortality levels were recorded even at intermediate concentrations, whereas in F3 and F4 the highest doses were required to achieve comparable levels of control. The dose-response trend and differences in population responses are clearly illustrated in Figure 3, which compares mortality dose-response curves among farms. These differences reflect intra-regional variability in acaricide efficacy associated with the presence of tick populations exhibiting different resistance levels, and with differentiated sanitary management practices among the evaluated livestock systems.

Local tick control practices and their relationship with the Resistance Ratio (RR_{50}) to cypermethrin

Descriptive patterns were observed between farm-level management practices and the Resistance Ratio (RR_{50}). Farms reporting product rotation and access to technical assistance, as well as lower treatment frequency (≤6 applications per year), tended to show lower RR_{50} values. In contrast, higher RR_{50} values were observed in farms with larger herd sizes and higher treatment frequency (>6 applications per year). Farm-level management characteristics and their relationship with RR_{50} values are presented in Table 4. However, given the limited number of farms evaluated, these patterns should be interpreted as exploratory and not as evidence of independent causal relationships.

The patterns observed in Table 4 suggest that resistance levels vary according to farm-level management conditions, highlighting differences among the evaluated production systems. From a management perspective, this variability in RR_{50} values suggests differences in acaricide selection pressure among farms. Production systems implementing rotation strategies and technical guidance tended to show reduced selection pressure, whereas farms with larger herd sizes and higher treatment frequency may increase the exposure of tick populations to the same active ingredient.

Table 3. Probit model estimates, lethal concentrations, and resistance ratios of *Rhipicephalus microplus* larval populations exposed to cypermethrin

ID	Slope ± SE	χ^2	p-value	LC_{50} (95% CI)	RR	LC_{99} (95% CI)	RR	Status
F1	1.409±0.109	21.755	0.001	0.009 (0.007-0.010)	1.00	0.394 (0.278-0.606)	1.00	Susceptible (Reference)
F2	1.838±0.100	23.132	0.001	0.028 (0.025-0.033)	3.11	0.524 (0.380-0.778)	1.33	Tolerant
F3	1.202±0.046	6.179	0.403	0.036 (0.032-0.040)	4.00	3.031 (2.183-4.429)	7.69	Tolerant
F4	1.571±0.053	90.314	0.001	0.037 (0.030-0.046)	4.11	1.121 (0.674-2.217)	2.84	Tolerant

Note: Slope values are presented with standard errors. χ^2 and p-values correspond to the Pearson goodness-of-fit test. LC_{50} and LC_{99} are shown with 95% confidence intervals. RR_{50} and RR_{99} were calculated using the most susceptible population as a reference

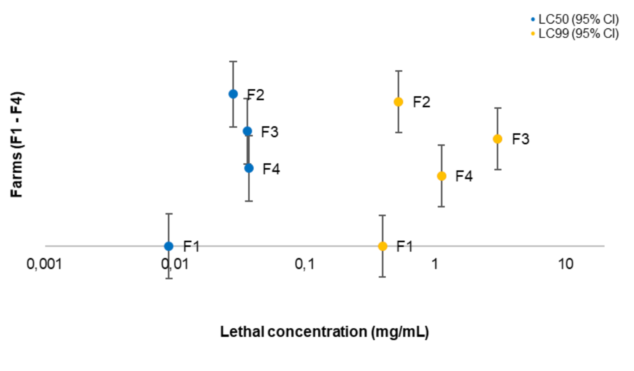

Figure 2. Comparison of LC_{50} and LC_{99} (95% CI) of cypermethrin in larval populations of *Rhipicephalus microplus* from four cattle farms in La Joya de los Sachas, Orellana Province, Ecuador. The x-axis is presented on a base-10 logarithmic scale (log_{10}). Points represent estimates derived from the Probit model, and horizontal bars indicate 95% confidence intervals

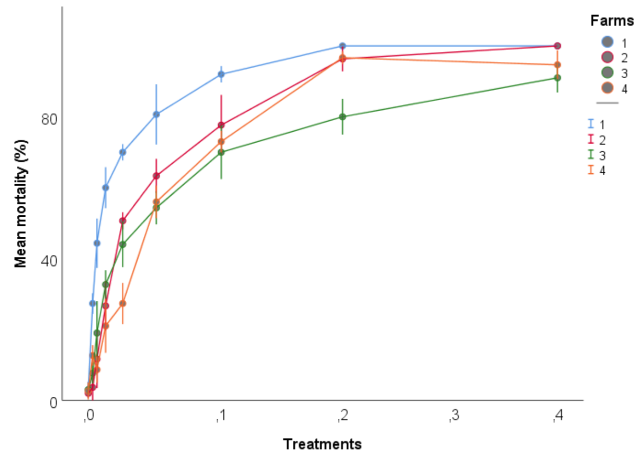

Figure 3. Dose-response curves of larval mortality (%) of *Rhipicephalus microplus* exposed to cypermethrin in four cattle farms in La Joya de los Sachas, Orellana Province, Ecuador. Blue line: farm 1; red line: farm 2; green line: farm 3; orange line: farm 4. Points represent the mean values (n=3 replicates per concentration and farm). Error bars indicate the 95% confidence Interval (95% CI). Cypermethrin concentrations are presented on a base-10 logarithmic scale (log_{10}). Treatments (doses): T0: 0% (control); T1: 0.00312%; T2: 0.00625%; T3: 0.0125%; T4: 0.025%; T5: 0.05%; T6: 0.1%; T7: 0.2% and T8: 0.4%

Table 4. Integration of farm-management characteristics, resistance ratios (RR_{50}), and resistance interpretation in *Rhipicephalus microplus* populations

Farm	Herd size	Treatment frequency	Product rotation	Technical assistance	RR_{50}	Resistance interpretation
F1	<25	≤6 times/year	Yes	Yes	1.00	Susceptible population associated with lower selection pressure and preserved cypermethrin susceptibility.
F2	25-60	≤6 times/year	Yes	No	3.11	Tolerant population, suggesting reduced susceptibility despite product rotation and low treatment frequency, possibly influenced by limited technical assistance.
F3	>60	>6 times/year	Yes	No	4.00	Tolerant population associated with larger herd size and higher treatment frequency, indicating increased selection pressure.
F4	>60	>6 times/year	No	No	4.11	Tolerant population with the highest RR_{50}, associated with high treatment frequency, absence of product rotation, and lack of technical assistance.

Note: Interpretations are descriptive and exploratory due to the limited number of farms evaluated

Discussion

Dose-response analysis and lethal concentrations

The variability observed in the susceptibility of *R. microplus* to cypermethrin suggests that resistance to this acaricide does not manifest homogeneously among the evaluated farms in La Joya de los Sachas. This pattern has been previously described in tropical livestock systems, where the selection pressure exerted by the continuous use of pyrethroids generates populations with different tolerance levels within the same geographic region (Pérez-Otáñez et al. 2024; Rojas-Cabeza et al. 2025). The dose-response pattern recorded in the bioassays indicates that cypermethrin maintains efficacy at higher concentrations; however, reduced mortality at lower doses suggests the presence of less susceptible individuals, which constitutes an early indicator of resistance development (Khating et al. 2024).

Similar variability in cypermethrin susceptibility among cattle tick populations has been reported in other tropical regions where chemical control remains the main strategy for tick management. Studies conducted in Latin America, Asia, and Africa have documented heterogeneous resistance patterns in *R. microplus*, reflecting differences in acaricide use history, management practices, and environmental conditions affecting tick population dynamics (Sindhu et al. 2022; Khating et al. 2024). These findings support the idea that resistance development is a gradual and spatially heterogeneous process influenced by both biological and management-related factors.

The Probit analysis allowed for a more accurate estimation of the lethal concentrations (LC_{50} and LC_{99}), revealing an increase in the LC_{50} in certain populations. This pattern could indicate a progressive decline in susceptibility, even when the product appears to be effective at higher concentrations. This phenomenon has been identified as a critical phase in the development of resistance where treatment failures may not yet be evident in the field, but the future effectiveness of the acaricide could be compromised due to the ongoing selection of less susceptible individuals (Dzemo et al. 2023; Lema 2024).

Acaricide resistance in cattle ticks has been associated with multiple mechanisms, including target-site mutations and metabolic detoxification processes (Abbas et al. 2014; Cossio-Bayugar et al. 2024). However, as no molecular analyses were performed in this study, the observed patterns in LC_{50} and LC_{99} values should be interpreted as phenotypic responses, which these underlying mechanisms may influence.

Resistance ratio and population classification

The Resistance Ratio (RR_{50}) allowed the classification of populations as susceptible or tolerant according to previously established thresholds, indicating variability in susceptibility. Similar findings have been reported in recent studies, suggesting that the coexistence of populations with different resistance levels within the same area reflects variation in acaricide use and sanitary management practices implemented by producers (Pérez-Otáñez et al. 2024).

From a practical livestock management perspective, the presence of tolerant populations suggests that cypermethrin may still be effective under certain conditions; however, the increase in LC_{50} values indicates a trend toward reduced susceptibility. This scenario represents an early stage in the development of resistance, where continued use of the same active ingredient without rotation could accelerate the selection of more resistant populations.

At the farm level, these patterns may be related to local pest management conditions that influence selection pressure on *R. microplus* populations, and consistent with the interactions between biological processes and management factors documented in previous studies (Rodríguez-Vivas et al. 2018; Obaid et al. 2022; Paucar-Quishpe et al. 2024). These results highlight the importance of the rational use of acaricides, particularly through rotation strategies and technical guidance to reduce such pressure. In contrast, systems lacking these practices could favor the progressive selection of less susceptible ticks, compromising the long-term effectiveness of control.

Local tick control practices and their relationship with the Resistance Ratio (RR_{50}) to cypermethrin

Although the small number of farms evaluated (n=4) prevented formal statistical inferences; descriptive patterns were observed between management practices and the Resistance Ratio (RR_{50}). Lower resistance ratios tended to be associated with farms reporting product rotation and access to technical assistance, which are key components of integrated tick management strategies reported to reduce the risk of acaricide resistance (Rodríguez et al. 2023; Lagunes-Quintanilla et al. 2024). In contrast, production systems with larger herd sizes and higher treatment frequency (>6 applications per year) showed comparatively higher resistance ratios, suggesting increased selection pressure due to repeated exposure of tick populations to the same active ingredient, as described in previous studies conducted in medium and large-scale livestock systems (Paucar-Quishpe et al. 2024; Makwarela et al. 2025).

The strong interrelationships observed among certain management variables may reflect structural and operational characteristics of the evaluated production systems rather than independent causal effects. Such dependencies are commonly reported in studies based on sanitary management surveys, where technical decisions such as access to veterinary assistance and product rotation are often implemented jointly as part of integrated management strategies (Lema 2024; Rojas-Cabeza et al. 2025). This descriptive pattern supports an exploratory resistance-risk framework in which farm-management practices may influence acaricide selection pressure and cypermethrin susceptibility in *R. microplus*, although causal relationships cannot be inferred from the limited number of farms evaluated.

Implications for management

Overall, the results indicate that variability in cypermethrin susceptibility in *R. microplus* may be associated with multiple factors, including parasite biology, acaricide use history, and farm-level management practices, highlighting the importance of integrated management strategies aimed at preserving the efficacy of available acaricides (Rodríguez-Vivas et al. 2018).

The integration of in vitro resistance diagnosis with information on farm management practices may contribute to a better understanding of resistance patterns and support the development of sustainable tick control strategies that consider both scientific evidence and farm-level knowledge, in order to preserve acaricide efficacy and improve the sustainability of cattle production in the Ecuadorian Amazon.

In conclusion, *R. microplus* populations from cattle farms in the Ecuadorian Amazon showed heterogeneous susceptibility to cypermethrin. The evaluated populations showed LC_{50} values ranging from 0.009 to 0.037 mg/mL and RR_{50} values between 1.00 and 4.11, indicating the coexistence of susceptible and tolerant populations under field conditions, one farm remained within the susceptible category ($RR_{50}<3$), whereas three farms were classified as tolerant ($RR_{50}=3-5$). Variability in resistance ratios (RR_{50}) appears related to certain farm-level management practices. The farms that showed lower levels of resistance were associated with practices such as: product rotation, received technical assistance, and applied treatments less frequently, whereas farms with larger numbers of animals and more frequent treatments showed higher RR_{50} values.

The use of cypermethrin in the Ecuadorian Amazon is still in its early stages because of integrated strategies are needed to combat this ectoparasite. However, the study was limited by the small number of farms included and the lack of molecular studies to determine the presence of mutations associated with the resistance process.

ACKNOWLEDGEMENTS

The authors thank the participating cattle farmers for their collaboration during field sampling and data collection. The authors declare no conflicts of interest regarding the publication of this manuscript.

REFERENCES

Abbas RZ, Zaman MA, Colwell DD, Gilleard J, Iqbal Z. 2014. Acaricide resistance in cattle ticks and approaches to its management: The state of play. Vet Parasitol 203 (1-2): 6-20. https://doi.org/10.1016/j.vetpar.2014.03.006.

Álvarez J. 2020. Desempeño relativo de la productividad física de la ganadería de Nueva Zelanda y Uruguay, 1870-2010. História agraria: Revista de agricultura e história rural. Sociedad EspaÃ±ola de Historia Agraria (80): 107-144. https://doi.org/10.26882/histagrar.080x06a. [Spanish]

Anderson JF, Magnarelli LA. 2008. Biology of ticks. Infect Dis Clin North Am 22 (2): 195-215. https://doi.org/10.1016/j.idc.2007.12.006.

Cabrera-Jiménez D, Rosado-Aguilar J, Rodríguez-Vivas R, García-Vázquez Z. 2008. Evaluación de la resistencia a la cipermetrina en cepas de campo de *Boophilus microplus* obtenidas de ranchos bovinos del estado de Yucatán, México. Técnica Pecuaria en México 46 (4): 439-448. [Spanish]

Cossio-Bayugar R, Martínez-Ibáñez F, Aguilar-Díaz H, Miranda-Miranda E. 2024. Relationship between acaricide resistance and acetylcholinesterase gene polymorphisms in the cattle tick *Rhipicephalus microplus*. Parasite 31: 3. https://doi.org/10.1051/parasite/2024003.

Daniele M, Dadé M, Álvarez JD, Reynaldi FJ, Errecalde JO, Rodríguez-Vivas RI. 2021. Current status of resistance to ivermectin in *Rhipicephalus sanguineus* sensu stricto infesting dogs in three provinces in Argentina. Vet Parasitol Reg Stud Rep 26: 100624. https://doi.org/10.1016/j.vprsr.2021.100624.

Dantas F, Martins TF, Muñoz-Leal S, Onofrio VC, Barros-Battesti DM. 2019. Ticks (Ixodida: Argasidae, Ixodidae) of Brazil: Updated species checklist and taxonomic keys. Ticks Tick Borne Dis 10: 101252. https://doi.org/10.1016/j.ttbdis.2019.06.012.

Dzemo WD, Vudriko P, Ramatla T, Thekisoe O. 2023. Acaricide resistance development in *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) populations against amitraz and deltamethrin on communal farms of the King Sabata Dalindyebo municipality, South Africa. Pathogens 12 (7): 875. https://doi.org/10.3390/pathogens12070875.

Food and Agriculture Organization (FAO). 2004. Guidelines Resistance Management and Integrated Parasite Control in Ruminants. Module 1: Ticks: Acaricide Resistance: Diagnosis, Management and Prevention. Food and Agriculture Organization, Rome.

Galván D, Vieyra P, de Oca RM, Ortega J, Martínez S, Rivas B, Vázquez J. 2023. Proteína Bm86 y su potencial uso como vacuna contra garrapatas en el ganado bovino. Revisión. Revista Mexicana De Ciencias Pecuarias 14 (3): 672-695. https://doi.org/10.22319/rmcp.v14i3.6255. [Spanish]

Guerrero FD, Lovis L, Martins JR. 2012. Acaricide resistance mechanisms in *Rhipicephalus (Boophilus) microplus*. Rev Bras Parasitol Vet 21: 1-6. https://doi.org/10.1590/S1984-29612012000100002.

Instituto Nacional de Meteorología e Hidrología (INAMHI). 2023. Información Meteorológica e Hidrológica del Ecuador. INAMHI, Quito.

Jongejan F, Berger L, Homminga L, Hulsebos I, Petersen A, Ferreira PT, Klafke G. 2024. Resistance Intensity Test (RIT): A novel bioassay for quantifying the level of acaricide resistance in *Rhipicephalus microplus* ticks. Parasit Vector 17 (1): 480. https://doi.org/10.1186/s13071-024-06561-6.

Khating S, Jadhav N, Vijay M, Sharma AK, Srivastava A, Jadhao S, Kumar S, Kalwaghe S, Sddiqui MFMF, Narawade M, Dhabale A, Chigure G. 2024. Current profile of phenotypic pyrethroid resistance in *Rhipicephalus microplus* (Acari: Ixodidae) populations sampled from the Marathwada region of Maharashtra state, India. Parasitol Res 123 (7): 282. https://doi.org/10.1007/s00436-024-08306-x.

Klafke GM, Golo PS, Monteiro CM, Costa-Júnior LM, Reck J. 2024. Brazil's battle against *Rhipicephalus* (*Boophilus*) *microplus* ticks: Current strategies and future directions. Rev Bras Parasitol Vet 33 (2): e001423. https://doi.org/10.1590/S1984-29612024026.

Lagunes-Quintanilla R, Gómez-Romero N, Mendoza-Martínez N, Castro-Saines E, Galván-Arellano D, Basurto-Alcántara FJ. 2024. Perspectives on using integrated tick management to control *Rhipicephalus microplus* in a tropical region of Mexico. Front Vet Sci 11: 1497840. https://doi.org/10.3389/fvets.2024.1497840.

Lema ED. 2024. Evaluación de la resistencia de *Rhipicephalus* spp. a la ivermectina y de factores condicionantes en bovinos del cantón Joya de los Sachas. [Tesis de pregrado]. Escuela Superior Politécnica de Chimborazo, Riobamba. [Spanish]

LeOra Software. 2004. POLO-Plus: Probit and Logit Analysis User's Guide. LeOra Software, Berkeley, CA, USA.

Makwarela TG, Seoraj-Pillai N, Malatji DP, Nangammbi TC. 2025. Adaptation and invasion dynamics of *Rhipicephalus microplus* in South Africa: Ecology, resistance, and management implications. Insects Insects 16 (12): 1204. https://doi.org/10.3390/insects16121204.

Masaquiza DA, Rivera SA, Zapata JE, Cabezas-Andrade LD, Daniele M, Romero D. 2025. *Rhipicephalus microplus*: Estado actual de la resistencia a la ivermectina en el ganado bovino de la Amazonía ecuatoriana. Agrisost 31: 1-10. https://doi.org/10.5281/zenodo.15856329.

Obaid MK, Islam N, Alouffi A, Khan AZ, Vaz IDS, Tanaka T, Ali A. 2022. Acaricides resistance in ticks: Selection, diagnosis, mechanisms, and mitigation. Front Cell Infect Microbiol 12: 941831. https://doi.org/10.3389/fcimb.2022.941831.

Paucar-Quishpe V, Pérez-Otáñez X, Rodríguez-Hidalgo R, Pérez-Escalante C, Cepeda-Bastidas D, Grijalva J, Ron-Garrido L. 2024. Farmers' adoption, knowledge, and perceptions of tick control measures on dairy farms in subtropical areas of continental Ecuador. Transboun Emerg Dis 2024 (1): 5023240. https://doi.org/10.1155/2024/5023240.

Pérez-Otáñez X, Orozco G, Arciniegas S, Ron L, Rodríguez R. 2024. Widespread acaricide resistance and multi-resistance in *Rhipicephalus microplus* in Ecuador and associated environmental and management risk factors. Ticks Tick Borne Dis 15 (1): 102274. https://doi.org/10.1016/j.ttbdis.2023.102274.

Pérez-Otáñez X, Paucar-Quishpe V, Saegerman C, Grijalva-Olmedo J, Pérez-Escalante C, Jácome L, Enríquez S, Ron-Garrido L, Rodríguez-Hidalgo R, Vanwambeke SO. 2024. Drivers and evolution of acaricide resistance and multi-resistance in two Ecuadorian subtropical livestock farming areas. Vet Ital 60 (4): Special Issue GeoVet 23. https://doi.org/10.12834/VetIt.3471.23969.2.

Pérez-Otáñez X, Rodríguez R, Enríquez S, Celi-Erazo M, Benítez W, Saegerman C, Vanwambeke SO. 2024. High-resolution prediction models for *Rhipicephalus microplus* and *Amblyomma cajennense* s.l. ticks affecting cattle and their spatial distribution in continental Ecuador using bioclimatic factors. Exp Appl Acarol 92 (3): 439-462. https://doi.org/10.1007/s10493-023-00883-3.

Rivera G, Ramírez A, Mancera B, Muñoz S, Hernández A, Cervantes P. 2009. Resistencia de *Rhipicephalus* (*Boophilus*) spp. an ixodicidas en ranchos ganaderos del sur de Veracruz, México. XXVII Reunión Científica-Tecnológica Forestal y Agropecuaria 1: 603-612. [Spanish]

Rodríguez R, Tinoco T, Ron-Garrido L, Pérez-Otañez X. 2023. Preliminary evaluation of the effectiveness of the GAVAC® immunogen and the rational use of acaricides as an alternative for an integrated tick control program in Ecuador. ESPOCH Congrs Ecuador J STEAM 3 (1): 793-806. https://doi.org/10.18502/espochv3i1.14487.

Rodríguez-Vivas RI, Jonsson NN, Bhushan C. 2018. Strategies for the control of *Rhipicephalus microplus* ticks in a world of conventional acaricide and macrocyclic lactone resistance. Parasitol Res 117 (1): 3-29. https://doi.org/10.1007/s00436-017-5677-6.

Rojas-Cabeza JF, Moreno-Córdova E, Ayala-Zavala JF, Ochoa-Terán A, Sonenshine DE, Valenzuela JG, Sotelo-Mundo RR. 2025. A review of acaricides and their resistance mechanisms in hard ticks and control alternatives with synergistic agents. Acta Trop 261: 107519. https://doi.org/10.1016/j.actatropica.2024.107519.

Sánchez-Hernández Z, Galina-Hidalgo C, Vargas-Leitón B, Rojas-Campos J, Estrada-König S. 2020. Sistemas de información de gestión de rebaños para apoyar la investigación sobre la población bovina: El caso VAMPP®. Agronom Mesoameric 31 (1): 141-156. https://doi.org/10.15517/am.v31i1.37062. [Spanish]

Sindhu ZU, Naseer MU, Raza A, Aslam B, Ahmad J, Abbas RZ, Khan MK, Imran M, Zafar MA, Khattak B. 2022. Resistance to cypermethrin is widespread in cattle ticks (*Rhipicephalus microplus*) in the province of Punjab, Pakistan: In vitro diagnosis of acaricide resistance. Pathogens 11 (11): 1293. https://doi.org/10.3390/pathogens11111293.