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APPLIQUÉES AUX SCIENCES VÉTÉRINAIRES

Développement de méthodes pour comprendre l'épidémiologie de l'infestation par les tiques, les maladies transmises par les tiques et la résistance aux acaricides dans le bétail équatorien subtropical

Development of methods to understand the epidemiology of tick infestation, tick-borne diseases, and acaricide resistance in sub-tropical Ecuadorian livestock

Adriana Valeria PAUCAR QUISHPE

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Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.

Marie Curie

Luck is what happens when preparation meets opportunity

Seneca

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ACRONYMS

ADI	Acceptable daily intake
AGROCALIDAD	*Agencia de Regulación y Control Fito y Zoosanitario*
AIC	Akaike information criterion
ARES	Academy of research and higher education
AUC	Area under the curve
BCS	Body condition scale
BMPs	Best Management Practices
BS	Blood smear
Bw	Body weight
CART	Classification and regression tree
CAT	Card agglutination test
cELISA	Competitive-inhibition enzyme-linked immunosorbent assay
CFSPH	Center for Food Security and Public Health (Iowa State University)
CFT	Complement fixation test
CI	Confidence interval
CIZ	*Instituto Internacional de Zoonosis*
COVID-19	Coronavirus disease 2019
CPM	Costs of a litre of milk
DC	Daily consumption
DNA	Deoxyribonucleic acid
ECDE	Estimate Chronic Dose Exposure
EDI	Estimated daily intake
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
FAO	Food and Agriculture Organization of the United Nations
G	Gametocytes
GCDE	Global Chronic Dose Exposure
GDP	Gross domestic product
GAHP	Good Animal Husbandry Practices
Ha	Hectare
HCA	Hierarchical cluster analysis
HPLC	High Performance Liquid Chromatographic

ICT	Immunochromatographic test
IFAT	Indirect fluorescent antibody test
ITM	Integrated Tick Management
INEC	*Instituto Nacional de Estadística y Censos*
INIAP	*Instituto Nacional de Investigaciones Agropecuarias*
JECFA	Joint FAO/WHO Expert Committee on Food Additives
K	Kinete
LIT	Larval dip test
LOD	Limit of detection
LOQ	Limit of quantification
LPT	Larval package test
Mab	Monoclonal antibody
MAE	*Ministerio del Ambiente, Agua y Transición Ecológica*
MAGAP	*Ministerio de Agricultura, Ganadería, Acuacultura y Pesca*
MRL	Maximum residue limit
MCA	Multiple Correspondence Analysis
MLR	Multiple logistic regression
mPCR	Multiplex polymerase chain reaction
MSE	Mean squared error
MSP5	Major surface protein 5
OIE	World Organization for Animal Health
OMS	*Organización Mundial de la Salud*
NGO	Non-governmental organization
OR	Odds ratio
OWS	Overall weighted score
P	Annual Profit
PPE	Personal Protective Equipment
PCR	Polymerase Chain Reaction
rMSP5	Recombinant major surface protein 5
RBCs	Red blood cells
ROC	Receiver Operating Characteristic
Se	Sensibility
Sk	Strahlenkörper
Sp	Specificity
Sz	Sporozoites

T	Trophozoites
TBDs	Tick-borne diseases
TC	Total livestock production cost
TCM	Cost of milk production
TR	Total revenue
UCE	*Universidad Central del Ecuador*
USD	United States dollar
VIF	Variance inflation factor
WHO	World Health Organization
Z	Zygote

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RÉSUMÉ

Introduction : Le secteur de l'élevage est une source importante de revenus pour le développement de l'économie équatorienne. Dans le relief accidenté de l'Équateur, la production animale est concentrée dans trois zones géographiques. Les hautes terres andines ont un climat tempéré et utilisent un modèle intensif pour la production laitière. Les deux autres régions, la région côtière et la région amazonienne, ont un environnement tropical et utilisent un modèle extensif et semi-extensif pour la production de lait et de viande dans des exploitations mixtes (Sánchez *et al.*, 2019; Torres *et al.*, 2014). Environ 75 % des troupeaux de bétail se trouvent dans les zones côtières tropicales et subtropicales (Pourrut *et al.*, 1983; Torres *et al.*, 2014). Cependant, l'introduction de pâturage à cycle court et les conditions environnementales rendent le bétail sensible aux infestations à tiques, principalement *Rhipicephalus microplus*. Pour lutter contre les infestations de tiques, les agriculteurs ont recours à l'emploi d'acaricides, mais leur utilisation abusive a entraîné une résistance des tiques, ce qui aggrave le problème (Betancur & Giraldo-Ríos, 2019). Dans la quête d'un développement agricole durable de l'Equateur, la recherche, la collaboration interdisciplinaire, l'investissement dans la recherche et les initiatives de surveillance sont essentiels pour améliorer la gestion des infestations à tiques et des maladies à tiques (*tick-borne diseases* ou TBDs). Cette approche protégera les moyens de subsistance des agriculteurs tout en promouvant la santé interconnectée des humains, des animaux et de l'environnement dans le cadre du paradigme *One Health*.

Méthodologie : Cette étude a été réalisée dans le cadre d'un projet intitulé "Socio-éco-épidémiologie des tiques, des parasites transmis par les tiques, de la résistance aux acaricides et des effets résiduels des acaricides dans le bétail tropical équatorien : impacts sur l'environnement, les animaux et la santé publique" financée par L'Académie de recherche et d'enseignement supérieur (ARES). Cette étude se compose de 4 parties, pour lesquelles 6 activités de terrain ont été menées (**Annexe 1 et tableau 1**). Dans l'activité de terrain I, une enquête transversale a été menée dans 139 fermes de deux régions tropicales et subtropicales de l'Équateur au moyen de questionnaires. Dans l'activité de terrain II, le niveau d'infestation par les tiques a été évalué, des échantillons de sang ont été prélevés et des tiques ont été collectées pour des études de résistance et l'identification morphologique et afin d'investiguer la présence de pathogènes transmis par les tiques. Dans le cadre de l'activité de terrain III, des entrepôts agricoles ont été interrogés afin de déterminer le coût des intrants agricoles. Dans le cadre de l'activité de terrain IV, des réunions participatives ont été organisées avec des agriculteurs dans les zones étudiées. L'activité de terrain V a été menée dans des fermes locales où des échantillons de lait, d'urine et de fèces ont été prélevés pour mesurer les résidus d'ivermectine. En outre, des échantillons de viande et de foie ont été prélevés dans les abattoirs locaux afin de rechercher les teneurs en résidus d'ivermectine. Enfin, dans l'activité de terrain VI, une enquête transversale a été menée pour déterminer les quantités de produits et sous-produits bovins consommées. Les informations

recueillies ont permis de réaliser cinq études. L'**étude 1** a permis d'identifier les espèces de tiques présentes dans la zone d'étude, de déterminer le niveau d'infestation par les tiques et d'identifier les facteurs de risque associés au niveau d'infestation des tiques. L'**étude 2** a rapporté le niveau de résistance à trois acaricides (amitraz, alpha-cyperméthrine et ivermectine), la typologie de production des fermes étudiées a été réalisée et l'influence du traitement acaricide sur les coûts de production de la ferme laitière a été étudiée. L'**étude 3** a décrit les perceptions, les connaissances et les pratiques de contrôle des tiques, communes ou non, utilisées et a mis ces variables en relation avec le niveau d'infestation des tiques et la présence d'une résistance aux acaricides. L'**étude 4** a permis de déterminer la prévalence de l'anaplasmose bovine et la proportion d'animaux naturellement immunisés contre cette maladie. Cette étude a également permis de déterminer les caractéristiques diagnostiques (sensibilité et spécificité) de trois tests utilisés pour le diagnostic de l'anaplasmose : réaction en chaîne de la polymérase multiplex (mPCR), test immuno-enzymatique par inhibition compétitive (cELISA), et frottis sanguin (BS) en utilisant un modèle probabiliste et une approche bayésienne. Dans l'**étude** 5, une chromatographie liquide à haute performance (CLHP) a été utilisée pour déterminer la présence de résidus d'acaricides dans la viande, le foie, le lait, l'urine et les fèces de bovins. En outre, une évaluation des risques a été estimée sur base de la quantité d'ivermectine présente dans le lait, le foie et la viande, de la consommation de produits bovins dans les zones étudiées et des données sur l'apport alimentaire établies par l'Organisation des Nations unies pour l'alimentation et l'agriculture (FAO).

Résultats : L'**étude 1** a montré que *R. microplus* était la principale tique présente sur les bovins des zones étudiées. L'étude a également montré un niveau élevé d'infestation par les tiques à la fois à l'échelle de la ferme (41%) et au niveau de l'individu (animal) (38 %). Les **études 2** et **3** décrivent que, pour lutter contre les infestations de tiques, la principale méthode est la lutte chimique par pulvérisation à l'aide d'un pulvérisateur à dos. Les acaricides utilisés par pulvérisation (amides, organophosphates et alpha-cyperméthrine) ont souvent été administrés en surdosage en raison de leur prix abordable. En revanche, les acaricides à verser (*pour on*) étaient souvent administrés en doses insuffisantes en raison de leur coût élevé. Lorsque le traitement chimique échouait, certains agriculteurs avaient recours au surdosage, au mélange de différents acaricides et à d'autres pratiques risquées telles que le mélange de différents acaricides commerciaux ou l'application d'acaricides par écouvillonnage. Des méthodes alternatives telles que la gestion des pâturages, le retrait manuel des tiques, la lutte biologique ou les extraits de plantes se sont avérées utiles pour réduire les niveaux élevés d'infestation des animaux par les tiques et le niveau de résistance aux acaricides dans les exploitations. L'**étude 2** a révélé, chez *R. microplus*, des taux de résistance de 49 %, 37 % et 53 % à l'amitraz, l'ivermectine et l'alpha-cyperméthrine, respectivement. L'étude a également montré qu'à l'échelle de l'animal, l'âge (plus avancé), l'état corporel (maigreur) et la lactation étaient associés à de fortes infestations par les tiques, tandis que les bovins de type *Bos indicus* et leurs croisements voyaient réduite la probabilité de fortes infestations. A l'échelle de l'exploitation, l'élevage bovin en tant qu'activité principale, l'utilisation

d'enclos extérieurs, l'utilisation d'amitraz et le manque de technification de l'exploitation ont été associés à des infestations élevées par les tiques. Lorsque les connaissances et les perceptions des agriculteurs ont été prises en compte (**étude 3**), un niveau élevé d'infestation par les tiques était lié à l'utilisation d'acaricides organophosphorés, à des niveaux de connaissances moyens sur les tiques et les TBDs, à des pratiques de pâturage extensif, à la déclaration de cas de TBDs dans l'exploitation et à une application fréquente (toutes les 1 ou 2 semaines) ou irrégulière (toutes les 5 semaines ou plus) de traitements acaricides. La résistance aux acaricides était liée au fait de déléguer la préparation des traitements acaricides à des employés et de ne pas les superviser, ainsi qu'à l'application d'acaricides par écouvillonnage. Pour quantifier les pertes indirectes liées à la présence de tiques, l'**étude 2** a montré que le traitement acaricide variait en fonction du niveau de technification, de la taille du troupeau et des pratiques mises en œuvre dans les exploitations. Les exploitations technicisées dépensent moins pour les traitements acaricides (1,30 % du budget de production) que les exploitations semi-technicisées (3,43 %) et non technicisées (6,24 %). Les exploitations à fort taux d'infestation de tiques dépensent plus (4,28%) que les exploitations à faible taux d'infestation (2,74%). Enfin, l'**étude 4** a déterminé que la prévalence réelle de l'infection par *A. marginale* était estimée à 32 % et que 70 % des bovins possédaient des anticorps protecteurs contre *A. marginale*. La séroprévalence élevée et la rareté des foyers cliniques dans ces régions suggèrent que l'agent pathogène a atteint une stabilité endémique. L'**étude 5** a mis en évidence la présence de résidus d'ivermectine dans 68 % des échantillons de fèces et dans 3 % des échantillons de lait, d'urine et de foie. Aucun résidu n'était présent dans les échantillons de viande. Les résultats obtenus à partir de l'estimation de la dose journalière d'ivermectine montrent que la consommation d'ivermectine est faible et que le risque est jugé négligeable.

Conclusions : Dans un premier temps, une enquête a permis de recueillir des informations sur les pratiques d'élevage dans l'exploitation, la gestion économique et les mesures sanitaires et de lutte contre les tiques. Cela nous a permis d'identifier les pratiques communes (contrôle chimique : acaricides par pulvérisation) et peu communes (contrôle alternatif : plantes médicinales, champignons entomopathogènes, retrait manuel des tiques, etc.) employées par les agriculteurs pour contrôler les infestations à tiques et de déterminer les facteurs qui contribuent à la présence d'une infestation à tiques et d'une résistance aux acaricides. Les résultats de cette étude ont fourni des informations précieuses sur la situation du bétail tropical et peuvent servir de base à de futures recherches sur l'impact d'une mauvaise gestion des acaricides sur l'environnement. Nous avons largement partagé notre travail avec les institutions académiques et les agriculteurs équatoriens par le biais d'ateliers, de conférences et de la distribution de manuels (**Annexe 2**) sur les tiques et les maladies à tiques. En outre, cette recherche a donné lieu à la publication de trois articles, et nous en avons soumis un autre, qui contribuera à la recherche dans ce domaine.

SUMMARY

Background: The livestock sector is an important source of income for the development of the Ecuadorian economy. In the rugged land of Ecuador, livestock production is concentrated in three geographical areas. Andean highlands have a temperate climate and use an intensive model for milk production. The other two regions, i.e. the Coastal and Amazon regions, have a tropical environment and use an extensive and semi-extensive model for milk and meat production on dual-purpose farms (Sánchez *et al.*, 2019; Torres *et al.*, 2014). Around 75% of livestock herds are found in the tropical and subtropical areas (Pourrut *et al.*, 1983; Torres *et al.*, 2014). However, the introduction of short-cycle pastures and environmental conditions make cattle susceptible to tick infestations, mainly *Rhipicephalus microplus*. To combat tick infestations, farmers use acaricides, but the misuse and overuse of these chemicals has led to resistance, which worsens the problem (Betancur & Giraldo-Ríos, 2019). In the quest of Ecuador for a sustainable agricultural development, research, interdisciplinary collaboration, investment in research, and surveillance initiatives are essential to improving the management of ticks and tick-borne diseases (TBDs). This approach will protect farmers' livelihoods while promoting the interconnected health of humans, animals, and the environment within the One Health paradigm.

Methodology: This work was conducted as part of a project called "Socio-eco-epidemiology of ticks, tick-borne parasites, acaricide resistance and residual effects of acaricides in tropical Ecuadorian livestock: environmental, animal, and public health impacts" funded by the Academy of Research and Higher Education (ARES). This study consists of 4 parts, for which 6 field activities were carried out (**Appendix 1 and Table 1**). In field activity I, a cross-sectional questionnaire survey was conducted on 139 farms in two tropical and subtropical areas of Ecuador. In field activity II, the level of tick infestation was evaluated, blood samples were taken, and ticks were collected for resistance studies and morphological identification and to investigate the presence of tick-borne pathogens. In field activity III, agricultural warehouses were interviewed to determine the cost of agricultural inputs. As part of field activity IV, participatory meetings were conducted with farmers in the study areas. Field Activity V was carried out in local farms where milk, urine, and faeces samples were collected to measure ivermectin residues. In addition, meat and liver samples were collected from local slaughterhouses to determine ivermectin residues. Finally, in field activity VI, a cross-sectional survey was conducted to determine the amount of consumption of bovine products and by-products. With the information collected, five studies were conducted. **Study 1** identified the tick species present in the study area, determined the level of tick infestation, and identified risk factors associated with the level of tick infestation. **Study 2** reported the level of resistance to three acaricides (amitraz, alpha-cypermethrin, and ivermectin), the production typology of study farms was carried out, and the influence of acaricide treatment on the production costs of the dairy farm was studied. **Study 3** described the perceptions, knowledge, and common and uncommon tick control practices employed and related these variables to

the level of tick infestation and the presence of acaricide resistance. **Study 4** determined the prevalence of bovine anaplasmosis and the proportion of animals naturally immunized against this disease. This study also allowed us to determine the diagnostic characteristics (sensibility and specificity) of three tests used to diagnose anaplasmosis: multiplex polymerase chain reaction (mPCR), competitive inhibition enzyme-linked immunosorbent assay (cELISA), and blood smear (BS) by using a probabilistic model and a Bayesian approach. In **study 5,** a high-performance liquid chromatography (HPLC) was used to determine the presence of acaricide residues in bovine meat, liver, milk, urine, and faeces. In addition, the risk assessment was estimated on the basis of the amount of ivermectin present in milk, liver, and meat, the consumption of bovine products in the study areas, and the dietary intake data established by the Food and Agriculture Organization of the United Nations (FAO).

Results: Study 1 identified *R. microplus* as the main tick species present on cattle in the study areas. The study also showed a high level of tick infestation at both farm (41%) and individual (animal) (38%) levels. **Studies 2** and **3** described that, to combat tick infestations, the main control method was the spraying of chemicals (by using a backpack sprayer). The acaricides used in spraying (amides, organophosphates, and alpha-cypermethrin) were often overdosed due to their affordability. In contrast, pour-on acaricides were often underdosed due to their high cost. When the chemical treatment was failing, some farmers resorted to overdosing, mixing different acaricides, and applying other risky practices such as mixing different commercial acaricides or applying acaricides by swabbing. Alternative methods such as grazing management, manual tick removal, biological control, or herbal extracts were demonstrated to be helpful in reducing high tick infestation levels on animals and the level of acaricide resistance in farms. **Study 2** revealed in *R. microplus* resistance rates of 49%, 37%, and 53% to amitraz, ivermectin, and alpha-cypermethrin, respectively. The study also found that at the animal level, age (old), body condition (thin), and lactation were associated with high tick infestations, while *Bos indicus* cattle and their crosses reduced the likelihood of high tick infestations. At the farm level, cattle farming as the main activity, and the use of external paddocks, the use of amitraz, and the lack of farm technification were associated with high tick infestations. When knowledge and farmers' perceptions were considered (**Study 3**), a high level of tick infestation was related to the use of organophosphate acaricides, fair levels of knowledge about ticks and TBDs, extensive grazing practices, report of TBDs cases in the farm, and a frequent application (every 1 or 2 weeks) or an irregular application (every 5 or more weeks) of acaricide treatments. Acaricide resistance was related to delegating the preparation of acaricide treatments to employees and not supervising them, and by applying acaricides by swabbing. To quantify the indirect losses associated with the presence of ticks, **Study 2** found that the acaricide treatment varied according to the level of technification, herd size, and practices carried out in the farms. Technified farms had a lower expenditure on acaricide treatments (1.30% of production budget) compared to semi-technified (3.43%) and non-technified farms (6.24%). Highly infested farms spend more (4.28%) than farms with a reduced tick infestation rate (2.74%).

Lastly, **Study 4** determined that the actual prevalence of *A. marginale* infection was estimated at 32% and that 70% of cattle harboured protective antibodies against *A. marginale*. The high seroprevalence and infrequent clinical outbreaks in these areas suggest that the pathogen has reached an endemic stability. **Study 5** evidenced the presence of ivermectin residues in 68% of the faeces samples and 3% in milk, urine, and liver samples. No residues were found in meat samples. The results obtained from the estimated daily intake of ivermectin show that the consumption of ivermectin is low, and the risk is assessed as negligible.

Conclusions: Initially, our research carried out a survey to gather information about the livestock practices on the farm, economic management, and measures for sanitary and tick control. This helped us identifying the common (chemical control: acaricides by spraying) and uncommon practices (alternative control: medicinal plants, entomopathogenic fungi, manual removal of ticks, among others) employed by farmers to control tick infestations and determining the factors that contribute to the presence of tick infestation and acaricide resistance. The findings of this study have provided valuable insights into the situation of tropical livestock and can serve as a foundation for future research on the impact of mismanagement of acaricides on the environment. We have shared our work widely with academic institutions and farmers in Ecuador through workshops, conferences, and the distribution of manuals (**Appendix 2**) on ticks and TBDs. Additionally, this research has resulted in the publication of three articles, and we have one more submitted, which will contribute to research in this field.

RESUMEN

Antecedentes: El sector ganadero es una importante fuente de ingresos para el desarrollo de la economía ecuatoriana. En la accidentada orografía ecuatoriana, la producción ganadera se concentra en tres zonas geográficas. La Sierra con un clima templado y utilizando un modelo intensivo se centra en la producción lechera. Las otras dos regiones, Costa y Amazonía tienen un ambiente tropical y utilizan un modelo extensivo y semi-extensivo para la producción de leche y carne en granjas de doble proposito (Sánchez *et al.*, 2019; Torres *et al.*, 2014). Alrededor del 75% de los rebaños de ganado se encuentran en estas áreas tropicales y subtropicales (Pourrut *et al.*, 1983; Torres *et al.*, 2014). Sin embargo, la introduccion de pastos de ciclo corto y las condiciones ambientales hacen que el ganado sea susceptible a las infestaciones por garrapatas, principalmente *Rhipicephalus microplus*. Para combatir las infestaciones de garrapatas, los ganaderos utilizan acaricidas, pero el uso indebido y excesivo de estos productos químicos ha provocado resistencias, lo que agrava el problema (Betancur & Giraldo-Ríos, 2019). En la búsqueda del desarrollo agrícola sostenible de Ecuador, la investigación, la colaboración interdisciplinaria, la inversión en investigación y las iniciativas de vigilancia son esenciales para mejorar la gestión de las garrapatas y las enfermedades transmitidas por garrapatas (*tick-borne diseases* ou TBDs). Este enfoque protegerá los medios de vida de los agricultores al tiempo que promueve la salud interconectada de los seres humanos, los animales y el medio ambiente dentro del paradigma de Una Salud.

Metodología: Este estudio se realizó como parte de un proyecto denominado "Socio-eco-epidemiología de garrapatas, parásitos transmitidos por garrapatas, resistencia a acaricidas y efectos residuales de acaricidas en la ganadería tropical ecuatoriana: impactos ambientales, animales y de salud pública", fundado por la Academia de Investigación y Enseñanza Superior (ARES). Este estudio consta de 4 partes, para las cuales se realizaron 6 actividades de campo (**Anexo 1 y Tabla 1**). En la actividad de campo I, se realizó una encuesta transversal mediante un cuestionario en 139 explotaciones de dos zonas tropicales y subtropicales del Ecuador. En la actividad de campo II se evaluó el nivel de infestación por garrapatas, se tomaron muestras de sangre y se recogieron garrapatas para realizar estudios de resistencia e identificación morfológica y evaluar la presencia de patógenos transmitidos por garrapatas. En la actividad de campo III se entrevistaron almacenes agropecuarios, para determinar el costo de los insumos agropecuarios. Como parte de la actividad de campo IV, se realizaron reuniones participativas con los ganaderos de las zonas de estudio. La actividad de campo V se llevó a cabo en granjas locales donde se recogieron muestras de leche, orina y heces para determinar los residuos de ivermectina. Además, se recogieron muestras de carne y de hígado en mataderos locales para investigar la presencia de residuos de ivermectina. Por último, en la actividad de campo VI se realizó una encuesta transversal para determinar la cantidad de productos y subproductos bovinos consumidos. Con la información recopilada se realizaron cinco estudios. El **Estudio 1** identificó las especies de garrapatas

presentes en la zona de estudio, determinó el nivel de infestación por garrapatas e identificó los factores de riesgo asociados al nivel de infestación por garrapatas. El **Estudio 2** informó del nivel de resistencia a tres acaricidas (amitraz, alfa-cipermetrina e ivermectina), se realizó la tipología productiva de las explotaciones de estudio y se estudió la influencia del tratamiento acaricida en los costes de producción de la explotación lechera. El **Estudio 3** describió las percepciones, conocimientos y prácticas habituales y no habituales de control de garrapatas empleadas y relacionó estas variables con el nivel de infestación de garrapatas y la presencia de resistencia a acaricidas. El **Estudio 4** determinó la prevalencia de la anaplasmosis bovina y la proporción de animales inmunizados naturalmente contra esta enfermedad. Este estudio también permitió determinar las características diagnósticas (sensibilidad y especificidad) de tres pruebas utilizadas para diagnosticar la anaplasmosis: reacción en cadena de la polimerasa múltiple (mPCR), ensayo de inmunoadsorción ligado a enzima por inhibición competitiva (cELISA), y frotis sanguíneo (BS), utilizando un modelo probabilístico y un enfoque bayesiano. El **Estudio 5**, cromatografía líquida de alta resolución (HPLC), se utilizó para determinar la presencia de residuos de acaricidas en carne, hígado, leche, orina y heces de bovinos. Además, la evaluación del riesgo se estimó sobre la base de la cantidad de ivermectina presente en la leche, hígado y carne, el consumo de productos bovinos en las zonas de estudio y los datos de ingesta alimentaria establecidos por la Organización de las Naciones Unidas para la Agricultura y la Alimentación (FAO).

Resultados: El **Estudio 1** reveló que *R. microplus* era la principal especie de garrapata en el ganado bovino presente en las zonas estudiadas. El estudio también mostró un alto nivel de infestación por garrapatas tanto a nivel de granja (41%) como a nivel individual (animal) (38%). Los **Estudios 2 y 3** describieron que, para combatir las infestaciones de garrapatas, el principal método de control de garrapatas es el control químico mediante aspersión utilizando un pulverizador de mochila. Aquí, los acaricidas utilizados (amidas, organofosforados y alfa-cipermetrina) se administraban a menudo en sobredosis debido a su asequibilidad. Por el contrario, los acaricidas de pour-on se administraron a menudo en dosis subdosis debido a su elevado costo. Cuando fallaba el tratamiento químico, algunos ganaderos recurrían a la sobredosificación, a la mezcla de diferentes acaricidas y aplicaban otras prácticas arriesgadas, como la mezcla de diferentes acaricidas comerciales o la aplicación de acaricidas mediante frotado. Métodos alternativos como la manejo de potreros, la eliminación manual de las garrapatas, el control biológico o los extractos de hierbas demostraron ser útiles para reducir los altos niveles de infestación de garrapatas en los animales y el nivel de resistencia a los acaricidas en las explotaciones. El **Estudio 2** reveló tasas de resistencia del 49%, 37% y 53% en *R. microplus* al amitraz, la ivermectina y la alfa-cipermetrina, respectivamente. El estudio también descubrió que, a nivel animal, la edad (viejo), la condición corporal (delgado) y la lactancia se asociaban con infestaciones elevadas de garrapatas, mientras que el ganado *Bos indicus* y sus cruces reducían la probabilidad de infestaciones elevadas de garrapatas. A nivel de explotación, la ganadería como actividad principal, el uso de potreros externos, el uso de amitraz y la falta de tecnificación de las explotaciones se asociaron con un alto nivel

de infestación por garrapatas. Cuando se consideraron los conocimientos y las percepciones de los ganaderos (**Estudio 3**), un alto nivel de infestación de garrapatas se relacionó con el uso de acaricidas organofosforados, un nivel de conocimientos aceptable sobre las garrapatas y las TBDs, prácticas de pastoreo extensivo, notificación de casos de TBDs en la explotación, y una aplicación frecuente (cada 1 o 2 semanas) o irregular (cada 5 o más semanas) de tratamientos acaricidas. La resistencia a los acaricidas se relacionó con delegar la preparación de los tratamientos acaricidas en los empleados y no supervisarlos, y con la aplicación de acaricidas mediante frotado. Para cuantificar las pérdidas indirectas asociadas a la presencia de garrapatas, el **Estudio 2** halló que el tratamiento acaricida variaba según el nivel de tecnificación, el tamaño del rebaño y las prácticas llevadas a cabo en las granjas. Las granjas tecnificadas tuvieron un menor gasto en tratamientos acaricidas (1,30% del presupuesto de producción) en comparación con las granjas semitecnificadas (3,43%) y no tecnificadas (6,24%). Las explotaciones con una infestación elevada de garrapatas gastaron más (4,28%) que las explotaciones con una carga de infestación reducida (2,74%). Por último, el **Estudio 4** determinó que la prevalencia real de la infección por *A. marginale* se estimaba en un 32% y que el 70% de los bovinos albergaban anticuerpos protectores contra *A. marginale*. La elevada seroprevalencia y la escasa frecuencia de los brotes clínicos en estas zonas sugieren que el patógeno ha alcanzado la estabilidad endémica. El **Estudio 5** evidenció la presencia de residuos de ivermectina en el 68% de las muestras de heces y en el 3% de las muestras de leche, orina e hígado. No había residuos en las muestras de carne. Los resultados obtenidos de la ingesta diaria estimada de ivermectina muestran que el consumo de ivermectina es bajo, y el riesgo se evalúa como insignificante.

Conclusiones: Inicialmente, nuestra investigación realizó una encuesta para recabar información sobre las prácticas ganaderas en la explotación, la gestión económica y las medidas de control sanitario y de garrapatas. Esto nos ayudó a identificar las prácticas comunes (control químico: acaricidas por aspersión) y no comunes (control alternativo: plantas medicinales, hongos entomopatógenos, retiro manual de garrapatas entre otros) empleadas por los ganaderos para controlar las infestaciones por garrapatas y a determinar los factores que contribuyen a la presencia de infestación por garrapatas y a la resistencia a los acaricidas. Los resultados de este estudio han aportado valiosos conocimientos sobre la situación de la ganadería tropical y pueden servir de base para futuras investigaciones sobre el impacto de la mala gestión de los acaricidas en el medio ambiente. Hemos compartido ampliamente nuestro trabajo con instituciones académicas y ganaderos de Ecuador mediante talleres, conferencias y la distribución de manuales sobre garrapatas (**Anexo 2**) y TBDs. Además, esta investigación ha dado lugar a la publicación de tres artículos, y tenemos uno más sometido, que contribuirán a la investigación en este campo.

General preamble

With its equatorial position in the tropics, Ecuador is an ideal environment for the proliferation of tick populations, posing significant challenges to the livestock industry (Pourrut *et al.*, 1983; Sánchez *et al.*, 2019; Torres *et al.*, 2014). These small, blood-sucking mites are capable of spreading a wide variety of diseases to both animals and humans (Enríquez *et al.*, 2020; Guglielmone & Robbins, 2018).

According to the most recent Ecuadorian Agricultural Census conducted in 2000, agricultural activities use up to one-third of the national territory (Brassel & Hidalgo, 2007). About 75% of livestock herds are located in tropical and subtropical areas. Livestock farming in these areas is typically extensive and dual-purpose with low levels of technification (Bustillos & Rodríguez, 2014; Rodríguez-Hidalgo *et al.*, 2017; Torres, 2012). These extensive livestock systems increase the interaction between ticks and cattle, which not only exposes animals to the risk of tick-borne diseases (TBDs) but also results in economic losses due to reduced productivity and expenses related to tick and TBDs control (Betancur & Giraldo-Ríos, 2019; Minjauw & Mcleod, 2003). Despite various control measures to manage tick infestations, the livestock sector in Ecuador depends heavily on chemical acaricides for tick control. This dependence has led to the development of acaricide resistance in *R. microplus* ticks that parasitize cattle and has already been reported in several provinces of continental Ecuador (Maya-Delgado *et al.*, 2020; Rodríguez-Hidalgo *et al.*, 2017).

Despite their profound impact on animal health in Ecuador, ticks and TBDs often languish in the shadows of public health priorities, neglected by policy and funding initiatives. This negligence exacerbates problems in rural communities, where access to health services and resources can be limited and awareness of TBDs remains low.

Given the global challenge posed by ticks, academia and international agencies have united to tackle the issues of sustainable tick management on livestock and acaricide resistance. In this collaborative effort, the Food and Agriculture Organization of the United Nations (FAO) has taken a leading role by establishing a Community of Practice (CoP) on Acaricide Resistance Management of Livestock Ticks, which focuses on managing acaricide resistance in cattle ticks. In Ecuador, collaboration and support from financial institutions and international research organisations are vital in the comprehensive effort to study ticks. This partnership has given rise to the "Socio-eco-epidemiology of ticks, tick-borne parasites, acaricide resistance, and residual effects of acaricides in tropical Ecuadorian livestock: environmental, animal and public health impacts, Acronym: Ticks & TBDs" project. It is funded by the *L'Académie de Recherche et d'Enseignement supérieur* (ARES).

This study, part of the Ticks & TBDs Project, focuses on developing methods to understand the impact of ticks and tick-borne diseases in tropical Ecuador. We evaluate farmers' practices, the economic impact of ticks, the presence of TBDs, and the risks associated with consuming ivermectin-contaminated bovine products. Six field activities (**Table 1**) were conducted to meet the objectives of the four study parts

(**Table S1**). This work follows a One Health approach by identifying the effects of ticks and TBDs on cattle and their implications for farmers' economics and consumer health, particularly regarding ivermectin contamination due to improper acaricide use. This study provides an overview of the tick and TBDs situation in Ecuador, highlighting potential threats to animal and human health.

Table 1: Fieldwork activities used in this thesis

FIELDWORK ACTIVITIES	STUDY UNIT	SAMPLES	ANALYSIS	STUDY
I. Cross-sectional survey: Socio-eco epidemiology of ticks and TBDs				
A. Farm general information and herd management	Farm	139	Acaricide management	Study 1 & Study 3
B. Tick management	Farm	139		
C. Inputs, outputs, and labour force	Farm	105	Economic analysis	Study 2 & Study 3
D. Pharmacological inputs and farming practices	Farm	105		
II. Animal sampling				
Male and female tick samples	Farm	133	Morphological Identification	Study 1
Engorged female tick samples	Farm	126	Acaricide resistance	Study 1, Study 2 & Study 3
Individual health record	Animal	883	Level of tick infestation	
Blood samples	Animal	620	TBDs diagnosis	Study 4
Faeces samples	Animal*	40	Ivermectin residues	Study 5
Urine samples	Animal*	39		
III. Interview of agricultural warehouses				
Drug prices	Warehouses	16	Economic analysis	Study 2

Table 1. *Cont.*

FIELDWORK ACTIVITIES	STUDY UNIT	SAMPLES	ANALYSIS	STUDY
IV. Participatory Meeting				
Brainstorming	Farmer	40	Perceptions of ticks	Study 3
Proportional piling	Farmer	40		
V. Bovine products sampling				
Milk samples	Milk can*	70	Ivermectin residues	Study 5
Meat samples	Animal	46		
Liver samples	Animal	30		
VI. Cross-sectional survey				
Consumption of bovine products	Households	631	Consumption of bovine products	Study 5

Legend: *The samples of faeces and urine consisted of a pool of 6 cows from the farm; the milk samples consisted of a pool of 5-7 cows from the farm.

Chap. 1 - Introduction

1. Livestock context in tropical and subtropical areas of Ecuador

Livestock plays a crucial role in both the nutrition and the economy of Ecuador. In many areas, livestock provides essential protein sources through meat and dairy products, contributing significantly to local diets (Franco Crespo *et al.*, 2019). Additionally, livestock farming serves as a vital source of income for many Ecuadorian families, particularly to small-scale farmers. The sale of livestock and related products not only sustains livelihoods but also stimulates economic activity within communities, generating sources of employment (MAGAP, 2019). It is worth noting that the Ecuadorian agriculture and livestock production covers an impressive 63% of the national territory, showcasing its significant reach (Brassel & Hidalgo, 2007). In addition, the agricultural sector during 2021 contributed by 8.2% to the country's overall gross domestic product, emphasizing its crucial place in the nation's economic structure (INEC, 2023).

The ecological division of continental Ecuador into three macro-regions, i.e. Coast, Highlands, and Amazonia, as well as the consequent differences in climatic conditions (**Figure 1**), has caused a certain degree of specialization with regards to cattle farming.

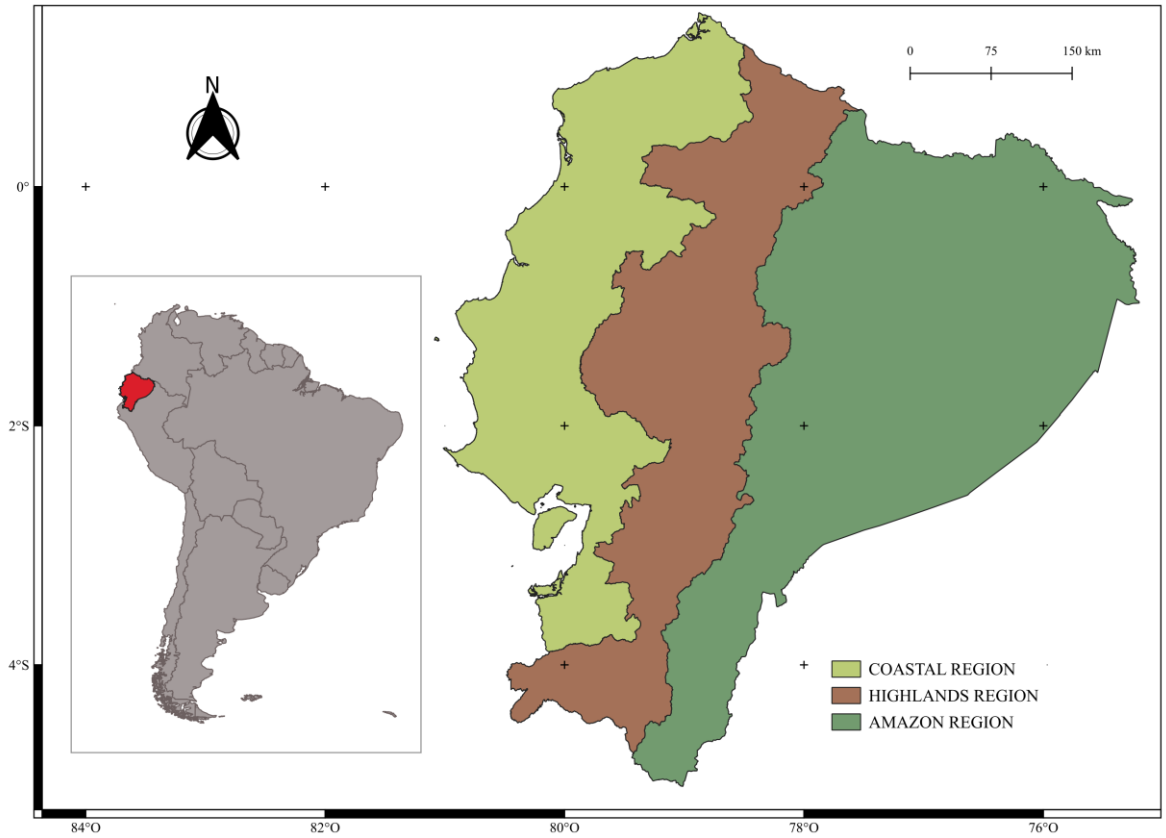

Figure 1. Geographic regions of continental Ecuador

Legend. Created by the author, 2024

In the country, dairy production is mainly located in the Andean highlands. The region has a temperate climate, and the temperature is linked to altitude; the average values vary between 8 and 20ºC. This region accounts for 52% of the cattle population and is dominated by crossbred cattle with Holstein or Brown Swiss crossed with local breeds (creole cattle). In contrast, the Coastal (39% of the cattle population) and Amazon regions (9% of the cattle population) consist mainly of beef and dual-purpose cattle. These regions predominantly feature crossbred cattle and hybrids between zebu (*Bos indicus*) and taurine (*Bos taurus*) breeds. The climate in these regions is humid and tropical, with temperatures around 25°C (INIAP, 1975; Roche *et al.*, 2022; Sánchez *et al.*, 2019; Torres *et al.*, 2014; Varela & Ron, 2018). The western and eastern flanks of the Andean Highlands, located in the foothills of the cordillera, are characterised by a very strong and irregular relief (**Figure 2**), as well as soils with low to medium fertility (Calvache, 2016). Agriculture (including sugar cane, banana, naranjilla, plantain, and other crops) and livestock farming converge in these regions (Vera, 2006). Depending on the location, dairy, dual-purpose, or beef cattle may predominate. Cattle are generally crossbred, involving combinations of zebu (Brahman) or dairy breeds (Holstein, Brown Swiss, Jersey, Normande, among others) depending on the purpose of the farm (Grijalva, Arevalo, & Wood, 2004; Meunier, 2007; Vargas *et al.*, 2011). These cattle graze year-round with little or no supplementation (Vera, 2006).

Figure 2. Tropical livestock in Ecuador

Legend. Photo taken by the author, 2021

In 2021, the cattle population in Ecuador was 4.01 million heads distributed among 280,000 cattle farms, two-thirds of which are smallholders, working on 20 hectares or less (Agrocalidad, 2018; Barahona & Beillard, 2015; Roche *et al.*, 2022). Due to Ecuador location on the equator, most of the

country, except for the high parts of the Highlands region, experiences a humid tropical climate (**Figure 3**), so about 75% of cattle herds are located in areas with environmental conditions conducive to the development of ectoparasites such as ticks (Pourrut *et al.*, 1983; Rodríguez-Hidalgo *et al.*, 2017; Torres *et al.*, 2014).

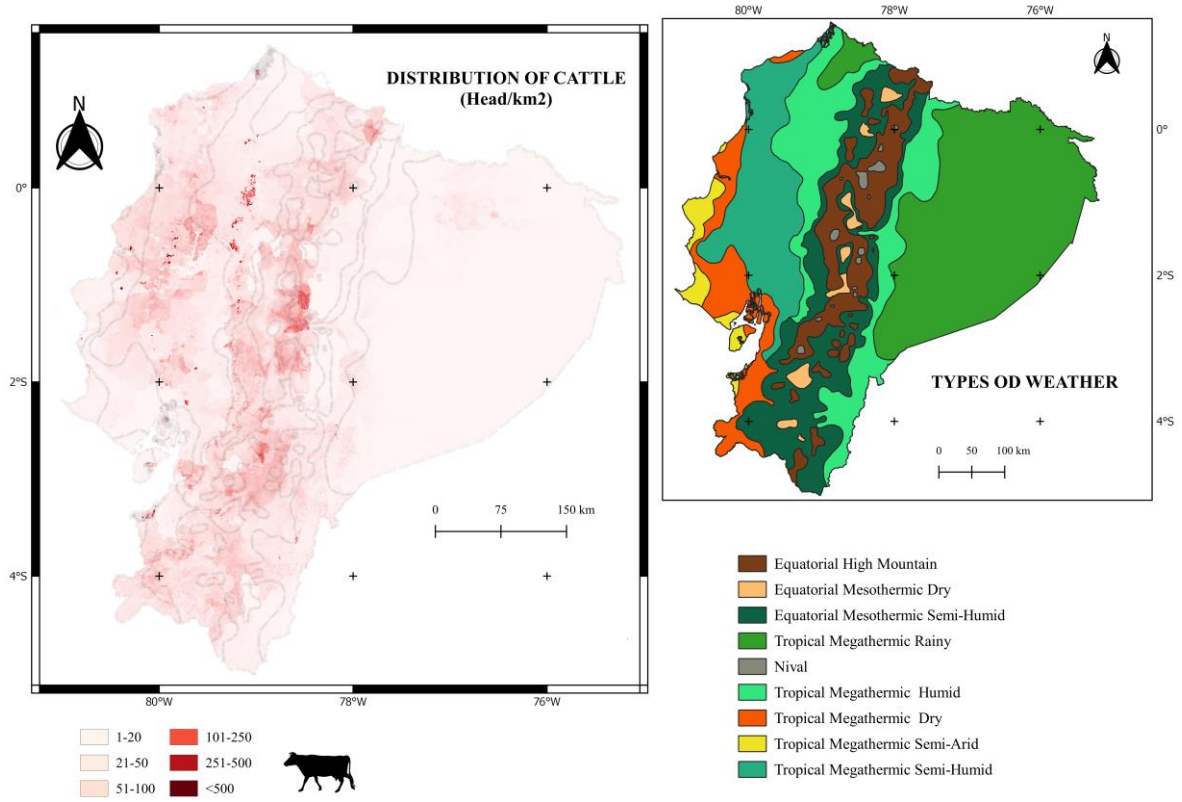

Figure 3. Distribution of the cattle population in continental Ecuador

Legend. Created by the author based on Gilbert *et al.*, 2018; INAMHI, 2017

Livestock farming in tropical regions of Latin America has historically suffered from low productivity and ecological vulnerability, with tropical herds being particularly inefficient (Guamán, 2010). In Ecuador, cattle production in tropical and subtropical zones began in the mid-twentieth century as part of the government's colonization and settlement policies, which aimed to incentivize migration to sparsely populated areas by offering land concessions and subsidies to colonists. The opening of roads for oil exploration also encouraged the growth of population centres that began to produce crops like naranjilla and sugar cane. However, these areas have nutrient-depleted soils and fragile ecosystems, which made them unsuitable for long-term crop production and led to their use by livestock producers (FAO, 2010; Grijalva, 2010; Grijalva, Arévalo, & Wood, 2004; Vizcarra, 2015).

Initially, tropical cattle farming focused on meat production. However, with the introduction of dairy breeds from the Highland Andean Region and the development of the dairy agroindustry, milk production surged (Vizcarra, 2015). Today, the extensive systems in Ecuadorian tropical and subtropical

zones rely on pasture as the primary food source for their animals (**Figure 4**). Despite low levels of technification and infrastructure, these cattle ranches produce 25% and 75% of the nation's milk and meat, respectively (Bustillos & Rodríguez, 2014; Gordillo & Gordillo, 2013; Rodríguez-Hidalgo *et al.*, 2017; Torres, 2012).

Figure 4. Breeding systems in tropical livestock farming

Legend. With the courtesy of Jacome, 2021

Unfortunately, high temperatures and humidity, along with the use of susceptible breeds (*Bos primigenious taurus*) and foreign short-cycle pastures (Bolaños, 2016; Ferrari, 2002; Torres *et al.*, 2023), have created an ideal environment for tick development in tropical livestock systems. This has led to a rapid increase in tick populations, infestations of animals, and the spread of TBDs among cattle (Acebo, 2016; Hüe & Fontfreyde, 2019; Kerario *et al.*, 2018).

2. Ticks

Ticks are a group of arthropods that have been around for millions of years and have changed very little over time. They are known to transmit a wide variety of infectious agents to humans and animals. These blood-sucking ectoparasites feed on a wide range of hosts, including mammals, birds, reptiles, and sometimes even humans (Betancur & Giraldo-Ríos, 2019; De La Fuente, Kocan, & Contreras, 2015). Ticks transmit a wider range of pathogenic microorganisms, including protozoa, rickettsiae, spirochaetes, and viruses, than any other group of arthropod vectors. They are among the

most important vectors of diseases that affect humans, livestock, and companion animals (Jongejan & Uilenberg, 2004).

Almost 981 species of ticks have been described, and they are divided into three families based on their physical characteristics. The first family is Ixodidae, which are hard-bodied ticks and consist of 762 species (Guglielmone, Nava, & Robbins, 2023). The second family is Argasidae, which are soft-bodied ticks and counts 218 species (Dantas-Torres *et al.*, 2019). The third family is Nuttalliellidae, in which only one species is reported; it has intermediate characteristics of the other two families.

The Ixodidae constitutes a diverse family within the order Ixodida, encompassing 78% of the world tick fauna. This family comprises 762 species gathered in 17 genera, with different ecological preferences and host ranges (Guglielmone *et al.*, 2010, 2023). Of these, the genera with veterinary and medical importance are *Amblyomma* spp., *Dermacentor* spp., *Haemaphysalis* spp., *Hyalomma* spp., *Ixodes* spp., and *Rhipicephalus* spp. (Barker & Murrell, 2004; Kassiri & Nasirian, 2021; Lotfi & Karima, 2021).

Ticks of the family Ixodidae have a one-, two- or three hosts-life cycle.

- *One-host ticks:* The larva, nymph, and adult feed on a single host. The engorged females drop off the host to oviposit on the ground (Basu & Charles, 2017; Nejash, 2016). *Rhiphicephalus microplus* is an example of this group.
- *Two-host ticks:* The larva moults into a nymph, and both stages feed on the same host. The nymph falls to the ground, where it moults from nymph to adult, and the adults feed on another host (Basu & Charles, 2017; Nejash, 2016). *Rhiphicephalus eversi* is an example of this group.
- *Three-host ticks:* The ticks require three animals to complete their life cycle. For each stage, the larva, the nymph, and the adult drop off the host after feeding to moult or oviposit on the ground (Basu & Charles, 2017; Nejash, 2016). *Amblyoma mixtum* is an example of this group.

In Ecuador, 33 species of the Ixodidae family have been identified, of which nine species correspond to the genus *Ixodes* spp., 20 species to the genus *Amblyomma* spp., two species to the genus *Rhipicephalus* spp., and one species to the genera *Dermacentor* spp. and *Haemaphysalis* spp. (Guglielmone, Nava, & Robbins, 2021; Guglielmone *et al.*, 2023).

2.1. Species of ticks on cattle in Ecuador

In Ecuador, ten species of ticks have been documented in cattle (**Table 2**), with *Rhipicephalus microplus* being the most important (Rodríguez-Hidalgo *et al.*, 2017).

Table 2. Species of ticks reported in cattle in Ecuador

Specie	Genus	Cycle life	Principal Host
Ixodes boliviensis	*Ixodes*	3 hosts	Domestic dog
Ixodes montoyanus	*Ixodes*	3 hosts	Deer
Amblyomma maculatum	*Amblyomma*	3 hosts	Domestic dog
Amblyomma mixtum	*Amblyomma*	3 hosts	Domestic and wild mammals
*Amblyomma ovale**	*Amblyomma*	3 hosts	Domestic/wild canines and felines
*Amblyomma triste**	*Amblyomma*	3 hosts	Rodents
Haemaphysalis juxtakochi	*Haemaphysalis*	3 hosts	Domestic and wild mammals
*Dermacentor nitens**	*Dermacentor*	1 host	Horses
Rhipicephalus microplus	*Rhipicephalus*	1 host	Cattle

Legend: Adapted from Defense Pest Management Information Analysis Center (U.S.). Forest Glen Section 1998; Guglielmone et al. 2021; Lapo 2019. *Unpublished data, Instituto Internacional de Zoonosis

2.1.1 Rhipicephalus microplus

The cattle tick, *Rhipicephalus microplus* (**Figure 5**), is a major problem for cattle breeding in tropical and subtropical regions worldwide. Due to its ability to adapt and reproduce quickly, it has spread to several geographical areas (Betancur & Giraldo-Ríos, 2019; Rodriguez-Vivas, Jonsson, & Bhushan, 2018). In Ecuador, *R. microplus* ticks have been found at altitudes ranging from sea level to 2,469 meters (Chávez Larrea *et al.*, 2021).

Figure 5. *Rhipicephalus microplus*

Legend: Female on the left side and male on the right side. With the courtesy of Enríquez, 2024

Life Cycle

Rhipicephalus microplus ticks are known as one-host ticks, and their life cycle is divided into both parasitic and non-parasitic phases. The duration of the parasitic phase is relatively constant, with a mode of approximately 23 days (Canevari *et al*., 2017). It begins when the larva attaches to the host and completes in three stages: larvae, nymphs, and adults (**Figure 6**).

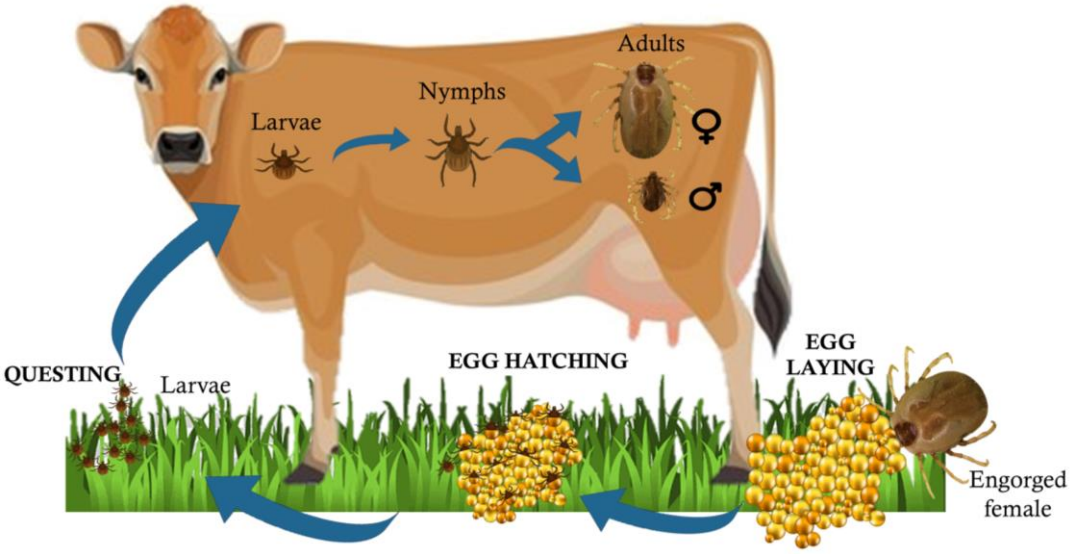

Figure 6. The life cycle of cattle tick *Rhipicephalus microplus*

Legend. Created by the author based on Alonso & Fernández, 2021

The larvae usually feed on the host for about 7-9 days before moulting into a nymph. The nymphs then feed and change into the juvenile adult stage in approximately 9 to 13 days. During this stage, male and female ticks become sexually dimorphic. The male is very mobile and walks around the host looking for females to mate. It is common to find male ticks below semi-engorged females. On the other hand, the female tick is not as mobile as the male and remains attached to the host throughout her life cycle (Cruz *et al.*, 2021; Senbill *et al.*, 2018). After mating, the females suck blood rapidly and swell enormously; the engorged females drop off the host to oviposit in the environment, beginning the non-parasitic phase (Sonenshine, Lane, & Nicholson, 2002).

The non-parasitic phase of ticks involves pre-ovipositional development and oviposition of engorged females, as well as the incubation of eggs and the search for a host by larvae. Female ticks can lay around 2,000-3,000 eggs in approximately two weeks before dying. These eggs typically hatch in about 43 days as larvae, which then climb the grass in search of grass tips while waiting for a new host (questing) to start the cycle again. The non-parasitic phase is not constant and is strongly influenced by environmental factors such as climate and vegetation. The larvae can survive for as long as 3 to 4 months without feeding in summer and up to 6 months in cooler temperatures (Mastropaolo *et al.*, 2017; Sonenshine *et al.*, 2002).

The life cycle of *Rhipicephalus microplus* is completed in a few weeks in tropical climates with frequent rainfall. It can require up to several months in regions with alternating dry and rainy seasons (O'Hara 2008). In tropical areas, the average life cycle is 70.20 days per generation, resulting in 5.19 tick generations per year (Cruz *et al.*, 2020; O'Hara, 2008).

2.2. Control and prevention of ticks

Tick control strategies aim to reduce infestations and to mitigate the risks posed to animals due to their role in the transmission of numerous diseases. Effective control measures include the right combination of strategies targeting the parasitic and free-living phases of the life cycle (FAO, 2004a). The most common tick control methods used are briefly described as follows:

2.2.1. Chemical tick control

Chemical control methods have the function of breaking the tick life cycles. Acaricides can act systemically, after absorption of the compound into the host tissues, or by direct contact with the target parasites after external application. With the exception of growth-regulating acaricides, most ectoparasiticides are neurotoxins that target the nervous system of the ectoparasites (Rodríguez-Vivas *et al.*, 2014; Taylor, 2001). Various types of acaricides are available for veterinary use, each with different mechanisms of action and application forms. The acaricides come in the form of wettable powders, emulsifiable concentrates, or flowable products. They can be applied to livestock through methods such as hand sprayers, spray races, immersion of animals in a dipping bat, or injection.

Additionally, more recent delivery systems include pour-on formulations, acaricide-impregnated ear-tags, intraruminal boluses, and pheromone/acaricide-impregnated tail-tags (George, Pound, & Davey, 2021; Rajput *et al.*, 2006). Commonly used acaricides such as organochlorines, organophosphates, amidines, synthetic pyrethroids, macrocyclic lactones, and fipronil work by affecting the nervous system of ticks. In contrast, fluazuron inhibits tick growth by interfering with chitin formation (Obaid *et al.*, 2022).

2.2.2. Biological methods of tick control

Biological control includes the introduction of natural predators, parasites, and bacterial and fungal pathogens. Ants (*Solenopsis germinata*, *S. saevissima*, and *Ectatomma cuadridens*), beetles, spiders, and many bird species (chickens, oxpeckers, and cattle egrets) are predators that affect tick population size in nature (Samish, Ginsberg, & Glazer, 2004; Samish & Rehacek, 1999). Fungi of the genera *Beauveria* and *Metarhizium* and nematodes of the genera *Heterorhabditis* spp. and *Steinernema* spp. kill ticks by penetrating through the integument. Other methods include releasing male ticks

sterilized by irradiation or crosses by mating between *R. annulatus* × *R. microplus* (Basu & Charles, 2017; Rodriguez-Vivas *et al.*, 2018; Samish *et al.*, 2004).

2.2.3. Herbal acaricides

Herbal acaricides have been shown to be a viable alternative to chemical acaricides for controlling ticks. However, their use is hindered by the lack of evaluation of their quality, efficacy, and safety, which could pose a risk to consumers. There are several herbs and medicinal plants that have acaricidal activities, including *Ocimum gratissimum* (clove basil), *Cinnamomum verum* (true cinnamon), *Curcuma longa* (turmeric), *Nicotiana tabacum, Chrysanthemum cinerariaefolium, Azadirachta indica (*neem tree*),* and *Allium sativum (*garlic), among others (Jain, Satapathy, & Pandey, 2020; Khare *et al.*, 2019). These plant extracts are typically applied through spraying and are available in powder form or diluted with a solvent (Habeeb, 2010; Klafke *et al.*, 2021).

2.2.4. Ecological tick control

Ecological tick control is used for habitat and host-linked treatment. Control of ticks in habitat and vegetation requires modification of vegetation cover by removing vegetation that provides refuge for ticks in order to interrupt the tick life cycle. Pasture rotation, use of grasses as repellents (*Melinis minutiflora*, species of *Stylosanthes* and *Cassia absus*), and pasture burning are also used as tick control strategies (Kasaija *et al.*, 2021; Pegram *et al.*, 1993; Rodriguez-Vivas *et al.*, 2018).

2.2.5. Intensive cattle systems (zero-grazing)

Intensively confining cattle provides a non-chemical method for reducing tick infestation. In these systems, the lack of pasture cover and high cattle density is enough to ensure that engorged females are squashed after dropping from the host. The feedlot environment, being too dry and lacking pasture, results in low survival rates for tick eggs and larvae. However, while costs associated with chemical treatments decrease or may disappear, there is an increase in feed and infrastructure costs. Additionally, there may be a higher incidence of pathologies associated with confinement systems, such as mastitis or foot problems (Jonsson & Piper, 2007; ParaBoss, 2022). It is also uncertain whether this system is completely effective, as in areas where animals were confined but fed forage cut from communal grazing areas, this could serve as an important reservoir for both ticks and tick-borne diseases (Jonsson & Piper, 2007; Ogden *et al.*, 2005).

2.2.6. Genetic tick control

Some cattle breeds exhibit natural resistance or tolerance to ticks, meaning they are less susceptible to infestations and to the diseases ticks may transmit. *Bos indicus* breeds or their crossbreeds show the greatest resistance to ticks than *B. taurus* animals (Bock et al. 1997). Resistance to ticks is

manifested by skin thickness, coat type, coat colour, hair density, and skin secretions (Rodriguez-Vivas *et al.*, 2018; Shyma, Gupta, & Singh, 2015). Animal breeding programs have been implemented in several countries to find genetic resistance against cattle ticks. This possibility seems feasible given a relative good heritability of this trait helping to reduce tick loads (Shyma *et al.*, 2015).

2.2.7. Vaccination

Vaccination has the potential to be a sustainable and eco-friendly approach to controlling ticks and TBDs. TickGARD and Gavac vaccines are two commercially available vaccines that are commonly used in various countries to manage R. *microplus* infestations. These vaccines contain a mid-gut membrane-bound recombinant protein BM86 of *R. microplus*. When cattle are vaccinated with BM86, it reduces the number, weight, and fecundity of engorged female ticks. The use of these vaccines can reduce the tick population by up to 74%, and their overall efficacy ranges from 51% to 91%. The vaccine effectiveness varies depending on the tick population, the cattle nutritional status and the compliance with the vaccine protocol (Abbas *et al.*, 2023). In Ecuador, the commercial vaccine Gavac® (Heber Biotec S.A., CIGB, Camagüey, Cuba) entered the market at the end of the year 2021, with a retail price of 3.92 per dose; however, effectiveness studies carried out in the country have not been conclusive about the vaccine's efficacy (Tinoco, 2022).

Products available in the Ecuadorian market for the control of ticks (**Table 3**):

Table 3. Tick control products in Ecuador

PRODUCT	COMPOSITION	APPLICATION		
		Spraying	Injection	Pour-on
CHEMICAL ACARICIDES				
Amides	Amitraz*	x		
Benzoylphenyl ureas	Fluazuron			x
Macrocyclic Lactones	Eprinomectin			x
	Doramectin		x	
	Ivermectin		x	x
	Moxidectin		x	
Organophosphates	Chlorpyrifos	x		
	Dichlorvos	x		
	Ethion	x		
	Trichlorfon	x		

Table 3. *Cont.*

PRODUCT	COMPOSITION	APPLICATION		
		Spraying	Injection	Pour-on
Phenylpyrazoles	Fipronil			x
Pyrethroids	Alpha-cypermethrin	x		
	Cypermethrin*	x		x
	Cypermethrin, Alpha-cypermethrin	x		
	Deltamethrin	x		
	Flumethrin			x
Amides + Phenylpyrazoles	Amitraz, Fipronil	x		
Benzoylphenyl ureas + Macrocyclic Lactones	Fluazuron, Abamectin**			x
Benzoylphenyl ureas + Phenylpyrazoles	Fluazuron, Fipronil			x
Benzoylphenyl ureas + Pyrethroids	Fluazuron, Flumethrin			x
Organophosphates + Pyrethroids	Dichlorvos, Cypermethrin	x		
	Chlorpyrifos, Cypermethrin*	x		x
	Fenitrothion, Cypermethrin			x
	Trichlorfon, Cypermethrin			x
	Ethion, Cypermethrin	x		x
Organophosphates + Phenylpyrazoles + Pyrethroids	Dichlorvos, Fipronil, Cypermethrin	x		
HERBAL ACARICIDE	Neem oil	x		
VACCINE	Bm86 antigen		x	

Legend: Some commercial presentations with * citronella oil and; **neem oil. Source: AGROCALIDAD (App Agromovil), 2024.

2.3. Acaricide resistance

The most common method used to control ticks in cattle is the application of chemical acaricides. However, the improper use of these chemicals, such as incorrect dilution, inappropriate application, persistent use, and overdosing, has led to the development of resistance to various pesticides in ticks (Castro-Janer *et al.*, 2010). Acaricide resistance results from the exposure of tick populations to acaricides, their survival, and the reproduction of less susceptible ticks, which usually carry resistance genes (FAO, 2004a).

A variety of bioassay methods have been developed to evaluate tick susceptibility to acaricides, but the most commonly used are the larval package test (LPT), the larval dip test (LIT), and the Drummond test (DT). The Food and Agriculture Organization of the United Nations (FAO)

recommended the LPT for use as a standard tick bioassay method (FAO, 2004a; Rodriguez-Vivas *et al.*, 2018). The larval packet test is considered to be the most repeatable, although it is limited by the length of time that it takes (until 6 weeks). In this test, 14 to 21-day-old tick larvae are exposed to chemically-impregnated filter papers, and their subsequent mortality is quantified after 24 hours (FAO, 2004a).

The lapse of time between identifying a resistance problem and receiving bioassay results, along with the challenge of obtaining an adequate amount of sample for a reliable analysis, is a significant issue with most current bioassays. As an alternative to bioassay methods, molecular methods offer the possibility of working with small DNA quantities and obtaining results within just one or two days, as opposed to the weeks required by conventional methods. However, these methods necessitate extensive knowledge of resistance mechanisms at the molecular level, and molecular tests may not be appropriate for all resistance mechanisms. Additionally, they are more costly and require qualified personnel (George *et al.*, 2021).

In Ecuador, resistance to 3 commonly used acaricides has been studied in 9 of the country's 24 provinces (Paucar-Quishpe *et al.*, 2023; Pérez-Otáñez *et al.*, 2024; Rodríguez-Hidalgo *et al.*, 2017). Furthermore, Maya-Delgado *et al.*, (2020) studied resistance to amitraz, reporting resistance to this acaricide in 8 additional provinces (**Table 4 and Figure 7**).

Table 4. Acaricide resistance studied in Ecuador

Province	Acaricides Studied	Acaricide Resistance reported						
		AM	CY	IV	OP	FP	FL	Total
Azuay	0	-	-	-	-	-	-	NA
Bolivar	0	-	-	-	-	-	-	NA
Cañar	0	-	-	-	-	-	-	NA
Carchi	3	YES	YES	YES	-	-	-	3
Chimborazo	1	YES	-	-	-	-	-	1
Cotopaxi	1	YES	-	-	-	-	-	1
El Oro	1	YES	-	-	-	-	-	1
Esmeraldas	3	YES	YES	YES	-	-	-	3
Galapagos	0	-	-	-	-	-	-	NA
Guayas	1	YES	-	-	-	-	-	1
Imbabura	3	YES	YES	YES	-	-	-	3
Loja	1	YES	-	-	-	-	-	1
Los Rios	1	YES	-	-	-	-	-	1
Manabi	3	YES	YES	YES	-	-	-	3

Table 4. *Cont.*

Province	Acaricides Studied	Acaricide Resistance reported						
		AM	CY	IV	OP	FP	FL	Total
Morona Santiago	0	-	-	-	-	-	-	NA
Napo	3	YES	YES	YES	-	-	-	3
Orellana	3	YES	YES	YES	-	-	-	3
Pastaza	0	-	-	-	-	-	-	NA
Pichincha	3	YES	YES	YES	-	-	-	3
Santa Elena	1	YES	-	-	-	-	-	1
Santo Domingo de los Tsachilas	3	YES	YES	YES	-	-	-	3
Sucumbios	3	YES	YES	YES	-	-	-	3
Tungurahua	0	-	-	-	-	-	-	NA
Zamora Chinchipe	1	YES	-	-	-	-	-	1

Legend: Amitraz (AM); alphacypermethrin (CY); ivermectin (IV); fipronil (FP); fluazurón (FL); organophosphates (OP); No information (-)**.** Source**:** Maya-Delgado *et al.*, 2020; Paucar-Quishpe *et al.*, 2023; Pérez-Otáñez *et al.*, 2024; Rodríguez-Hidalgo *et al.*, 2017.

2.4. Impact of livestock

Approximately 80% of the world's cattle population is at risk of tick infestations and TBDs, leading to various economic losses in the livestock industry (FAO, 2004a). The effects of tick infestation on livestock will depend on factors such as tick species and local climatic conditions, as well as the susceptibility of the regional livestock. The global costs of tick and TBDs control have been estimated at US$ 7 billion. However, this amount is tentative and may vary depending on the region, fluctuating exchange rates, inflation, and variables that were taken into account for its calculation (Jongejan & Uilenberg, 2004). Ticks are responsible for both direct and indirect economic losses in the livestock industry. Direct losses stem from the damage caused by ticks feeding on blood. In contrast, indirect losses result from the transmission of infectious agents during feeding, as well as the expenses incurred in treatment and acaricide control (Betancur & Giraldo-Ríos, 2019).

Tick bites on cattle can result in skin irritation and wounds, which can decrease the value of hides and skins used for leather manufacturing by 20-30% in the market. Ticks with long and massive hypostomes, such as *Amblyomma*, can cause abscesses due to secondary bacterial infections, which can even lead to teat loss or lameness (Jongejan & Uilenberg, 2004; Minjauw & Mcleod, 2003; Nath *et al.*, 2018). When ticks feed on cattle, an adult female tick can cause blood loss of 0.5-2 ml, which can result in anaemia and significant milk and weight losses in heavy infestations (Basu & Charles, 2017). For

instance, cows infested with an average of 105 ticks have a 23% reduction in milk production per day, and 40 ticks per animal per day can cause a loss of 20 kg of weight per year (Frisch, O'Neill, & Kelly, 2000; Manjunathachar *et al.*, 2014). Annual losses due to the cattle tick *Rhipicephalus microplus*, related to milk and beef production in Mexico and Brazil, were estimated at US$573.61 million and US$3.24 billion per year, respectively (Grisi *et al.*, 2014; Rodríguez-Vivas *et al.*, 2017).

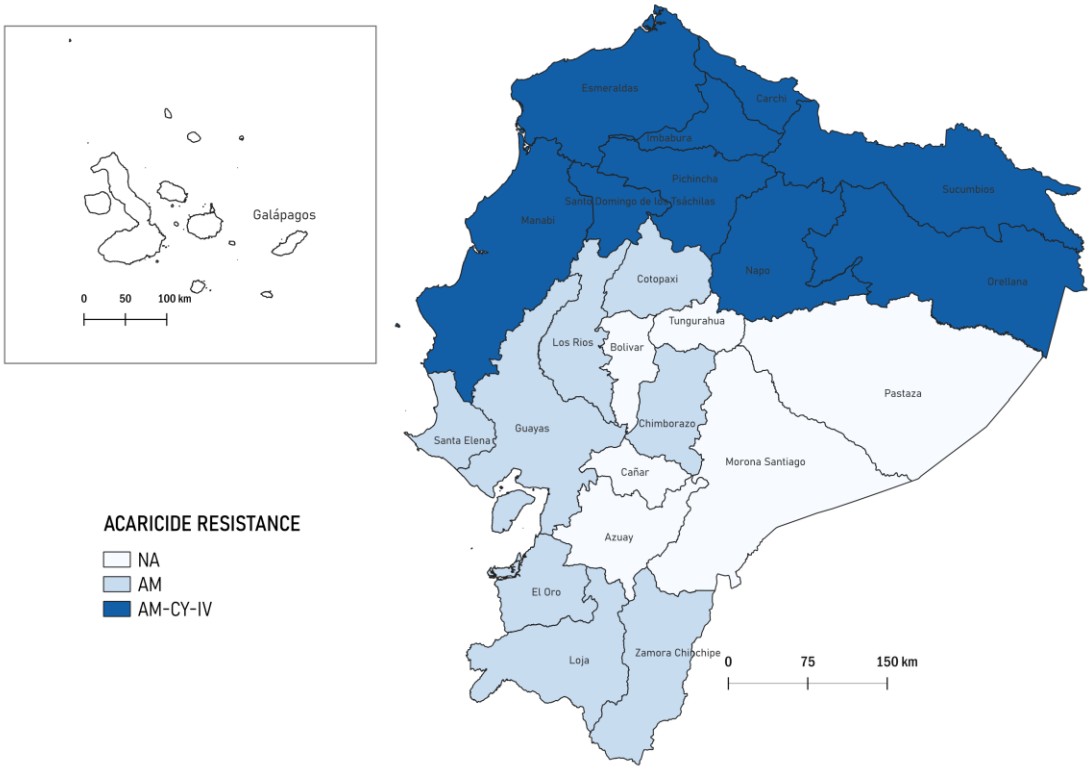

Figure 7. Acaricide resistance studied in Ecuador

Legend. Amitraz (AM); alphacypermethrin (CY); ivermectin (IV); no information (NA). Created by the author, 2024

Expenses associated with treating TBDs include chemotherapy and the cost associated with mortality or prolonged recovery leading to loss of body weight, loss of milk, and immunization costs (Jongejan & Uilenberg, 2004; Ocaido, Muwazi, & Opuda, 2009). On the other hand, controlling tick infestation involves the use of acaricides, labour, and infrastructure. The cost of using acaricides to control ticks is around $2.9 per treatment, considering an average tick burden of 40 ticks per day (Frisch *et al.*, 2000; O'Hara, 2008). Controlling ticks in livestock with chemicals is crucial, but their misuse can be dangerous to both the environment and consumers if recommended withdrawal time for animal foods are not adhered to (Kivaria, 2006).

3. Tick-borne diseases (TBDs) in cattle

Tick-borne diseases and pathogens transmitted by ticks are a global phenomenon, particularly prevalent in tropical and subtropical regions, affecting, among others, livestock, humans, and companion

animals (Regitano & Prayaga, 2010). Specifically, TBDs impact substantially 80% of the world cattle population, resulting in significant economic losses. The associated expenses include parasite control, declines in fertility, body weight, and milk production (Minjauw & Mcleod, 2003; Ogata *et al.*, 2021). Four groups of TBDs significantly impact livestock production: theileriosis, babesiosis, anaplasmosis, and cowdriosis (**Table 5**) (Minjauw & Mcleod, 2003; Regitano & Prayaga, 2010).

Table 5. Main tick-borne diseases that affect cattle around the world

Disease	Agent	Type of pathogen	Principal vectors*	Main distribution
Babesiosis	*Babesia bovis*	Parasite	*R. microplus, R. annulatus* and *R. geigyi*	Most tropical and subtropical regions
	Babesia bigemina	Parasite	*R. microplus, R. decoloratus, R. annulatus, R. geigyi* and *R. evertsi evertsi*	Most tropical and subtropical regions
	Babesia divergens	Parasite	*Ixodes spp.*	Europe
Anaplasmosis	*Anaplasma marginale*	Bacteria	Most tick species and mechanical transmission by biting flies	Tropical and subtropical regions
	Anaplasma centrale	Bacteria	*R. simus*	Most tropical and subtropical regions
Tropical theileriosis, Mediterranean Coast fever	*Theileria annulata*	Parasite	*H. detritum, H. anatolicum* and *H. dromedarii*	Northern Africa, Sudan, Southern Europe, the Middle East, the Indian subcontinent, and part of China.
Cowdriosis, Heartwater	*Ehrlichia ruminantium*	Bacteria	*Amblyomma* spp.	Sub-saharan Africa, Caribbean islands, Madagascar, Comoros
East Cost Fever	*Theileria parva*	Parasite	*R. appendiculatus*	Eastern, central and southern Africa.

Legend: Adapted from Madder & Horak, 2001; Minjauw & Mcleod, 2003; Qiu *et al*., 2021; Regitano & Prayaga, 2010. * Tick genera: A = *Amblyomma,* H = *Hyalomma,* O = *Ornithodorus*, R = *Rhipicephalus.*

In Ecuador, anaplasmosis and babesiosis are TBDs affecting cattle (Guglielmone, 1995). Despite reports of Q fever (*Coxiella burnetii*) (Echeverría *et al.*, 2019) and trypanosomiasis

(*Trypanosoma vivax* and *Trypanosoma theileri*) (Chávez-Larrea *et al.*, 2021, 2023; De la Cadena *et al.*, 2023) in cattle, the involvement of ticks in their transmission remains uncertain (Koual *et al.*, 2023; Krige *et al.*, 2021; Latif *et al.*, 2004; Martins, Leite, & Doyle, 2008; Varela-Castro *et al.*, 2018).

3.1. Anaplasmosis

Anaplasmosis, commonly known as yellow fever, is a TBDs caused by various species of *Anaplasma* spp. The term "Anaplasma" is derived from the Greek words "*an*" and "*plasma*," meaning "without" and "molded," respectively. Organisms within the genus *Anaplasma* spp. are gram-negative bacteria functioning as obligate intracellular parasites of eukaryotic cells (Abdisa, 2019; Battilani *et al.*, 2017; Zobba *et al.*, 2014).

Belonging to the family *Anaplasmataceae* in the order Rickettsiales, the genus *Anaplasma* has encompassed six species since 2001: *Anaplasma marginale*, *A. centrale*, *A. ovis*, *A. phagocytophilum* (previously known as *Ehrlichia phagocytophila*, *Ehrlichia equi*), *A. bovis* (previously known as *Ehrlichia bovis*), and *A. platys* (previously known as *Ehrlichia platys*) (Dumler *et al.*, 2001; Koual *et al.*, 2023; Woldehiwet, 2010). In addition to these species, two new species tentatively named *A. capra and A. odocoilei*, and many new unclassified candidate species and genovariants have been identified (Rar, Tkachev, & Tikunova, 2021). Of the seven species, *A. marginale*, *A. bovis*, *A. centrale*, and *A. phagocytophilum* are known to cause bovine anaplasmosis, being *A. marginale* the most important pathogen in cattle (Makgabo *et al.*, 2023). In Ecuador, several studies have reported the presence of *A. maginale* in cattle, in addition to reports of *A. ovis*, *A. phagocytophilum*, and *Anaplasma platys* in *R. microplus* ticks collected from cattle (**Table 6**).

3.1.1 Clinical signs

Clinical manifestations increase as a function of host factors: low immune status, *Bos taurus* breeds, and host age (adult animals), generally causing high mortality in adult animals and mild disease in calves (Parodi *et al.*, 2021; Sisson *et al.*, 2023). Mild, acute, peracute, and chronic forms of anaplasmosis used to occur. The disease is generally **mild** in calves up to 1 year old and usually without clinical signs; acute and occasionally fatal in bovines up to 2 years old, presenting moderately severe signs; acute and frequently fatal in bovines over three years of age (Ristic, 2012; Tabor, 2022; Young, 2020). The main signs of **acute** anaplasmosis are fever, depression, weakness, icterus, and anaemia. In addition, rapid loss of body condition, infertility, and severe decrease in milk production may be present. Abortions in pregnant cattle and temporary infertility in males may occur (Bock, DeVos, & Molloy, 2006; Rodning & Navarre, 2008). Animals with the **peracute** form die within a few hours of the onset of clinical manifestations. This is most common in purebred, older cattle, or in high-producing milk cows. **Chronic** forms occur in affected animals that survive the disease; these animals develop a

lifelong carrier state and are free of clinical anaplasmosis. However, they may relapse when immunosuppressed (Rodning & Navarre, 2008; Tabor, 2022).

Table 6. Species of *Anaplasma* reported in cattle in Ecuador

Agent	Vectors [a]	Reference
Anaplasma ovis	*Rhipicephalus microplus**	Guglielmone *et al.*, 2023; Matysiak *et al.*, 2016; Maya-Delgado *et al.*, 2020
Anaplasma phagocytophilum	*Rhipicephalus microplus**	Guglielmone *et al.*, 2023; Matysiak *et al.*, 2016; Pesquera *et al.*, 2015
Anaplasma platys	*Rhipicephalus microplus**	Gioia *et al.*, 2018; Guglielmone *et al.*, 2023; Lu *et al.*, 2022
Anaplasma marginale	*Rhipicephalus microplus** *Rhipicephalus sanguineus* *Amblyomma cajennense* s.l* *Amblyomma maculatum* *Dermacentor nitens* *Tabanus* spp. *Psorophora* spp. *Stomoxis calcitrans* *Haematobia irritans*	Buestan, Navarrete, & Mejia, 2007; Carballo, Colombo, & Heinzen, 1992; Cotes-Perdomo, Oviedo, & Castro, 2020; Escobar *et al.*, 2015; García-Ruilova *et al.*, 2020; Gioia *et al.*, 2018; Guglielmone *et al.*, 2023; Kemp, 1994; Kocan *et al.*, 2003; Maya-Delgado *et al.*, 2020; Moreira, 2018; Rey-Valeirón *et al.*, 2018; Rezende Araújo *et al.*, 2021; Soto, 2010; Tana-Hernández *et al.*, 2017

Legend: [a] Vectors of *Anaplasma* spp. present in Ecuador. * Reported in Ecuador as a vector of *Anaplasma* spp.

3.1.2 Transmission

Bovine anaplasmosis caused by *Anaplasma marginale* can be transmitted in three routes: biological, mechanical, and transplacental. Ticks are responsible for **biological** transmission; around 20 species of ticks are known vectors of this disease. However, *Rhipicephalus* spp. are clearly important vectors of anaplasmosis in countries such as Australia and countries in Africa and Latin America (Kocan *et al.*, 2003; OIE, 2024; Rar *et al.*, 2021). **Mechanical** transmission of *A. marginale* can be realised by biting flies or blood-contaminated fomites (surgical instruments or needles) during routine management practices such as vaccination, castration, ear tagging, and dehorning (Aubry & Geale, 2011; Orenge, 2021). Hematophagous diptera of the genera *Tabanus, Haematobia*, *Stomoxys* and mosquitoes of the genera *Psorophora* have also been reported to transmit the pathogen but less efficiently than ticks (Kemp, 1994; Kocan *et al.*, 2003, 2010; OIE, 2024). In addition, *A. marginale* can be transmitted from the mother to the calf during gestation via the **transplacental** route. This transmission may play a role

in maintaining the disease within herds. Calves infected *in utero* rarely show clinical signs but become persistent carriers of the infection (Kocan *et al.*, 2003, 2010).

Although several studies have determined the presence of *A. marginale* in several tick species present in Ecuador (**Table 5**), its presence has only been reported in *R. microplus* and *Amblyomma cajennense* s.l. ticks (Escobar *et al.*, 2015; Gioia *et al.*, 2018; Guglielmone *et al.*, 2023; Maya-Delgado *et al.*, 2020; Moreira, 2018; Soto, 2010; Tana-Hernández *et al.*, 2017). In addition, the literature refers as vectors of *Anaplasma* spp. to several species of hematophagous diptera such as *Tabanus* (horseflies), *Haematobia irritans* (horn fly), *Stomoxis calcitrans* (stable fly), and mosquitoes of the *Psorophora* genus that are present in Ecuador (Buestan *et al.*, 2007; Carballo *et al.*, 1992; García-Ruilova *et al.*, 2020; Kocan *et al.*, 2003).

3.1.3 Life cycle

Erythrocytes or red blood cells (RBCs) infected with *A. marginale* are ingested by ticks. Once in the tick gut cells, the initial bodies are released and infect the cells of the intestinal epithelium and other tick tissues, including the salivary glands, from where the pathogen is transmitted to vertebrates during feeding. The reticulate (vegetative) form divides by binary fission, forming large colonies, and subsequently condenses into dense forms, which is the infective form and can survive outside of cells (**Figure 8**). The dense form is transmitted during tick feeding through the salivary glands (Kocan *et al.*, 2003).

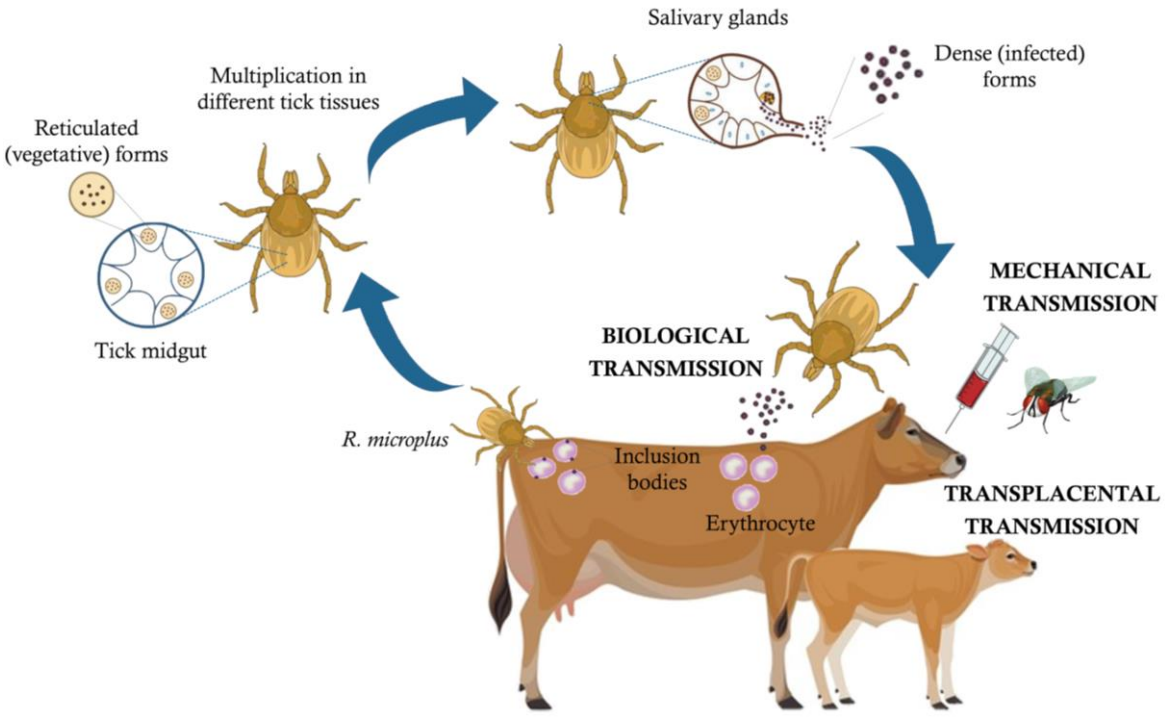

Figure 8. *Anaplasma marginale* life cycle

Legend. Created by the author based on Kocan *et al.*, 2003; Marcelino *et al.*, 2012; Salinas *et al.*, 2023.

Ticks at all stages of life - larvae, nymphs, and adults - can transmit the disease. When *A. marginale* enters bovine erythrocytes, it replicates by forming a type of morula called an inclusion body, which contains 4 to 8 initial bodies. These initial bodies then spread to other red blood cells, leading to high infection rates where 70% or more of the erythrocytes can become infected during acute infections. Infected erythrocytes are phagocytized by bovine reticuloendothelial cells, resulting in anaemia and jaundice without haemoglobinemia or haemoglobinuria. The length of the incubation period can vary based on the number of organisms in the infective dose, ranging from 7 to 60 days with an average of 28 days. Once the acute phase has passed, cattle that recover can become carriers and transmit the infection to other animals (Kocan *et al.*, 2003, 2004).

3.2. Babesiosis

Babesiosis, commonly known as tick fever, cattle fever, red water disease, or piroplasmosis, is a tickborne disease caused by intraerythrocytic protozoan parasites of the genus *Babesia;* they are the second most common blood-borne parasites of mammals after trypanosomes (Jacob *et al.*, 2020; Telford *et al.*, 1993). The name of the disease and the parasite "Babesia" derives from the name Victor Babes, who discovered the organisms in the late 19th century in the red blood cells of cattle (Hunfeld, Hildebrandt, & Gray, 2008)

The *Babesia* genus, which is part of the *Babesiidae* family and the *Piroplasmida* order, comprises over 100 species. Six species of parasites cause bovine babesiosis worldwide: *Babesia bigemina*, *Babesia bovis*, *Babesia divergens*, *Babesia major*, *Babesia occultans*, and *Babesia Argentina* (Jacob *et al.*, 2020; Levine, 1971; Schnittger *et al.*, 2012). Among these species, *B. bigemina* is the most widely prevalent, while *B. bovis* is the most pathogenic (Ibrahim *et al.*, 2013; Jacob *et al.*, 2020). In Ecuador, several studies have reported the presence of *B. bovis* and *B. bigemina* in cattle (**Table 7**).

Table 7. Species of *Babesia* reported in cattle in Ecuador

Agent	Vectors	Reference
Babesia bovis	*Rhipicephalus microplus*	Bock *et al.*, 2004; Chávez Larrea *et al.*, 2021; Guglielmone *et al.*, 2023; Hernández, 2012
Babesia bigemina	*Rhipicephalus microplus*	Bock *et al.*, 2004; Chávez Larrea *et al.*, 2021; Guglielmone *et al.*, 2023; Vasco, 2014

3.2.1 Clinical signs

Babesia infections increase in severity with host age (adult animals), low immune status, genetic factors (*Bos taurus* breeds), and the presence of coinfections (Schnittger *et al.*, 2012; Stuhr & Wood,

2007). Acute, subacute, and chronic forms of babesiosis occur. **Acute** babesia infections can lead to various symptoms such as fever, malaise, lethargy, prostration, anaemia, jaundice, and even death. In some countries, the disease is referred to as "red water" due to haemoglobinuria that frequently occurs. The presence of fever can also cause abortions and temporary infertility in males. Cerebral babesiosis can cause damage to the capillary endothelium of the brain, resulting in aggressiveness and incoordination (Bock *et al.*, 2004; Everitt *et al.*, 1986; Ozubek *et al.*, 2020; Schnittger *et al.*, 2012).

In **subacute** infections, clinical signs may be less pronounced and more difficult to detect. Subclinical infection is common in calves infected before nine months of age (Bock *et al.*, 2004). Bovines that survive acute infections can develop **chronic** babesiosis, leading to lifelong protective immune responses (Suarez *et al.*, 2019). With *Bos taurus* cattle, tick-transmitted *B. bovis* infection lasts for at least four years but with *B. bigemina* it is usually less than 6 months (Bock *et al.*, 2004; Goff *et al.*, 2008).

3.2.2 Transmission

The major vectors for *B. bigemina* and *B. bovis* are *R. microplus* and, in some areas, *R. annulatus*. *Babesia* can be transmitted transovarially. Transmission of *B. bovis* and *B. bigemina* occurs when adult female ticks become infected and transmit to their progeny through their eggs. In the case of *B. bovis*, the disease is transmitted by the larvae of the next generation, while in the case of *B. bigemina*, it is transmitted by the nymphal and adult stages of the next generation (Friedhoff, 2017; Pal & Chakravarty, 2020). Transplacental transmission of *B. bovis* and *B. bigemina* has been reported. However, these are rare cases and have mostly been isolated cases (Costa *et al.*, 2016). Calves develop innate immunity to babesiosis between 3 and 9 months of age, following the passive resistance gained from colostrum that lasts for approximately two months. Calves exposed during this period rarely exhibit clinical symptoms and develop a long-lasting immunity (Bock *et al.*, 2004).

3.2.3 Life cycle

During the tick feeding, *Babesia* sporozoites (Sz) are inoculated into the host and invade erythrocytes, where they transform into trophozoites (T). These grow and divide by binary fission into two merozoites (M), which in turn are able to infect new erythrocytes and restart the replicative cycle. A few merozoites stop division and transform into gametocytes (G). Ticks ingest erythrocytes infected with gametocytes. Gametogony and sporogony occur in the tick gut. Gametocytes develop into "Strahlenkörper" (Sk), which fuse to form a zygote (Z), developing into a kinete (K). The kinete, which accesses the haemolymph, replicates and invades various organs of the tick, including the ovaries, where transovarial transmission occurs, producing eggs with infected embryos. Kinetes enter the salivary glands and are transformed into sporozoites. Infected larvae (*B. bovis*) hatch and attach to a bovine, and

during feeding, the sporozoites are liberated with saliva into the animal's circulatory system (Hunfeld, Hildebrandt, & Gray, 2008; Schnittger et al., 2012) (**Figure 9**).

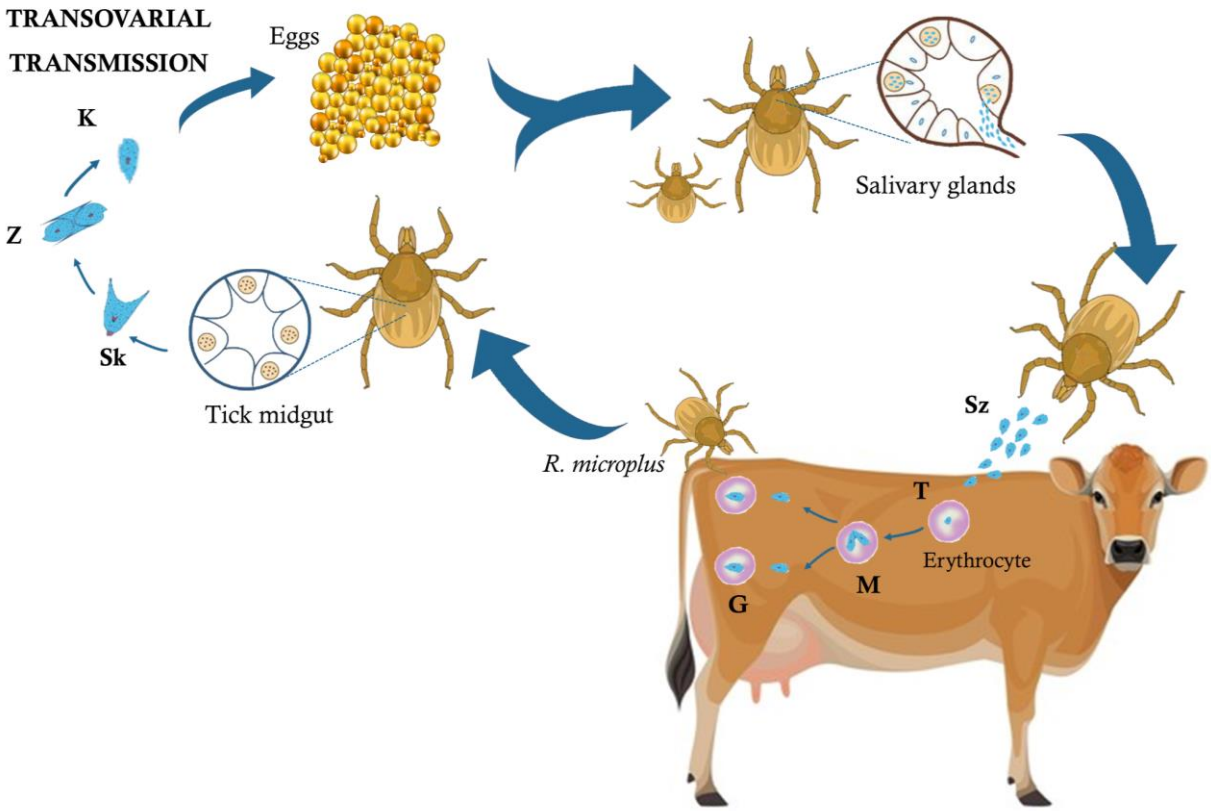

Figure 9. *Babesia bovis* and *Babesia bigemina* life cycle

Legend: Sporozoites (Sz); trophozoites (T); merozoites (M); gametocytes (G); Strahlenkörper (Sk); zygote (Z); kinete (K). Created by the author based on Hunfeld et al. 2008; Schnittger et al., 2012

In *B. bigemina*, infective sporozoites take about 9 days to appear, so only the nymphal and adult stages of the tick are potentially infective (Hodgson, 1992; Hunfeld et al. 2008; Mosqueda *et al.*, 2012; Schnittger et al., 2012). The spleen, with its function as a lympho-reticular filter, eliminates infected erythrocytes from the circulation. This immune-mediated destruction, together with the lysis of red blood cells due to the proliferation of parasites, causes hemolytic anaemia, which in turn can cause jaundice and dark urine. The incubation period can be as short as 4-5 days for *B. bigemina* and 10-12 days for *B. bovis* (CFSPH, 2012; Hunfeld et al. 2008; Rasoulzadeh *et al.*, 2021).

3.3. Diagnosis of anaplasmosis and babesiosis

Clinical diagnosis can be made tentatively based on geographic location, season, presence of vectors, anamnesis, clinical signs, and necropsy findings observed in infected animals. When a presumptive diagnosis is made, then it is necessary to confirm it with laboratory tests. Infections can be detected directly by PCR or blood smears or indirectly by serology (OIE, 2021, 2024).

3.3.1 Direct

The first option is the **microscopic observation** of the pathogen in blood smears stained with Giemsa (**Figure 10**). *A. marginale* and *A. centrale* appear as spherical, dark blue or purple intraerythrocytic bodies, 0.3 to 1.0 µm in diameter. The inclusion bodies of *A. marginale* are located near the periphery of the erythrocytes, and those of *A. centrale* are more centrally located in the erythrocyte (OIE, 2024; Richey, 1991). The single forms of *B. bigemina* are irregular and large, measuring about 2-3 µm in diameter. The paired form of *B. bigemina*, usually pear-shaped, is often found in pairs forming an acute angle to each other, measuring approximately 3- 3.5 µm long and 1-1.5 µm wide (Bock *et al.*, 2006). The single form of *B. bovis* is round, usually located in the centre of the erythrocyte, with a diameter of 1 to 1.5 µm. The paired form is usually pear-shaped, separated at obtuse angles, with a rounded distal end, and measures approximately 1.5 to 2.5 µm (Laha, Das, & Sen, 2015; OIE, 2024).

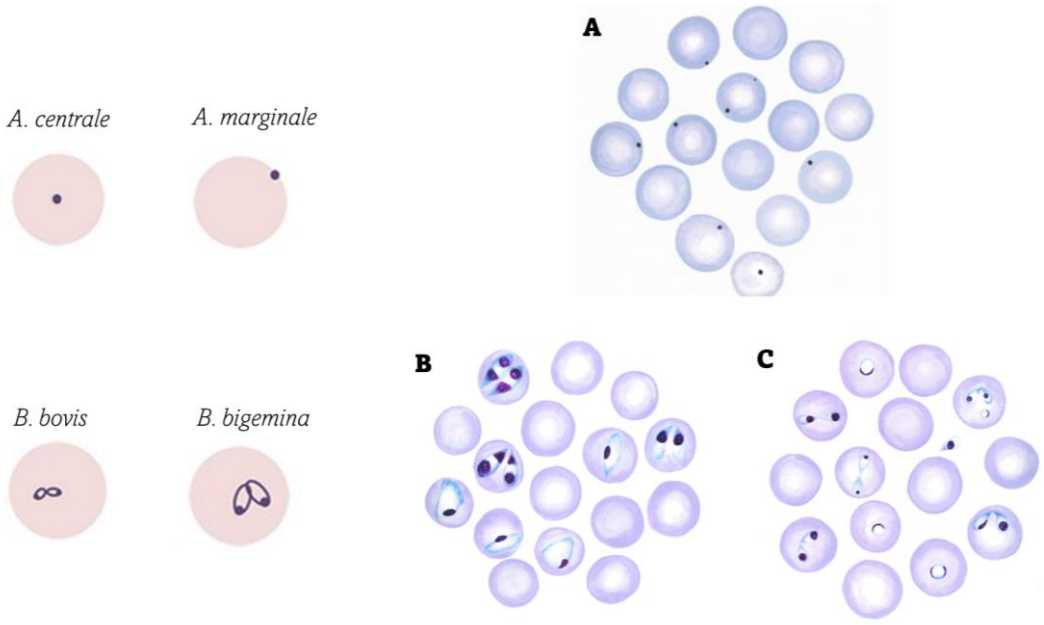

Figure 10. Schematic of a blood smear from a cow infected

Legend. A. *Anaplasma marginale*, **B.** *Babesia bigemina*, **C.** *Babesia bovis*. Adapted by Tick Fever Centre, 2022

The blood smear allows the diagnosis of anaplasmosis in clinically infected animals during the acute phase of the disease. Disadvantages of this technique include: 1) it requires experienced personnel, 2) is not reliable for detecting infection in pre-symptomatic or carrier animals, and 3) difficult in species differentiation (Aubry & Geale, 2011; OIE, 2021, 2024; Richey, 1991).

The **polymerase chain reaction** (PCR) can be used to diagnose clinical cases and distinguish pathogen species (Eshetu, 2015). Although nucleic acid-based tests have been developed to detect *Anaplasma* spp. infection in carrier cattle, they are not yet fully validated. In the diagnosis of anaplasma,

the analytical sensitivity of PCR has been estimated at 0.0001% of infected erythrocytes (1 infected RBCs in 10^6 RBCs), which would allow detection of only a proportion of carrier cattle (OIE, 2024). More sensitive nested PCR (nPCR) was capable of identifying a 0.000001% (1 infected RBCs in 10^8 RBCs) (Torioni de Echaide *et al.*, 1998). In the case of babesiosis, PCR-based techniques can detect parasitaemia levels ranging from 0.001% to 0.0000001% (1 parasite in 10^5 to10^9 RBCs, respectively) (OIE, 2021). The disadvantages of PCR are: 1) it cannot be performed at a large scale, generally used as confirmatory tests, and 2) it requires expensive equipment and preventive maintenance (OIE, 2021, 2024).

3.3.2 Indirect

Serological tests are recommended in three different situations: (i) to monitor the development of antibodies in cases of infection or immunization, (ii) for epidemiological purposes such as monitoring in an enzootic region or during an outbreak, and (iii) for commercial exchanges or movements of cattle, especially when they have no contact with other animals (Salinas-Estrella *et al.*, 2023).

Several serological tests have been developed for the detection of *Anaplasma* spp. antibodies in infected cattle. The card agglutination test (CAT), the indirect fluorescent antibody test (IFAT), the complement fixation test (CFT), and an enzyme-linked immunosorbent assay (ELISA) tests are routinely used in many laboratories. The two serological tests currently preferred to identify infected animals are competitive ELISA (cELISA) and CAT (Aubry & Geale, 2011; OIE, 2024; De Waal, 2000). The **cELISA** technique uses a recombinant antigen called rMSP5 and a monoclonal antibody (MAb) specific for MSP5. This technique is currently available in kit form and has proven to be very sensitive and specific for detecting *Anaplasma*-infected animals in non-endemic areas (Knowles *et al.*, 1996; Kocan *et al.*, 2012; OIE, 2024; Torioni de Echaide *et al.*, 2005). However, there are three main limitations to the cELISA: 1) low sensitivity for the detection of early infections, 3) insufficient specificity for identifying true negative cattle, and 3) cross-reactivity with other *Anaplasma* species (Aubry & Geale, 2011). The **card agglutination test** is the next most commonly used test. This test is sensitive and rapid and can be performed in the laboratory or the field. However, there is variability in test interpretation, the presence of nonspecific reactions, and the CAT antigen can be difficult to prepare and may vary from lot to lot and from laboratory to laboratory (Aubry & Geale, 2011; OIE, 2008).

Antibodies to *Babesia* are generally detected by indirect immunofluorescence assay, immunochromatographic test (ICT), and ELISA. ELISA has now replaced the IFAT because of its low specificity for the detection of *B. bigemina* and the presence of cross-reactions between *B. bigemina* and *B. bovis* in areas where the two parasites coexist (OIE, 2021). On the other hand, **ELISA** offers greater sensitivity, objectivity, and rapid adaptability to analyse samples on a large scale, making it widely used as a routine test. Indirect and competitive ELISAs have been developed using recombinant merozoite

surface and rhoptry-associated antigens of *B. bovis* and *B. bigemina* (OIE, 2021; Terkawi *et al.*, 2011), which have been validated in different laboratories, and antibodies from other parts of the world have recognized the antigen. Although there are promising results for its international application, the accuracy and reliability of ELISA in enzootic regions still need to be tested (Goff *et al.*, 2006, 2008). **Immunochromatographic tests** have also been developed with recombinant *B. bovis* and *B. bigemina* merozoite proteins. These are rapid, easy-to-use, inexpensive, and applicable diagnostic methods in the field or in developing countries where equipment and electricity are limited. However, further optimization is recommended to improve the specificity of ICT (Guswanto *et al.*, 2017; Lira-Amaya *et al.*, 2021; OIE, 2021; Tayebwa *et al.*, 2020).

3.4. Treatment of anaplasmosis and babesiosis

Successful treatment of TBDs depends on early diagnosis and the prompt administration of effective drugs (Suarez *et al.*, 2019; Vial & Gorenflot, 2006). Treatment of anaplasmosis can be done with tetracycline antibiotics (tetracycline, chlortetracycline, oxytetracycline) and imidocarb dipropionate. Imidocarb dipropionate and diminazene acetate are the usual treatments of choice for babesiosis (Mosqueda *et al.*, 2012; Suarez *et al.*, 2019; Vial & Gorenflot, 2006). In severe cases of babesiosis and anaplasmosis, supportive therapy such as blood transfusions, anti-inflammatory drugs, iron preparations, dextrose, vitamins of the B complex, and fluid replacements may be necessary (Mosqueda *et al.*, 2012).

Blood transfusions are recommended for cattle with acute anaemia. However, the effectiveness of transfusion therapy can be compromised if the procedure is delayed, insufficient blood is administered, the transfusion rate is excessively high, or if pyrogenic bacteria are inadvertently introduced during the process. The risk of an incompatibility reaction during the first transfusion is typically low in cattle. However, if a second transfusion is required, it is crucial to use the same donor to minimize the risk of adverse reactions (Divers, 2005; Zintl *et al.*, 2003).

The treatment does not eliminate persistent *A. marginale* infections (Aubry & Geale, 2011; Kocan *et al.*, 2010; Tabor, 2022), but *Babesia* parasites can be cleared from carrier animals (Carter Phillip & Rolls Peter, 2022; WOHA, 2021).

3.5. Zoonotic potential

Babesia bigemina, *B. bovis*, and *Anaplasma marginale* are widely distributed among cattle in multiple tropical and subtropical areas of Ecuador (CFSPH, 2012; OIE, 2024). Although cases of human infection by these pathogens are generally rare, Calvopiña et al. in 2023 identified a human case of *B. bigemina* in the Ecuadorian Amazon region.

4. Methodologies and statistics used in the thesis

4.1. Regression Analysis

Regression analysis is a statistical method for estimating how a dependent variable (*y*) changes for an independent variable (*x*) (Merrill & Timmreck, 2006).

4.1.1.Multiple Logistic Regression Analysis

Multiple logistic regression (MLR) is a statistical technique that extends logistic regression by including two or more independent variables in the model (Merrill & Timmreck, 2006). This method allows researchers to examine the simultaneous effects of multiple independent variables (*covariates*) on a dependent variable. The predictor variables can be of any data level, whether categorical, ordinal, or continuous. Multiple logistic regression is a valuable tool that can help identifying potential confounders. The objective of MLR is to develop the best-fitting, most efficient, and biologically reasonable model to describe the association between an outcome and a set of predictors (Beukelman & Brunner, 2016).

4.2. Multiple correspondence analysis

Multiple Correspondence Analysis (MCA) is a technique used to examine multivariate categorical data. It is mainly used to identify important relationships between categories of variables in a graphical format. MCA analyses reduce large sets of variables into smaller sets of components that summarize the information contained in the data (Mori, Kuroda, & Makino, 2016). In this method, the categories are represented visually, and their association is assessed based on their graphical proximity. The closer the categories are, the more likely they are to be associated with each other. MCA is particularly useful when the amount of data to be analysed is large, and a simple statistical analysis would not be sufficient to reveal the underlying data structure. However, as an exploratory methodology, the definition of what characterizes "closeness" is subjective and scale dependent. This sometimes makes data interpretation difficult or questionable (Almeida, Infantosi, & Costa, 2009).

4.3. Hierarchical Clustering Analysis (HCA)

Hierarchical cluster analysis (HCA) is a popular method for cluster analysis in big data research and data mining, aiming to establish clusters. It aims to group subjects with similar features into clusters. There are two types of strategies used in HCA: the agglomerative and the divisive strategy. In the agglomerative approach, each sample is initially considered a cluster, and subsequently, pairs of clusters are merged. On the other hand, the divisive clustering approach starts with one cluster, including all samples, and recursively splits it into smaller clusters (Granato *et al.*, 2018; Zhang *et al.*, 2017).

4.4. Classification and regression tree analysis

Classification and regression trees (CART) are defined as a graphical method of expressing, in chronological order, the relationship between a sequence of decisions, events outcomes, or resource costs, and utility (Chambers, O'Sullivan, & Gates, 2020; Kurhade & Wankhade, 2016; Rahman *et al.*, 2023). CART can be used to analyse either categorical (classification trees) or continuous (regression trees) response variables (Speybroeck, 2012). CART consists of four main components: the root node, decision node, branch node, and terminal (leaf) node. The decision tree begins with a root node (entire population/sample), once the first split is chosen, each of the two subsets (branch/Sub Tree) is split again using the same approach, and the process continues iteratively. A decision node is a subset of the set of variables, and it can be a terminal or non-terminal node. A non-terminal node (parent) is a node that splits into two left and right child nodes (**Figure 11**) (Dheri *et al.*, 2004; Krzywinski & Altman, 2017; Rahman *et al.*, 2023).

The goal of CART is to progressively create smaller and more homogeneous groups from the initial dataset by minimizing the variation in the response variable within each subset. Groups are created by identifying specific values of the predictor variables that separate an existing group into two other groups. Thus, each branching point on the tree is dichotomous, and the splitting criterion is always a single value of one of the predictor variables. The two groups created from the splitting usually do not have equal size (number of observations). Moreover, a predictor variable can be used at more than one splitting point, and not all predictor variables in the set might be used if they are not useful for creating homogeneous groups (Lawrence & Wright, 2001; Veech, 2021).

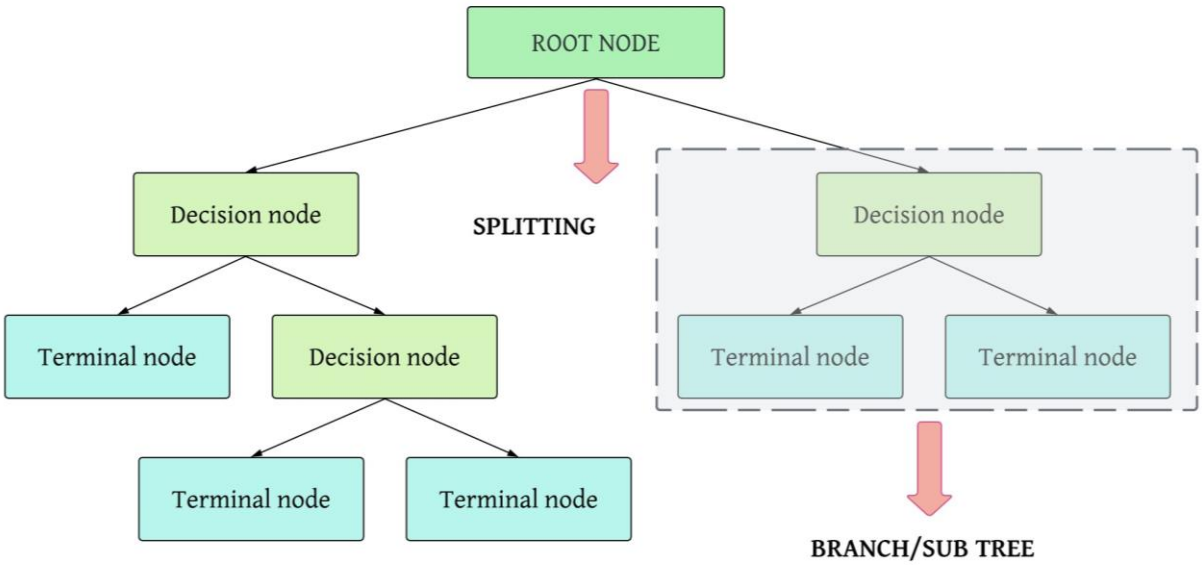

Figure 11. General Structure of a Decision Tree

Legend. Created by the author based on Javatpoint, 2022

4.5. Bayesian Analysis

Bayesian statistics is a methodology used for analysing data based on Bayes' theorem. This approach can work with smaller samples, enables the incorporation of prior knowledge into the analysis, unknown parameters are referred to as random variables in order to make probability statements about them. In contrast, frequentist statistics typically rely on large samples for reliable results, do not incorporate prior knowledge, and the unknown parameters are considered to be fixed (Van de Schoot *et al.*, 2014). In addition, these two approaches differ in the way they provide evidence to answer the research questions. Frequentist approaches make inferences concerning the probability (**P**) of observing a test statistic with a value that exceeds a certain threshold based on the data (**D**), assuming some specified hypothesis (**H**) is true, annotated as P(D|H). Bayesian determining how likely the specified hypothesis is to be true given the new data (**D**) from the current experiment, combined with prior evidence or data ($\mathbf{D_0}$) about the hypothesis, annotated as $P(H|D_0,D)$ (Ruberg *et al.*, 2023). **Table 8** presents a comparison between frequentist and Bayesian statistics, highlighting their similarities and differences.

Table 8. A brief comparison of frequentist and Bayesian statistics.

Category	Frequentist statistics	Bayesian statistics
Large samples	Usually	Not necessarily
Prior knowledge	No	Yes
Unknown parameters	Fixed	Random
Population parameter	One true value	A distribution of values reflecting uncertainty
Answers/calculates:	*P* (D|H)	*P* ($H|D_0$,D)
Estimated intervals	Confidence interval	Credibility interval

Legend: Probability (*P*); new data (D); prior data (D_0); hypothesis (H) Adapted from Rózsás, Kovács, and Sýkora 2016; Van de Schoot et al. 2014

The typical Bayesian workflow consists of three main steps (**Fig. 12**):

- Capturing available knowledge about a given parameter in a statistical model via the prior distribution, which is typically determined before data collection.
- Determining the likelihood function using the information about the parameters available in the observed data.
- Combining both the prior distribution and the likelihood function using Bayes' theorem in the form of the posterior distribution. The posterior distribution reflects one's updated knowledge,

balancing prior knowledge with observed data, and is used to conduct inferences (Van de Schoot *et al.*, 2021).

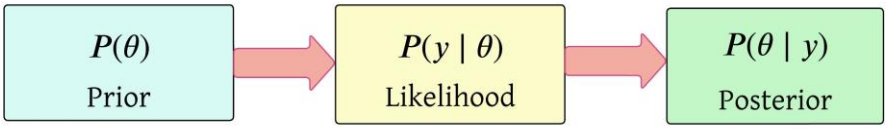

Figure 12. Bayesian workflow

Legend. Created by the author based on van de Schoot *et al.*, 2021

In the past, Bayesian methods were not commonly used due to their high computational requirements. At that time, computers were not powerful enough to perform the necessary calculations. However, with the advancement of statistical theory and computational technology, this challenge has been largely resolved. This has removed one significant obstacle to implementing Bayesian methods (Ruberg *et al.*, 2023).

4.6. Participatory Epidemiology

Participatory epidemiology is the systematic use of participatory approaches and methods to improve understanding of diseases and options for animal disease control, especially in low-income countries (Catley, Alders, & Wood, 2012). Participatory epidemiology involves accessing community knowledge systems, particularly the knowledge of livestock owners regarding the diseases affecting their animals. This includes clinical presentation, gross pathology, epidemiological features of the disease, associated risk factors, and treatment options (Tomaselli, 2022). This knowledge is collected through various data collection techniques in the field. The techniques range from semi-structured interviews (both individual and group) to interactive scoring and visual exercises such as proportional piling, ranking and scoring, seasonal calendars, and Venn diagrams. Direct observations are also used to gather data (Alders *et al.*, 2020; Tomaselli, 2022). The participatory tools used in this investigation are exposed in **Table 9** and **Figure 13**.

Table 9. Participatory tools used

Method	Tools	Examples of data gathered
Interviewing	Individual interviews Focus-group discussions	Personal and group accounts of disease history.
Ranking and scoring	Proportional piling	Importance of ticks in livestock. Economic losses caused by disease.

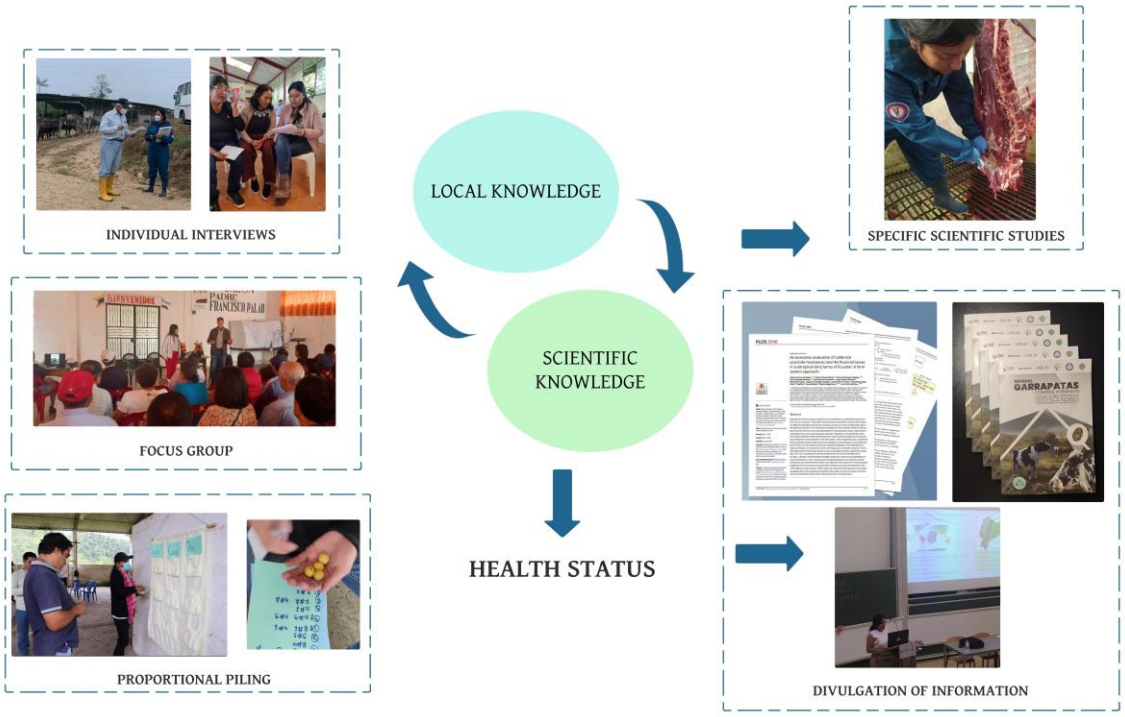

Figure 13. Schematic representation of the participatory tools

Legend. Created by the author

Chapter 2 –Objectives

1. General objective

The general objective of this thesis is to develop methods to understand the impact of ticks and tick-borne diseases in tropical Ecuador by assessing farmers' practices, the economic impact caused by ticks, the presence of TBDs and the risks of consuming ivermectin-contaminated bovine products.

2. Specific objectives

Tick infestations in livestock must be managed effectively to ensure the well-being and productivity of animals, while also preventing the transmission of TBDs. Unfortunately, in Ecuador, tick control is often overlooked by the national government, as it is considered a private issue. To safeguard the country's economy, food security, and public health, it is crucial to study the impact of ticks on livestock in Ecuador. This research will emphasize the importance of prioritizing research, surveillance, and control measures to minimize the impact of tick infestations on livestock. The thesis has been broken down into four parts for ease of understanding (**Appendix 1**).

Part 1. Assessment of farmers' knowledge, perceptions of ticks and TBDs and the different practices they use to control these parasites

The first study was conducted with the objective of identifying the problem in the study area. This was done by investigating the level of **tick infestation** on livestock farms, identifying the tick species present, and determining the tick control practices used (**Study 1**). Additionally, the study aimed to investigate the farmers' **perceptions** and **knowledge** of TBDs in cattle (**Study 3**).

Part 2. Quantitative assessment of the economic impact of ticks in Ecuador

The second part of this study help to determine the economic impact of tick control; for this, the expenses of inputs involved in milk production were determinate, and the **indirect losses** incurred due to **acaricide treatment** were calculated. These losses were assessed at both the animal and farm levels, taking into account the presence of **tick infestation** and **acaricide resistance** in the study area. Additionally, a typology of the farms and an economic analysis were carried out. Since the sampling was conducted during the pandemic, a descriptive analysis of the economic impact of COVID-19 on livestock production in the study areas was also included (**Study 2**).

Part 3. Determine the presence of several blood pathogens in cattle

The third part of this study consisted of evaluating the diagnostic performance (sensitivity and specificity) of three diagnostic tests (ELISA, PCR and blood smear) used to detect anaplasmosis in endemic areas. As part of this study, three diagnostic tests (ELISA, PCR, and blood smear) were

evaluated to determine their sensitivity and specificity in detecting anaplasmosis. The research also aimed to estimate the true prevalence and the proportion of animals naturally immunized against bovine anaplasmosis using a Bayesian approach that combined test results and previous information (**Study 4**). This was based on a previous study where, using a similar approach, two tests were evaluated for the diagnosis of bovine brucellosis in Ecuador (**Appendix 3**).

Part 4. Assessment of human exposure to acaricide residues in food of bovine origin

The study aimed to assess the risk of acaricide residues in bovine products and their impact on human health. Firstly, the management practices for acaricide application were reviewed to identify any bad practices. Subsequently, the presence of ivermectin residues in bovine products was assessed. Finally, the consumption of these products by the local population was also investigated. The results of all these assessments were integrated to determine the risk of consuming bovine products (meat, liver, and milk) contaminated with ivermectin residues (**Study 5**). In addition, this study determined the prevalence of faeces and urine samples contaminated by ivermectin residues.

Chapter 3 – Experimental section

Experimental section

Study 1

The Associated Decision and Management Factors on Cattle Tick Level of Infestation in Two Tropical Areas of Ecuador

Pathogens 2022, 11, 403.

Valeria Paucar, Ximena Pérez-Otáñez, Richar Rodríguez-Hidalgo, Cecilia Perez, Darío Cepeda-Bastidas, Jorge Grijalva, Sandra Enríquez, Susana Arciniegas-Ortega, Sophie O. Vanwambeke, Lenin Ron-Garrido and Claude Saegerman

Preamble

Ticks are small arachnids that feed on the blood of wild and domestic animals, including humans. Rhipicephalus microplus is considered the most important tick of cattle worldwide. It is crucial to control cattle tick infestations to maintain the well-being of cattle populations and ensure sustainable livestock production. Ecuador, due to its tropical location, provides an ideal habitat for the proliferation of diverse tick populations, including R. microplus. However, despite their prevalence and potential impact, there remains a notable lack of comprehensive studies on ticks infesting cattle in tropical Ecuadorian regions. The collected data would provide us with a better understanding of the extent of tick infestation at the animal and farm levels and help us characterize tick control practices and their possible association with the level of tick infestation.

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Article

The Associated Decision and Management Factors on CattleTick Level of Infestation in Two Tropical Areas of Ecuador

Valeria Paucar [1,2], Ximena Pérez-Otáñez [1,3], Richar Rodríguez-Hidalgo [1,4], Cecilia Perez [4], Darío Cepeda-Bastidas [5], Jorge Grijalva [4], Sandra Enríquez [1], Susana Arciniegas-Ortega [6], Sophie O. Vanwambeke [2], Lenin Ron-Garrido [1,4,†] and Claude Saegerman [2,*,†]

1 Instituto de Investigación en Zoonosis (CIZ), Universidad Central del Ecuador, Quito 170521, Ecuador; avpaucar@uce.edu.ec (V.P.); xfperez@uce.edu.ec (X.P.-O.); rrodriguez@uce.edu.ec (R.R.-H.); ienriquez@uce.edu.ec (S.E.); ljron@uce.edu.ec (L.R.-G.)
2 Research Unit of Epidemiology and Risk Analysis Applied to Veterinary Sciences (UREAR-ULiège), Fundamental and Applied Research for Animals & Health (FARAH) Center, Faculty of Veterinary Medicine, University of Liege, 4000 Liège, Belgium; sophie.vanwambeke@uclouvain.be
3 Georges Lemaitre Centre for Earth and Climate Research, UCLouvain, 1348 Louvain-la-Neuve, Belgium
4 Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, Quito 170521, Ecuador;bcperez@uce.edu.ec (C.P.); jgrijalva@uce.edu.ec (J.G.)
5 Facultad de Ciencias Agrícolas, Universidad Central del Ecuador, Quito 170521, Ecuador; dacepedab@uce.edu.ec
6 Facultad de Geología, Minas y Petróleo, Universidad Central del Ecuador, Quito 170521, Ecuador;srarciniegas@uce.edu.ec
* Correspondence: claude.saegerman@uliege.be
† These authors contributed equally to this work.

Abstract: Decision-making on tick control practices is linked to the level of knowledge about livestock farming and to the social context in which individuals practice them. Tick infestation is one of the main problems in tropical livestock production. The objective of this study was to characterize tick-control related practices in two tropical livestock areas and their potential association with the level of tick infestation. A total of 139 farms were included in this survey. To determine this association, a multivariate logistic regression model was used. A stepwise model selection procedure was used and model validation was tested. Cattle husbandry as a main activity, the use of external paddocks, the use of amitraz, and the lack of mechanization on the farm were related with high tick infestation. On the other hand, owner involvement in the preparation of acaricide solution was identified as a protective factor against high tick infestation. At animal level, age (old), body condition status (thin), and lactation were also associated with high tick infestations, while *Bos primigenius indicus* cattle and their crosses reduced the probability of high tick infestations. The factors studied, such as herdsize, education level of the owners, and veterinary guidance, varied from farm to farm. Nonetheless,these differences did not generate changes in the level of tick infestation. According to the area under the receiver operating characteristic curve (AUC-ROC), the model at farm level predicts a high level of infestation, with an accuracy of 72.00% and high sensitivity. In addition, at animal level, crossbreeding with indicus cattle and breeding selection for host resistance will be useful against high tick infestation. Likewise, the implementation of programs of capacitation and research on tickcontrol for farmers, cowboys, and vets in these areas is necessary.

Keywords: acaricide; cattle; Ecuador; protective factor; risk factor; tick; tick-borne diseases; tropical

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1. Introduction

Livestock is a major economic activity in Ecuador. It contributes substantially to local nutrition, providing milk, meat, and derivatives that are in high demand by the population [1]. The agriculture sector, including livestock farming, represents around 7.80% of the total of Ecuador's gross domestic product [2]. Ecuadorian cattle population is about 4.3 million heads, from 280,000 cattle farms nationwide [3]. Due to Ecuador's location, most of the country, except for certain parts of highlands (places above 2500 m), experiences a humid tropical climate [4,5], providing favorable environmental conditions for the development of ectoparasites such as ticks. In fact, more than 75.00% of cattle herds are found in areas either infested or potentially infested with ticks [6].

Tick infestation causes significant economic losses in the livestock industry. Ticks transmit a wide range of pathogens that can cause tick-borne diseases (TBDs). The most important TBDs of cattle in Ecuador are anaplasmosis caused by rickettsia of the genus *Anaplasma* [7] and babesiosis caused by protozoa of the genus *Babesia* [8]. In addition to spreading pathogenic microorganisms, ticks cause weight loss, reduced milk production, and cause skin injures that can lead to secondary bacterial and fungal infections and even myasis (*Cochlomyia hominivorax*) [9–11]. Additional losses include the cost of treatment for clinical cases and the expenses derived from the indiscriminate use of acaricides for tick control. Likewise, the indiscriminate use of chemical compounds has increased the problem of tick multiresistance to acaricides, which has already been reported in Ecuador [11].

The livestock production present in tropical areas is extensive, with a low level of mechanization used, and grazing is the main source of food for animals [12]. Consequently, to increase milk production, farmers tend to introduce exotic breeds (*Bos primigenious taurus*), which often are susceptible to TBDs. In addition, for improving farm profitability, natural ecosystems are incorporated into production, clearing forests to plant non-native pasture, in order to expand the agricultural frontier [13,14]. These environmental and host modifications have had a major impact on the ecology of these parasites, causing the encounter rate between tick and host to be higher, leading to an increase in tick infestation [15,16]. However, decisions on tick control practices are usually linked to the level of knowledge about livestock farming and to the social context in which individuals practice these strategies [17]. Understanding the reasons that lead farmers to use particular control measures will contribute to holding back the advance of the threat that acaricides pose to the environment and public health and also increase farm productivity.

The three objectives of this study were to (i) describe two tropical dairy production areas located on the eastern and western foothills of the Ecuadorian Andes; (ii) relate tick control practices at animal and farm levels on the level of tick infestation; and (iii) identify tick species infesting cattle in these areas.

2. Results

2.1. Tick Species

In total, 1905 adult ticks were collected from 133 farms, 1345 ticks (70.60%) were females and 560 (29.40%) were males. In six farms, we did not find ticks on the animals examined. Tick prevalence in farms was estimated to be 95.70% (95% confidence internal, CI: 90–98). Table 1 shows the number of farms with tick presence and the tick species reported. Four species of ticks were morphologically identified. *Rhipicephalus microplus* (female: 1328; male: 553) being the most common species in the Northwest of Pichincha and Quijos river valley.

Table 1. Ticks identified in the study areas.

Tick Species	Northwest of Pichincha (Number of Farms)	Quijos River Valley (Number of Farms)	Total of Farms
R. microplus	63 *	67 **	130
Ixodes boliviensis	1 *	2 **	3
Ixodes montoyanus	1 *	1 **	2
Amblyomma mixtum	1 *	0	1

* In two farms in the Northwest of Pichincha, different species were found on the same farm (Farm 1: *R. microplus* and *A. mixtum*; Farm 2: *I. boliviensis* and *I. montoyanus);* ** In one farm in the Quijos river valley, different species were found on the same farm (*I. boliviensis* and *I. montoyanus*).

2.2. Characteristics of Farming and Tick Control

A total of 139 farms were visited, 72 in the Quijos river valley, and 67 in the Northwest of Pichincha province (Table 2). According to the number of cattle, most of the farms visited in the two areas were medium farms (21 to 70 cattle; 54.17% in Quijos river valley, and 64.18% in Northwest of Pichincha), followed by small farms (1 to 20 cattle) in the Quijos river valley (38.89%) and large farms (more than 70 cattle; 23.88%) in Northwest of Pichincha. The principal activity in those areas is cattle husbandry, with a focus on milk production. Agriculture occupies the second place, being practiced at 22.20% in Quijos river valley and 37.31% in Northwest of Pichincha. In 19.44% (Quijos river valley) and 32.84% (Northwest of Pichincha) of farms these two activities are practiced jointly.

Table 2. Characteristics of farming and tick control in Quijos river valley and Northwest of Pichincha.

Parameter	Quijos River Valley		Northwest of Pichincha		*p*-Value of the Fisher's Exact Test
	Number of Farms	Percentage of Farms	Number of Farms	Percentage of Farms	
Tick infestation					
Low	42	58.30	40	59.70	1.00
High	30	41.67	27	40.29	
Level of education					
Without formal education	3	4.17	1	1.49	
Primary school	27	37.50	20	29.85	0.01 *
High school [a]	35	48.61	26	38.81	
University concluded	7	9.72	20	29.85	
Animal husbandry as principal activity	64	88.89	59	88.06	1.00
Who is the cowherd					
Employee	7	9.72	6	8.96	0.07
Owner	41	56.94	26	38.81	
Owner and Employees	24	33.33	35	52.24	
Herd size Small	28	38.89	8	11.94	<0.01 *
Medium	39	54.17	43	64.18	
Large	5	6.94	16	23.88	
Type of production					
Beef cattle	0	0.00	1	1.49	0.64
Dual purpose cattle	21	29.17	17	25.37	
Dairy cattle	51	70.83	49	73.13	
Level of mechanization					
Non-mechanized	47	65.28	38	56.72	0.37
Semi-mechanized	18	18.06	17	7.46	
Mechanized	7	16.67	12	35.82	

Table 2. *Cont.*

Parameter	Quijos River Valley		Northwest of Pichincha		*p*-Value of the Fisher's Exact Test
	Number of Farms	Number of Farms	Number of Farms	Number of Farms	
Veterinary support					
No	9	12.50	37	55.22	<0.01 *
Yes	63	87.50	30	44.78	
Acaricide					
Amide	45	62.50	58	86.57	0.08
Organophosphate	52	72.22	34	50.75	0.01 *
Pyrethroid	43	59.72	18	26.87	<0.01 *
Macrocyclic lactone	56	77.78	58	86.57	0.19
Phenylpyrazolone	1	1.39	2	2.99	0.61
Benzoylphenyl urea	8	11.11	22	32.84	<0.01 *
Pyrethroid + Organophosphate	48	66.67	32	47.76	<0.01 *
Pyrethroid + Organophosphate+ Phenylpyrazolone	22	30.56	12	17.91	0.11
Benzoylphenyl urea + Macrocyclic lactone	1	1.39	5	7.46	0.10
Benzoylphenyl urea + Phenylpyrazolone	20	27.78	16	23.88	0.70
Frequency of acaricide treatment application					
Less than 1 month	44	61.11	43	64.18	
Every 1 to 2 months	21	29.17	11	16.42	0.13
Every 3 to 6 months	6	8.33	12	17.91	
More of 6 months	1	1.39	1	1.49	

[a] High school—including farmers with unfinished university education. * Characteristics of farming and tickcontrol with *p*-Value ≤ 0.05.

With respect to the education level, the percentage of farmers with university education was higher in Northwest of Pichincha (29.85%) than in Quijos river valley (9.72%). In the two areas, most of the farms are non-mechanized (65.28% and 56.72%, respectively).However, the number of mechanized farms is twice as high (35.82%) in Northwest of Pichincha compared to Quijos river valley (16.67%). Only 43.06% in Quijos river valley and 37.31% in Northwest of Pichincha have a storage space to keep veterinary drugs. All the farms use grazing as the feeding method. In addition, 31.94% in Quijos river valley and 43.28% in Northwest of Pichincha cut and carry pasture, and this is mainly used to feed the lactating dairy cows. Fifty percent (36 out of 72 farms) of the farms in Quijos river valley and 25.37% (17 out of 67 farms) of the farms in Northwest of Pichincha do not have sufficient feeding paddocks in the total area on the farm and have to use external farm paddocks to feed their cattle. Most of the external paddocks in Quijos river valley are owned (63.89%) by the farmer but, in Northwest of Pichincha most of these are rented (64.71%), and the farmers pay an annual fee for their use.

This survey revealed that most cattle farms in Quijos river valley (87.50%) have veterinary support. Of these farms, 81.43% are managed by public veterinarians. In Northwest of Pichincha, in the majority of farms (55.22%) do not have the accompaniment of a veterinarian. On farms with veterinary accompaniment (44.78%), this service is private in half of the cases (50.91%).

All farms (100.00%) in the Quijos river valley use chemical treatment for tick control, and in the Northwest of Pichincha 95.52% used it. Only a few farms (4.48%) did not use chemical control, because they prefer uncommon control methods such as bath spray with entomopathogenic fungus, medicinal plants (*Azadirachta indica*), sulfocalcic broth, or sulfur supplementation in the diet. In most cases, the frequency of application of an acaricide treatment is less than once per month. The main

method of application of acaricides was spraying with a hand sprayer (96.32%). In spray solution, the most commonly used acaricides were amides and organophosphates. Amides (amitraz) were used in 80.56% and 67.16% of the farms in Quijos river valley and Northwest of Pichincha, respectively. Organophosphates were used by 50.75% of all farms in Quijos river valley and 72.22% of thefarms in Northwest of Pichincha. Ivermectin (macrocyclic lactone) was also commonly used for tick control by 77.78% of farms in the Quijos river valley and 86.57% in the Northwestof Pichincha (Table 2). This principle was administered parenterally (subcutaneously); however, in 19.65% and 6.90% of the farms in the Quijos river valley and Northwest of Pichincha it was applied topically (spraying). Among the farmers using ivermectin, 53.76% (Quijos river valley) and 50.00% (Northwest of Pichincha) use it on milking cattle. Although the two zones have different herd sizes and different levels of mechanization, both zones had similar percentages of high tick infestation (Quijos river valley with 41.67%; Northwestern of Pichincha with 40.29%) (p-Value > 0.05).

2.3 Tick-Infestation Associated Factors at Farm Level

Forty-one percent (95% CI: 32.84–49.68) (57/139) of farms had a high level of tick infestation. The variables included in the model are shown in Table 3. The initial model included 26 variables with (AIC: 206.40). The final model included eight factors significantlyassociated with high infestation level of ticks at farm level, selected for having the smallest value of AIC (AIC: 181.15).

Table 3. Risk and protective explanatory variables for a high level of tick infestation at the farm level using univariate analysis

Explanatory Variable		Number of Farms	Positive Farms	Proportion	OR (95% CI)	p-Value of the Fisher's Exact Test
Level of education [a]	High School	61	26	42.62	Reference	-
	Primary School	51	19	37.25	0.80 (0.35–1.83)	0.70
	University, concluded	27	12	44.44	1.08 (0.39–2.95)	1.00
Range of experience	1–5 years	15	9	60.00	Reference	-
	6–10 years	21	8	38.10	0.42 (0.08–1.93)	0.31
	11–20 years	25	9	36.00	0.85 (0.29–2.36)	0.82
	≥ 21 years	78	31	39.74	0.44 (0.12–1.56)	0.17
Who is the cowherd	Employees	13	7	53.85	Reference	-
	Owner	67	26	38.81	0.55 (0.14–2.14)	0.37
	Owner and Employees	59	24	40.68	0.59 (0.14–2.35)	0.54
Cattle husbandry as the principal activity	No	16	4	25.00	Reference	-
	Yes	123	53	43.09	2.26 (0.64–10.16)	0.19
Herd size	Large	21	8	38.10	Reference	-
	Medium	82	34	41.46	1.15 (0.39–3.57)	0.81
	Small	36	15	41.67	1.16 (0.34–4.10)	1.00
Level of mechanization	Mechanized	19	5	26.32	Reference	
	Semi-mechanized	35	15	42.86	2.07 (0.54–9.04)	0.26
	Non-mechanized	85	37	43.53	2.29 (0.71–8.82)	0.20
Cut and carry pasture	No	92	36	39.13	Reference	-
	Yes	47	21	44.68	1.25 (0.58–2.71)	0.58
Paddock maintenance	No	24	9	37.50	Reference	-
	Yes	115	48	41.74	1.19 (0.44–3.36)	0.82

Table 3. *Cont.*

Explanatory Variable		Number of Farms	Positive Farms	Proportion	OR (95% CI)	*p*-Value of the Fisher's Exact Test
Pasture rotation	No	32	15	46.88	Reference	-
	Yes	107	42	39.25	0.73 (0.31–1.76)	0.54
External paddocks	No	86	29	33.72	Reference	-
	Yes	53	28	52.83	2.19 (1.03–4.70)	0.03 *
Paddocks with dallis grass (*Paspalum dilatatum*)	No	41	17	41.46	Reference	-
	Yes	98	40	40.82	0.97 (0.44–2.20)	1.00
Knowledge of the life cycle of ticks	No	14	4	28.57	Reference	-
	Yes	125	53	42.40	1.83 (0.49–8.45)	0.4
Correct knowledge of the location of ticks in the grass	No	48	17	35.42	Reference	-
	Yes	91	40	43.96	1.43 (0.66–3.16)	0.37
Veterinary support	No	46	15	32.61	Reference	-
	Yes	93	42	45.16	1.70 (0.77–3.86)	0.2
Prescription by a veterinarian	No	37	13	35.14	Reference	-
	Yes	102	44	43.14	1.40 (0.60–3.35)	0.44
Who prepares the acaricide solution	Employed	31	16	51.61	Reference	-
	Owner	108	41	37.96	0.58 (0.24–1.39)	0.21
Person who applies acaricide treatment	Employed	48	20	41.67	Reference	-
	Owner	82	33	40.24	0.55 (0.14–2.14)	0.37
	Owner and Employees	9	4	44.44	1.12 (0.20–5.94)	1.00
Has storage area	No	83	39	46.99	Reference	-
	Yes	56	18	32.14	0.54 (0.25–1.14)	0.11
Use of amitraz	No	36	11	30.56	Reference	-
	Yes	103	46	44.66	1.83 (0.77–4.57)	0.17
Dose of acaricide	Correct	44	18	40.91	Reference	-
	Incorrect	95	39	41.05	1.00 (0.46–2.23)	1.00
Frequency of acaricide treatment application	<1 month	87	41	47.13	Reference	-
	1–2 months	32	10	31.25	0.51 (0.19–1.29)	0.15
	3–6 months	20	6	30.00	0.48 (0.14–1.50)	0.21
Perception: predisposition for a breed	No	51	15	29.41	Reference	-
	Yes	88	42	47.73	2.17 (1.00–4.93)	0.05 *
Perception: predisposition for a color	No	78	30	38.46	Reference	-
	Yes	61	27	44.26	1.27 (0.61–2.65)	0.6
Perception: predisposition for a category	No	46	18	39.13	Reference	-
	Yes	93	39	41.94	1.12 (0.52–2.48)	0.86
Perception: ticks can affect the cattle	No	11	1	9.09	Reference	-
	Yes	128	56	43.75	7.70 (1.04–342.91)	0.03 *
Perception: economic loss	No	6	1	16.67	Reference	-
	Yes	133	56	42.11	3.61 (0.39–174.87)	0.4

[a] Primary school: including farmers without formal education; High school: including farmers with unfinished university education. [b] * Characteristics of farming and tick control with p-Value ≤ 0.05.

The final logistic regression model is presented in Table 4. The results of the model showed that cattle husbandry as the principal economic activity has a positive association with high levels of tick infestation. The OR when raising animals is the principal activity was 3.96 (95% CI: 0.97–16.10; p-Value 0.053, marginally significant) times higher than when cattle husbandry is not the principal activity.

Absence of mechanization on the farm has a positive association (risk explanatory variable) with high tick infestation. Indeed, semi-mechanized (OR = 4.48 with 95% CI: 1.02–19.53) and non-mechanized farms (OR = 5.11 with 95% CI: 1.14–22.86) had higher odds ratio to have high tick infestation than the mechanized farming.

Table 4. Risk and protective explanatory variables for a high level of tick infestation at the farm level using a multivariable binary logistic regression model.

Explanatory Variable		Final model	
		OR (95% CI)	p-Value of the Fisher's Exact Test
Cattle husbandry as the principal activity	No	Reference	-
	Yes	3.96 (0.97–16.10)	0.053 ***
Level of mechanization	Mechanized	Reference	-
	Semi-mechanized	4.48 (1.02–19.53)	0.05 *
	Non-mechanized	5.11 (1.14–22.86)	0.03 *
External paddocks	No	Reference	-
	Yes	2.08 (0.94–4.60)	0.07
Veterinary support	No	Reference	-
	Yes	2.09 (0.86–5.07)	0.10
Who prepared the acaricide solution	Employee	Reference	-
	Owner	0.19 (0.06–0.61)	<0.01 **
Has storage area	No	Reference	-
	Yes	0.52 (0.23–1.20)	0.12
Use of amitraz	No	Reference	-
	Yes	2.58 (0.92–7.20)	0.07
Perception: predisposition for a breed	No	Reference	-
	Yes	1.87 (0.83–4.20)	00.13

*risk explanatory variable. ** protective explanatory variable. *** marginally significant.

When the acaricide spray was prepared by the owner, there was less chance (OR = 0.19 with 95% CI: 0.06–0.061) of having animals with high tick infestation in the farms, in comparison with situations where the solution was prepared by employees.

2.4. Overall Weighted Score at Farm Level and Area under the Receiver Operating Characteristic Curve

The factors associated with a p-Value 0.10 in the final model (see Table 4) were used to calculate the weighted score. This threshold was used because of the relatively low number of farms sampled. Six covariates were aggregated as a unique overall weighted score (OWS) by farm, using the following formula:

$$\begin{aligned} OWS = {} & [(Presence\_a = 1) \times (OR\_a)] + [(Presence\_b2 = 1) \times (OR\_b2)] + \\ & [(Presence\_b3 = 1) \times (OR\_b3)] + [(Presence\_c = 1) \times (OR\_c)] + [(Presence\_d = 1) \times (OR\_d)] \\ & + [(Absence\_e = 1) \times (1/OR\_e)] + [(Presence\_f = 1) \times (OR\_f)] \end{aligned} \quad (1)$$

where a = cattle husbandry is the principal activity; b2 = semi-mechanized farm; b3 = mechanized farm; c = farm with external paddocks; d = farm with veterinary support; e = the person who prepares the acaricide solution is the owner; f = use of amitraz. With this formula, the minimum and the maximum

theoretical values of the OWS are 5.11 and 21.08. The probability that a farm has a low or high level of tick infestation as a function of the result of the OWS is represented in Table 5.

Table 5. The probability that a farm has a low or high level of tick infestation as a function of the overall weighted score.

	Level of tick infestation (Number of farms)		Total Farms	Probability of a level of tick infestation	
OWS	Low	High		Low	High
5–7	4	1	5	0.80	0.20
7–9	7	0	7	1.00	0.00
9–11	13	0	13	1.00	0.00
11–13	18	11	29	0.62	0.38
13–15	24	19	43	0.56	0.44
15–17	14	23	37	0.38	0.62
17–21	2	3	5	0.40	0.60
Total	82	57	139		

Legend: for the probability, the color scale is related to the increase of its value (red to blue; with blue color being low risk and red color being high risk).

The diagnostic discriminatory power of OWS was assessed by calculating the AUC-ROC (Figure 1). The AUC-ROC was 0.72 (95% CI: 0.63–0.80), with standard error = 0.043. Using the Youden index (0.34), the best cut-off to discriminate the level of tick infestation (high and low level) was OWS = 11.65. Applying this cut-off, the sensitivity was 94.74 and the specificity was 41.46%.

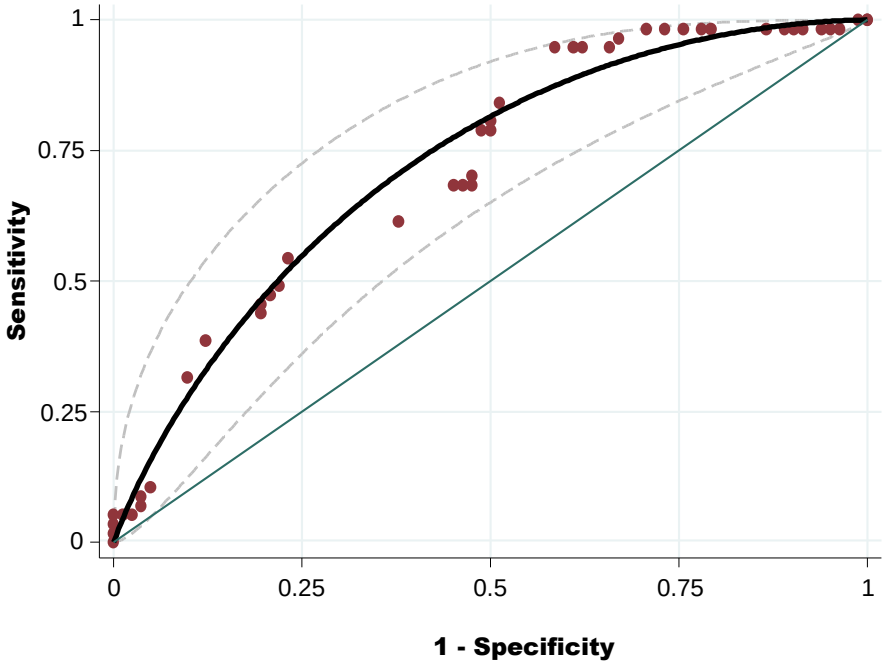

Figure 1. Receiver operating characteristic curve of the overall weighted score of a high level of tick infestation at farm level.

2.5. Tick-infestation Associated Factors at Animal Level

The covariates included in the analysis for tick infestation at animal level are shown in Table 6. In the univariate analysis six covariates were included. The sex of animals was discarded, given that only

27 out of 826 animals were males. For the final model, four covariates were associated with a high level of infestation at the animal level.

Table 6. Risk explanatory factors for a high level of tick infestation at the animal level using a univariate analysis.

Risk factor		Total animals	Positive animals	Proportion	OR (95% CI)	*p*-value Fisher test
Breed	*B. p. taurus*	769	283	0.37	Reference	-
	Crossbreed: B. p. taurus x B. p. indicus	47	18	0.38	1.07 (0.55–2.03)	0.88
	B. p. indicus	10	3	0.30	0.74 (0.12–3.26)	0.75
Color [a]	Black-White	313	122	0.39	Reference	-
	Black	142	53	0.37	0.93 (0.61–1.43)	0.76
	Brown	266	92	0.35	0.83 (0.58–1.18)	0.30
	Red	84	28	0.33	0.78 (0.45–1.33)	0.38
	White	21	9	0.43	1.17 (0.42–3.14)	0.82
Sex	Female	799	296	0.37	Reference	-
	Male	27	8	0.30	0.72 (0.27–1.74)	0.54
Age [b]	Young	40	11	0.28	Reference	-
	Young adult	600	216	0.36	1.48 (0.70–3.36)	0.31
	Adults over 7 years old	186	77	0.41	1.86 (0.84–4.83)	0.11
Cows in lactating status	No	157	40	0.25	Reference	-
	Yes	669	264	0.39	1.91 (1.27–2.90)	<0.01*
Body condition status	Fat	40	17	0.43	Reference	
	Good	551	191	0.35	0.72 (0.36–1.47)	0.31
	Thin	235	96	0.41	0.93 (0.45–1.97)	0.86

[a] Color coat: classification was based according to the coat color dominance. [b] Age: young (cattle with ≤ 23 months); young adult (cattle between 24 to 83 months); and adults over 7 years old (cattle with ≥84 months).

The final logistic regression model is presented in Table 7, and the results of the multivariable binary logistic regression model showed that cattle breed: Crossbreed: *B. primigenious taurus* x *B. primigenious indicus* (OR = 0.547 with 95% CI: 0.546–0.548) and *B. p. indicus* (OR = 0.539 with 95% CI: 0.538–0.540) were protective factors against a high level of infestation.

Table 7. Risk and protective factors for a high level of tick infestation at the animal level included in the final multivariable binary logistic regression model.

Risk factor		OR (95% CI)	*p*-value Fisher test
Breed	*B. p. taurus*	Reference	_
	Crossbreed: B. p. taurus x B. p. indicus	0.547 (0.546–0.548)	<0.01**
	B. p. indicus	0.539 (0.538–0.540)	<0.01**
Age	Young	Reference	_
	Young adult	1.050 (1.048–1.051)	<0.01*
	Adults over 7 years old	1.480 (1.478–1.482)	<0.01*
Lactating dairy cows	No	Reference	_
	Yes	2.287 (2.283–2.900)	<0.01*
Body condition status	Fat	Reference	–
	Good	1.212 (1.210–1.214)	<0.01*
	Thin	1.992 (1.990–1.995)	<0.01*

* risk explanatory variable. ** protective explanatory variable.

In comparison to young animals, young adult and adults over 7 years old had an OR = 1.050 (95% CI: 1.048-1.051) and OR = 1.480 (95% CI: 1.478–1.482), respectively. Cows in lactating status had an OR = 2.287 (95% CI: 2.283–2.900) in comparison to other categories. Finally, for body condition status, good (OR = 1.21 with 95% CI: 1.21–1.21) and thin (OR = 1.992 with 95% CI: 1.990–1.995) conditions were risk explanatory variables for high level of infestation in comparison to fat animals.

2.6. Overall Weighted Score (OWS) at Animal Level and Area Under the Receiver Operating Characteristic Curve

The risk and protective explanatory variables with a *p*-value ≤0.05 (see Table 6) were used to calculate the weighted score. Finally, four covariates were aggregated as a unique overall weighted score (OWS) by animal, using the following formula:

OWS = [(Absence_g1=1)*(1/OR_g2)] + [(Absence_g1=1)*(1/OR_g3)] +
[(Presence_h=1)*(OR_h2)] + [(Presence_h3=1)*(OR_h3)] + [(Presence_i=1)*(OR_i)] + (2)
[(Presence_j2=1)*(OR_j2)] + [(Presence_j3=1)*(OR_j3)]

where g1= *B. primigenious taurus* g2= breed is a Crossbreed*: B. primigenious taurus* x *B. primigenious indicus*; g3 = breed is *B. primigenious indicus*; h2 = young adult animal; h3 = adult over 7 years old; i = lactating dairy cows; j2 = animal with good body condition status; and j3 = animal with thin body condition status.

With this formula, the minimum and the maximum theoretical values of the OWS were 0.00 and 7.59.

The diagnostic discriminatory power was assessed by calculating the AUC-ROC (Figure 2). The AUC-ROC was 0.56 (95% CI: 0.52–0.60) with standard error = 0.021. Using both the Youden index (0.087) the best cut-off to discriminate the level of tick infestation (high and low level) was OWS = 3.34. Applying this cut-off, the sensitivity was 89.14%, and the specificity was 19.54%.

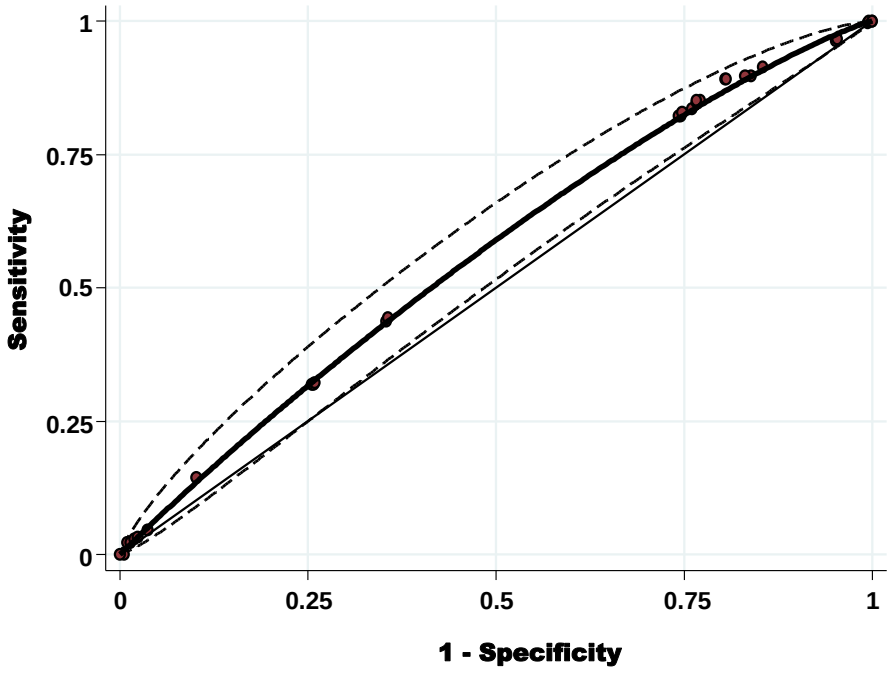

Figure 2. Receiver operating characteristic curve of the overall weighted score of high level of tick infestation at animal level.

3. Discussion

R. microplus was the most collected and identified tick in both zones in this study, which confirms that it is the most common species on cattle. *I. boliviensis, I. montoyanus,* and *A. mixtum* were also identified on a few farms. Previous studies carried out in Ecuador have determined the presence of the *R. microplus* tick in Santo Domingo de los Colorados, Los Bancos, and Napo province [18–24]. Unpublished results obtained from the 'Encuesta Nacional de Brucelosis, Tuberculosis y Garrapatas', reported *R. microplus* as the most abundant species in tropical and subtropical areas of Ecuador. Nava et al. [25] and Aguilar- Domínguez et al. [26] reported the presence of *A. mixtum* larvae in the vegetation of coastal Ecuadorian localities. In addition, Guillén and Muñoz [20] identified *Amblyomma* spp. and *Ixodes* spp. on cattle at Santo Domingo de los Colorados. On the other hand, studies carried out in different parts of the Amazonia region have reported the presence of *I. boliviensis* [27]and *I. montoyanus* [28] on cattle.

In Ecuador, according to reports of several projects carried out by the Institute of Research in Zoonoses (unpublished data), *Amblyomma maculatum*, *Amblyomma ovale*, *Haempahysalis juxtakochi*, and *Dermacentor nitens* were also present on cattle. Species of the genus *Amblyomma* were reported by Enriquez et al. [29], Voltzit [30], and Maya et al. [22], who reported the presence of *Amblyomma coelebs*, *Amblyomma triste,* and *Amblyomma cajennense,* respectively, on cattle.

When looking at the results of the number of farmers with university studies, mechanized farms and herd size (farms with more than 70 animals), it is evident that cattle husbandry is a more developed activity in the Northwest of Pichincha compared to the Quijos river valley. All this can be associated to the fact that the two zones had a different historical trajectory. The Northwest of Pichincha began to practice cattle husbandry over 50 years ago. This activity has expanded over time in relation to its location in a transit zone between the Coast region and the Highlands region, which facilitates the entrance of animals from other areas and the exit of livestock products to their destinations; becoming an area that supplies livestock products to the capital of Ecuador located in the Highlands region, and providing more than 200,000 L of raw milk per day [31–33]. On the other hand, cattle husbandry in the Quijos river valley produces 55,000 L of milk per day [33,34] and is a family activity that grew thanks to the construction of roads to the Amazon region for oil exploitation between 1968 and 1972 [35]. In addition, with the incursion of the multinational company Nestlé, cattle husbandry displaced agricultural production, which was previously the main source of income in the area [34,36,37]. Although the two zones had different herd sizes, farm mechanization, and education levels, these two zones had similar percentages of high tick infestation (Quijos river valley with 41.67%; Northwestern of Pichincha with 40.29%). This finding shows that some factors may not have been considered. Although the climate is very similar in both areas, and the sampling was done in the rainy season, the dry season in Northwestern of Pichincha is longer.

Regarding tick control methods, both zones mainly use chemical control. The range of acaricide products available on the market is wide. However, the site of action does not have much variety, as it can be seen that most of the acaricides used in this study belong to one of these families amide, organophosphate, pyrethroid, macrocyclic lactone, and phenylpyrazolone, whose mode of action is at the level of the nervous system [38,39]. The only acaricide with a different mode of action is fluazuron (benzoylphenyl urea). This acaricide is relatively new on the market. It is applied as a pour on that affects the molting process. However, it is expensive and has a long residual life in meat and milk [40,41]. The limited use of this acaricide in the study areas is associated with its

price. Ivermectin(macrocyclic lactone) also generates residues in milk and meat for several weeks after application [41], but it is used by 77.78% of farmers in the Quijos river valley and 86.57% in the Northwest of Pichincha. It is also used in lactating cows.

Acaricides were generally applied using hand sprayers, and the most usual acaricide applied in this survey was amitraz, an acaricide used extensively around the world, which entered in Ecuador in the 1960s [42]. Spraying consists of dissolving the correct dose of a wettable powder or flowable product in water [43]. However, this step is not followed by all farmers. Inadequate acaricide preparations (under-dosing or overdosing) and misapplications lead to the development of resistance [43,44], which has already been reported in Ecuador [11,22]. This fact would explain why farmers reduce the time between the treatments to less than one month in both zones (Quijos river valley, 80.56% and in Northwest of Pichincha, 67.16%). Bianchi et al., in 2003, [45] and Rodriguez-Vivas et al.in 2018 [39] reported that farmers used to apply control methods for ticks every month or whenever they observe a significant infestation level. Decreasing the interval between treatments is the first reaction when farmers observe that acaricide does not have the expected effect. Other common acaricides used are organophosphates, which despite being chemicals with a variable toxicity that can range from highly toxic to slightly toxic, and which have already been documented to cause neurological damage, are being used by farmers in the Quijos river valley in 77.78% and Northwest of Pichincha with 50.75% of farms. This is associated with the lack of knowledge that farmers have about the dangers of these acaricides. In addition to the fact that they are products freely sold in Ecuador, they do not need veterinary prescription [46], despite being banned in 32 countries (dichlorvos and trichlorfon) due to their harmful properties for the health and the environment [47].

At the animal level, the presence of crossbreed (*B. primigenious taurus B. primigeniousindicus*) and *B. primigenious indicus* breeds were protective to high levels of infestation in comparison with *B. primigenious taurus* cattle. This is because *B. primigenious indicus* cattle and their crossbreeds are genetically more resistant to ticks, which makes them adaptable to tropical climates [48–50]. The tolerance of *B. primigenious indicus* cattle is due to the coexistence and co-evolution of zebu cattle originating from Asia with *R. microplus* species also originating from the Asian continent, while European breeds (*B. p. taurus*) are more susceptible because they were less exposed in this evolution process, in addition to the fact that this type of cattle has thinner skin [14,51–53]. This is one of the main reasons why cattle breeds such as Jersey and Holstein have a higher level of tick infestation in tropical zones.Cattle in the studied areas with 'good' and 'thin' body conditions have 1.21 and 2.00 higher odds ratio of high level of tick infestation than cattle with 'fat' body condition.This is due to the fact that low nutrition causes metabolic, endocrine, and immunologicalconsequences that increase parasitism [54]. These results are consistent with those reported by Sutherst et al. [55], Tolleson et al. [56], and Abbas et al. [38], who associated poor nutrition in cattle with increased tick burdens. In addition, a study in sheep found that lean sheep had 50.00% more ticks than fat sheep [55]. However, this is a factor to be taken with caution, as the worse body condition is also a consequence of high tick infestation. An average of 40 ticks per day per animal may cause losses around of 20 kg of weight peryear [57,58].

Another factor associated with a high level of tick infestation was lactation, these animals had almost three times higher risk (OR = 2.29) of a high-level infestation compared to an animal that was not in production. This could be related to the fact that cows in this period have a high level of prolactin and progesterone, altering their immune system and making them more susceptible to infection. Indeed, infestation added to production stresses,such as pregnancy or lactation, decrease resistance to

infection [59–62]. Additionally, dry cows can be treated with ivermectin at higher doses, given the lifting of restrictions for ivermectin usage during the milking period.

Animal age also constitutes a risk explanatory variable; young adults (OR = 1.05) and adults over 7 years (OR = 1.48) animals had a higher risk of having high tick infestationthan young animals. This result is consistent with the work of Swai et al. in 2005 [63]and Rehman et al. in 2017 [61], who found that mature animals had a higher chance of carrying ticks compared to calves. The low infestation in young cattle is related to maternal immunity transmitted during suckling, maternal grooming, management, and also because in young cattle, farmers avoid free grazing, while the reduced size of calves reduces thearea of contact with ticks [64,65].

Farm mechanization was an important preventive factor for high level of tick infestation. The odds ratio of high tick infestation on cattle from semi-mechanized (OR= 4.48) and non-mechanized (OR = 5.11) farms was higher, compared to mechanized farming. This result is consistent with studies in which farms furnished with cattle handling systems were at less risk of having a higher tick infestation than unequipped farms; this fact has also been observed in farms with poor facilities, where incorrect use of acaricides led to acaricide resistance [66,67].

The lack of paddocks for livestock feeding causes farmers to mobilize their animals to external paddocks. Although this practice helps to maintain the production of cattle,the use of external paddocks increased the risk for high tick infestation by 2.08 times,with regard to the farms that did not move animals outside the farm. Even though these paddocks can be owned or rented, in most cases they are rented for only few months of the year and not only to one farm, but to several farms during the year. Causing them to be used by different animals than can easily transport ticks between farms. Studies carried out by Heath in 2016 [68] and Zannou et al. in 2020 [69] found that herd movements around and between farms and pasture management can all also have a bearing on the presence of high level of tick infestation in the animals and in the occurrence or progress of TBDs.

The person who prepares the solution for the acaricide spray is a critical factor for the risk to present a high level of tick infestation. When the acaricide solution was prepared by the owner, there was a lower level of tick infestation (OR = 0.19) compared to farms where the spray solution was prepared by employees. This could be related to the differences in the level of education between owners and employees. Additionally, when there are training events, the person who attends is in most cases the owner. The insufficient knowledge and lack of training leads to under or over-dosing acaricides, which results indecreased acaricide efficacy and an increase in resistant tick populations [42,66,70].

Cattle husbandry is an important activity in the studied areas. However, the practiceof cattle raising as the main activity was a significant explanatory variable for the presence of high level of tick infestation (OR = 3.96). This fact could be associated with several causes; among them, cattle raising is an activity that has been inherited from generationto generation [71]; therefore, correct and incorrect knowledge about the use of acaricides have also been transmitted between generation, fostering resistance to the commonly used acaricides [11,21,42]. Dairy farmers in Ecuador do not have access to research or tools to implement an integrated tick management control, which is based on the appropriate combination of at least two control tools as animal management practices, selection of cattle breeds resistant to ticks, use of plant extracts, pasture management, vaccination, or biological control [39]. The implementation of these tools requires economic resources, which are in part limited because the lack of clear government policies for the establishmentof basic milk prices [72]. Likewise, innovative control strategies are needed, because

theareas studied here are ecologically vulnerable, so that the impact of acaricides may decrease the biodiversity in the zone.

In addition, capacitation programs on livestock management systems and tick control are needed. Their implementation will help farmers to make decisions that will improve livestock production. Knowing the number of animals that can be fed (system carrying capacity) from the farm's paddocks and when animals can enter the paddocks will help to improve meat and milk production, obtaining quality products using the farm's own resources [36,73]. Avoiding that, the deficit of pasture causes farmers to look for other grazing sites. From renting outside paddocks that increases the presence of a high level of infestation, to using entire landscape areas that are part of a forest and cause serious problems to biodiversity and soil stability [74].

Receiver operating characteristic (ROC) graphs are used in signal detection theory to depict the tradeoff between hit rates and false alarm rates of classifiers. This technique visualizes, organizes, and selects classifiers based on their performance [75,76]. At farm level the AUC-ROC of the OWS of high level of tick infestation was 0.72. This model, according to the Swets [76] scale is useful and can help to predict the potential level of tick infestation with an accuracy of 72.00%. Similarly, the sensitivity of the model prediction was very high for detecting farms with high levels of tick infestation. Indeed, if a farm hasan OWS under 11, it has a high probability of having a low level of infestation. However, the specificity of the model was relatively low (41.00%), but this problem can be solved by a visit to the farm by a veterinarian, who can give appropriate guidance for the control of infestation, taking into account the specific context of the farm.

4. Materials and Methods

4.1. Study Area and Sampling Design

This cross-sectional survey was part of the project entitled 'Socio-eco-epidemiology of ticks, tick-borne parasites, acaricide resistance and residual effects of acaricides in tropical Ecuadorian livestock: environmental, animal and public health impacts'. Sampling was conducted from November 2020 to March 2021 in two tropical regions of Ecuador. Area 1: Northwest of Pichincha Province in the Western Andean foothill, and Area 2: Quijos river valley in the Eastern Andean foothills.

Area 1 is located in the Northwest of Pichincha and is crossed by Chocó Andino of

Pichincha Biosphere Reserve [77]. The Northwest of Pichincha is located on the western slopes of the Andes Mountains and has several altitudinal floors and microclimates [78–80]. Area 2 is located in the province of Napo and is located in the middle of two conservation areas, i.e., Cayambe Coca National Park and Sumaco Napo Galeras National Park [81]. Quijos river valley is located between the foothills of the eastern Andes Mountains and high jungle of the Amazon region [82].

The selection was made using snowball sampling techniques, where, with the help of community leaders and authorities, the farms were selected with an emphasis on small and medium herds.

4.2. Investigation of Risk Factors Socio-Eco-Epidemiological Survey

To identify risk factors associated with high levels of tick infestation, an epidemiological questionnaire called 'Socio-eco-epidemiological survey of ticks and TBDs' was administered in each farm (Supplementary File S1). The survey was validated by nationaland international experts in the field. It was also pilot-tested on three farms in each area (one small, one medium, and one large) to ensure that farmers understood all the questions. The questionnaire was divided into

four parts: (A) farm general information and herd management; (B) tick and acaricides related information; (C) inputs, outputs, and labor force used in the farm; and (D) pharmacological inputs and farming practices. This survey consisted of a personal interview with the person who knows the most about farm management, irrespective of gender or age. The data were collected using the Epicollect-5 mobile application [83], except for part D which was collected in physical form.

4.3. Farms Selected

The average minimum distance between farms in Quijos river valley is 1.35 km (0.08–12.80 km), and in Northwest of Pichincha 4.34 km (0.34–24.69 km). The difference in distance between each zone is due to the fact that the farms in Quijos river valley are small and medium farms, and in Northwest of Pichincha there is a majority of medium and large farms.

According to the information collected in the survey, the farms were classified on the basis of the number of animals, as small (1 to 20 cattle), medium (21 to 70 cattle), and large (more than 70 cattle). The level of mechanization on the farm was classified using three criteria: infrastructures availability (corrals and cattle handling systems), the use of automatic or manual milking, and the usage of artificial insemination or natural services as reproduction method. A farm was considered mechanized if it met three criteria, semi-mechanized if meeting two, and non-mechanized if it only met one of the criteria.

The usage of external paddocks was considered if the paddocks used for cattle feeding were outside of the farm borders, regardless of whether the paddock was rented (paddocks of neighboring farms) or owned (paddocks of the same owner but in different locations). Paddock maintenance was defined according to two criteria: paddock topping (by scythe or machete) and paddock cleaning (feces removed). A farm performs paddock maintenance when it complies with one criterion.

Veterinary support was evaluated in four levels according to the presence of a veterinarian on the farm: permanent, sometimes, rarely, and never. The presence of a veterinarian permanently or sometimes was classified as the presence of veterinary support.

Knowledge on the presence of tick larvae in paddocks was a dichotomous answer 'yes' or 'no'. With respect to the correct knowledge of tick location in the grass, this was classified as 'yes' if the farmer knows that tick larvae are located on flowers and the pasture canopy.

To identify the acaricide treatment used on the farm, acaricides were classified according to their active ingredient and the chemical group. The acaricide currently used was identified by inspection of the place where the drugs were stored and, with the help of a list of the main trade names, the acaricides used by the farmers in the 12 months prior to the visit were determined. Once the group to which the acaricide belongs was identified, the form and frequency of application, dosage, animals treated, and efficacy of the product, among others, were investigated.

4.4. Animals Sampled

The farms visited did not use acaricides for 10 days prior to the visit. In each farm, at least five animals were randomly selected, irrespective of breed and age. An individual health record form and general information about identification of animals (name or number), age, sex, and breed and clinical information, including a physical examination, behavior of the animal, and notations regarding observed physiological abnormalities (e.g., neoplasms and injuries), and level of tick infestation were filled out for each animal. A total of 883 individual health records were obtained.

The animals were classified according to their age into young, young adult, and adults over 7 years old. A young adult animal is between 24 and 83 months. The category adults over 7 years old, corresponds to cattle that are over the optimal culling age [84,85]. According to body condition, the animals were classified as thin (BCS 1-2), good (BCS 3), and fat (BCS 4-5), according to the body condition scale (BCS), which evaluates body condition from 1 to 5 points [86].

The areas where the study was carried out are dairy zones, and beef production is not significant, so this variable was not taken into account.

Color of the animals was categorized according to the most common colors present in the zones (black-white, black, brown, red, and white). Regarding breed, this was not used, because in the study areas there are no pure breeds, but rather farms use hybrids in the attempt to raise milk production. For this reason, macro groups were used: *B. primigenious taurus, Crossbreed: B. primigenious taurus B. primigenious indicus,* and *B. primigenious indicus.*

4.5. Level of Infestation

The level of infestation of the animals was evaluated by a semi-quantitative visual inspection of the total bovine body (head, neck, back, loin, rump, arms, legs, ribs, chest, front flank, and udder) for approximately 8 min. The bovine body was divided into two parts (medial plane): right half and left half. Each half was subdivided into three zones: front third (from the head to the thoracic perimeter), the middle third (from the thoracic perimeter to the sacral bone), and the back third (from the sacral bone to the perineum).One-third was infested when it had 20 or more ingested females [87]. The presence of ticks in animals was rated in four levels: null, low, medium, and high. It was considered a low level of infestation if it was one-third infested, medium level of infestation two-thirds, andhigh level of infestation three-thirds infested.

4.6. Morphological Identification of Ticks

The tick samples were transported to the Entomology Unit of the Institute of Research in Zoonoses, located at Central University of Ecuador in Quito. For further morphological identification, the dichotomous keys and tick species descriptions of Guerrero [88] and Barros et al. [89] were used. The specimens were identified under stereomicroscope.

4.7. Statistical Analyses

All data from the questionnaire were exported to Microsoft Excel®, to be organized andcleaned. Inconsistencies across the data base were checked and verified by the interviewer and if necessary, the farmers were contacted by phone again. For the comparison of farmingand tick control practices used in the survey areas, Fisher's exact test was used; in this case the response variable was the survey area.

For the level of tick infestation, ordinal categorical levels (null, low, medium, and high) were assigned to a numerical scale from 0 to 3, and the average of the entire-rounded part of the five animal records with the infestation level was taken as a representation of the farm (level of tick infestation at the farm level). Farms with a score of 0 or 1 were referred to as having a low level of infestation, and with a score of 2 or 3 as having a high level of infestation. Tick infestation at the animal level was considered high when it was recorded as medium or high. Covariates with little or no variability were discarded from the analysis. Finally, a cleaned database was obtained with information of 826 animals (93.54% sampled),belonging to 139 farms (100.00% sampled).

At farm level, a univariate association test was applied, with 26 covariates. To identify the

covariates for the final model, a stepwise procedure was practiced with a multiple logistic regression using the step AIC function from the MASS package [90] in the R environment [91]. For the analysis of explanatory factors at the animal level, six variables were included (age, sex, breed, color, body condition status, and lactating dairy cows). The glmer function from the lme4 package in R environment [91] was used to incorporate both fixed-effect parameters and random effects (farm to which they belong) in a linear predictor, through maximum likelihood [92]. The risk or protective explanatory variables with a *p*-Value ≤0.05 were associated with a high level of tick infestation.

A multivariate logistic model was used to combine and to evaluate the covariates. Variance inflation factor (VIF) was used to determine the degree of multicollinearity between the covariates. Function VIF from the REGCLASS package [93] in R [91] was used. If the explanatory variables were not redundant, then the VIF was equal to 1, but when the VIF value was greater than 5, they suggested the existence of multicollinearity [94,95].

A scoring system was developed using odds ratios (ORs) for each covariate (risk explanatory variable) in the final model. Each covariate was evaluated by its OR, and its presence/absence was coded as 1 or 0. When an OR was significantly less than one (protective explanatory variable), reverse coding of this variable was performed, its absence was recorded as 1, and the weight was 1/OR. All risk and protective explanatory variables were weighted as a single weighted overall score (OWS) by farm (farm-level risk explanatory variables) and by animal (animal-level risk explanatory variables). The area under the receiver operating characteristic curve (AUC-ROC) was used to measure performance for the OWS classification. The Youden index and ROC curve analysis were obtained by using Stata SE 14.2 [96]; likewise, this was used to estimate the best cut-off point. The Youden index was calculated to evaluate the performance of OWS in farms with high and low tick infestation levels. This Youden index was defined as sensitivity + specificity-1 [93]. The Swets [76] scale was used to qualify the usefulness of the model.

5. Conclusions

In conclusion, the two zones studied in the tropical part of Ecuador, had the same proportion of farms with high tick infestation, but different cattle management systems. Zone 1 of Noroccidente de Pichincha is a zone with a long history of cattle raising, better mechanization, and larger herd sizes than zone 2. Zone 2 (Quijos river valley) is a zone with a more recent establishment of livestock raising, where most farms are small and medium sized, and although it has a lower level of mechanization, they receive more support from public veterinarians. High level of infestation depends on management practices (use of amitraz with growing resistance, who prepares the acaricide solution, veterinary support, and cattle husbandry as the principal activity), infrastructure present on the farm (level of mechanization on the farm), and the usage of farm external paddocks. These factors can be considered as exploratory variables, which suggests that farmers try to generate income by practicing cattle raising as their main activity, even near natural areas. However, bad habits and practices, and lack of mechanization on the farm, can cause the level of tick infestation to be high, which makes them look for new forms of control that do not solve the problem, but cause extra expenses for production. In addition, the model found some associated factors helping to predict a high level of infestation with high sensitivity, which can contribute in a useful way to decision making on control of tick infestation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pathogens11040403/s1, File S1: Socio-Epidemiological Survey.

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Institutional Review Board Statement: Due to the nature of the study and the low risk to participants, no formal Ethics Committee approval was required. All animals were treated with care, and the usual farm management of sample collection was followed, without mistreatment and ensuring animal welfare.

Informed Consent Statement: The farmers were properly informed and gave their written consent prior to sampling their animals.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon request.

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Conflicts of Interest: The authors declare no conflict of interest.

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7. Supplementary File

S1: SURVEY SOCIO ECO EPIDEMIOLOGICAL SURVEY: TICKS AND TBDs

SOCIO ECO EPIDEMIOLOGICAL SURVEY: TICKS AND TBDs
UNIVERSITY OF LIEGE & CATHOLIC UNIVERSITY OF LOUVAIN
CENTRAL UNIVERSITY OF ECUADOR
Zoonosis Research Institute - CIZ

The researchers request their authorization to participate in the research project entitled: Socio-eco-epidemiology of ticks, tick-borne parasites, resistance to acaricides and residual effects of acaricides in the Ecuadorian tropical cattle: impacts on the environment, public health, and animal health. This project consists of Applying a socio-eco-epidemiological survey. Collect ticks on the animal. Extract blood samples from cattle. On certain farms, collect larvae of ticks, samples of urine, faeces, milk, and soil. The project is committed to delivering respective results once the samples have been analyzed.

INTRODUCTORY SECTION

Date: / / **Surveyor:** _____ **Survey Number:** _____
GPS (gg/mm/ss): **latitude** **longitude** **Altitude (masl):**
Farm Name: **Name of the Owner:**
Province: Pichincha ☐ Napo ☐ **Canton:** S. M. Bancos ☐ Pedro Vicente Maldonado ☐ Quito ☐ El Chaco ☐ Quijos ☐
Parish: Bancos ☐ PVM ☐ Nanegal ☐ Nanegalito ☐ Gualea ☐ Pacto ☐ Baeza ☐ Borja ☐ Sardinas ☐ El Chaco ☐
Locality: _____
Name of respondent: _____ **Age:**_____**Gender:** F ☐ M ☐ **Phone:** / / / / / / / / / /
Education level: No formal education ☐ Primary ☐ High School ☐ University ☐ **Profession/Activity:** _____

SECTION A: FARM GENERAL INFORMATION

1. **What is the total area of the farm? (hectares)**

Use	Quantity	Unit (m/ha)
House		
Cattle pen		
Pastures		
Crops		

Use	Quantity	Unit (m/ha)
Forests within the property		
Temporary fallow		
Other:		

2. **Are there forests outside the property?**
   Yes ☐ No ☐ How far are they from the property? _____
3. **Under what condition of possession does this property consist of?**
   Own ☐ Co-owner ☐ Rented ☐ Loan ☐ Other:_____
4. **Is the owner of the farm a member of any association?**
   Yes (which) _____No ☐
5. **Which is the milking mode?**
   Manual ☐ Mechanical ☐
6. **What is your method of reproduction?**
   Natural mating ☐ Artificial insemination ☐
7. **Do you use poultry manure or other organic fertilizer as soil fertilizer?** Yes ☐ No ☐
   Chicken manure Yes ☐ No ☐
   Mycorrhizae Yes ☐No ☐
   Biol Yes ☐ No ☐
   The biol used is: Prepared by you ☐ Bought ☐
8. **What is fertilized with organic fertilizer?**
   Crops ☐ Pastures ☐
9. **Are there any crops grown on your farm?**
   Yes ☐ No ☐
10. **What is the number of varieties grown?**
    One ☐ More than one ☐

11. Type of crops: Temporary crops ☐
Permanent crops ☐ Home and backyard gardens ☐

12. Crops

N°	Cultivated species	Area (ha)	HARVEST			DESTINATION		
			Quantity	Unit	Frequency	Self-consumption	For sale	Price (lb)

13. Type of pastures
Grazing pastures Yes ☐ No ☐

Type of pasture	Area (ha)	Grazing system	Bovine category in the pasture	N animals	Paddock occupancy (days)	Paddock rest (days)

Cutting pastures Yes ☐ No ☐

Type of pasture	Area (ha)	Cattle category consuming it	Where is the grass consumed?	How is the grass consumed?

14. In which pasture have you perceived that there is a greater presence of grackles?_____

15. Where in the pasture are the tassels located?
Stem base ☐ Stem ☐ Leaf intermediate part ☐ Leaf tip ☐ Flower ☐ Don't know ☐

SECTION A: HERD COMPOSITION

16. Total cattle inventory:

Category	N°	Category most affected by ticks
Female calves (<1 year)		
Male calves (<1 year)		
Heifers (13-18 months)		
Heifers (19 months-1 lactation)		
Dry cows		
Lactating cow		
Steers (>1 year)		
Bullocks (>1 year)		
Breeding bulls (>1 year)		
TOTAL		

17. Other types of animals on the farm

Species	Quantity
Buffalo	
Horses	
Mules	
Rabbits	
Guinea Pigs	
Hens	

18. Buy replacement animals? Yes ☐ No ☐

19. Annual replacement animals

Bovines	N°	Origin *	Price ($/animal)
Female calves (<1 year)			
Male calves (<1 year)			
Heifers (13-18 months)			
Heifers (19 months-1 lactation)			
Dry cows			
Lactating cow			
Steers (>1 year)			
Bullocks (>1 year)			
Breeding bulls (>1 year)			

20. Production parameters

Parameter	Quantity	Unit
Months of lactation (average)		Months
Milk production in Dry Season		Lt/cow/day
Milk production in Rainy Season		Lt/cow/day
Average number of cows milked per month		cows/month
Calving interval		Days

21. Is there a decrease in production of dairy cows with ticks?
No ☐ Yes ☐ Number of liters_____

22. Productive Parameters (In case of beef cattle or double purpose

Parameter	Quantity	Unit
Months of fattening (average)		Months
Average weight at deboning		Pounds
Average number of animals slaughtered annually		Animals

23. In fattening cattle with ticks, is there a decrease in growth or weight loss?
No ☐ Yes ☐ At what age and in what time do they reach selling weight? Age:_____ Weight at time of sale:____

SECTION B: TICK AND ACARICIDES RELATED INFORMATION

24. Are there other paddocks outside the farm limits? No ☐ Yes ☐

25. These paddocks are? Own ☐ Rented ☐

26. Are the animals moved to these paddocks?

27. Constantly throughout the year ☐ In certain months ☐ What months? _____

28. What category of animals are mobilized to these paddocks? _____

29. What kind of grass exists in these paddocks? _____
After the cattle return from these paddocks, do they have more or less ticks? More ☐ Less ☐

30. How long do the animals spend in these paddocks? _____

31. Do you perform veterinary support? Permanent ☐ Sometimes ☐ Never ☐ Hardly ever ☐

32. The veterinarian that provides technique attendance on your farm is? Private ☐ GAD ☐ MAG ☐

33. Who prescribes the treatment?
Owner ☐ Veterinarian ☐ Agricultural warehouse salesperson ☐ Farm steward ☐
Workers☐ Pharmaceutical Company ☐ Other_____

34. Who applies the treatments?
Owner ☐ Veterinarian ☐ Farm steward ☐ Workers☐ Other_____

35. Which of these diseases have you observed in your livestock?

Illness	N° animals affected/year	Mortality		Categories of cattle concerned
		No	%	
Internal parasitosis				
Tick fever				
Abortion				
Mastitis				
Metritis				
Placental retention				
Milk fever				
Dystocic labor				
Pneumonia				
Tympanism				
Myiasis				
Dermatobia hominis				
Abscess				
Lameness				
Heat stress				

36. What do you think is the degree of affection that ticks cause in your animals?
None☐ Low ☐ Medium☐ High☐

37. **Do you think the presence of ticks causes economic losses in your herd?** No ☐ Yes ☐

38. **Did you know that ticks can transmit disease? No ☐ Yes ☐ What diseases?**

39. **Which breeds of animals on your farm have been the most affected by ticks?** Breed: _____ Colour: _____

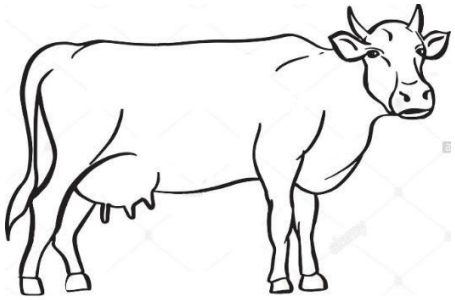
40. **Do you identify any of them?**

Rhipicephalus microplus

Amblyomma cajennense

Ixodes boliviensis

41. **In which third of the bovine body are ticks most frequent?**
Front third ☐ Middle Third ☐ Back Third ☐ All ☐



42. **When are there more ticks on the cattle on your farm?**
Rainy season ☐ Dry season ☐ All year round ☐
Specify in which months: _____

43. **Do you use bath spray for tick control on your livestock?** No ☐ Yes ☐

44. **Do you use chemical acaricides to control ectoparasites in livestock on your farm?** No ☐ Yes ☐

45. **Do you calibrate the spraying equipment?** No ☐ Yes ☐
Every so often?_____

46. **Who prepares the solutions with the acaricide?** Veterinarian ☐ Owner ☐ Workers ☐

47. **What water do you use to dissolve the acaricide?**
Drinking water☐ Irrigation water☐ River ☐ Bottled ☐ Bottled water ☐

48. What are the rotation dynamics of the acaricides used?_____

---

49. **Which of the following acaricides do you use or have you used on your farm?**

Organophosphates:

Trade name		Frequency of use		Application		This product was			
Nuvan	☐	Less than one month	☐	In immersion baths	☐	Lately used	☐		
Neguvon	☐	Between 1 and 2 months	☐	Bath spray	☐	Used some time ago	☐		
Asuntol	☐	Between 3 and 6 months	☐	Injected	☐	Used a long time ago	☐		
Diclorvos	☐	More than 6 months	☐	Pour on	☐	**Treated animals**			
Garafos	☐	**Dosage:**		Wipe cloth	☐	Only those affected	☐	All	☐
Matanuche	☐	**Duration of Tto.**		**Withdrawal time (days)**		Dry cows	☐	Calves	☐
		Efficacy (%)		Milk_____ Beef____		Lactating cows	☐	Bulls	☐

What do I use before this product?_____ **How long did you use it?**_____

Pyretroides:

Trade name		Frequency of use		Application		This product was			
Virkos	☐	Less than one month	☐	In immersion baths	☐	Lately used	☐		
Garrakill	☐	Between 1 and 2 months	☐	Bath spray	☐	Used some time ago	☐		
Abamectin	☐	Between 3 and 6 months	☐	Injected	☐	Used a long time ago	☐		
Garrapaticin	☐	More than 6 months	☐	Pour on	☐	**Treated animals**			
Cipermetrin	☐	**Dosage:**		Wipe cloth	☐	Only those affected	☐	All	☐
Bayticol	☐	**Duration of Tto.**		**Withdrawal time (days)**		Dry cows	☐	Calves	☐
Butox		**Efficacy (%)**		Milk_____ Beef____		Lactating cows	☐	Bulls	☐

What do I use before this product?_____ **How long did you use it?**_____

Amides:

Trade name		Frequency of use		Application		This product was			
Fulminado	☐	Less than one month	☐	In immersion baths	☐	Lately used	☐		
Amitraz	☐	Between 1 and 2 months	☐	Bath spray	☐	Used some time ago	☐		
Parex	☐	Between 3 and 6 months	☐	Injected	☐	Used a long time ago	☐		
Toril	☐	More than 6 months	☐	Pour on	☐	Treated animals			
Bovitraz	☐	Dosage:		Wipe cloth	☐	Only those affected	☐	All	☐
Effitick	☐	Duration of Tto.		Withdrawal time (days)		Dry cows	☐	Calves	☐
Singap		Efficacy (%)		Milk_____ Beef____		Lactating cows	☐	Bulls	☐

What do I use before this product?_____ How long did you use it?_____

Macrolitic Lactone:

Trade name		Frequency of use		Application		This product was			
Bovimec	☐	Less than one month	☐	In immersion baths	☐	Lately used	☐		
Ivermic	☐	Between 1 and 2 months	☐	Bath spray	☐	Used some time ago	☐		
Ivermec-JB	☐	Between 3 and 6 months	☐	Injected	☐	Used a long time ago	☐		
Vermectin	☐	More than 6 months	☐	Pour on	☐	Treated animals			
Doramectina	☐	Dosage:		Wipe cloth	☐	Only those affected	☐	All	☐
Doramectin	☐	Duration of Tto.		Withdrawal time (days)		Dry cows	☐	Calves	☐
		Efficacy (%)		Milk_____ Beef____		Lactating cows	☐	Bulls	☐

What do I use before this product?_____ How long did you use it?_____

Fluazuron:

Trade name		Frequency of use		Application		This product was			
Acatak	☐	Less than one month	☐	In immersion baths	☐	Lately used	☐		
_____	☐	Between 1 and 2 months	☐	Bath spray	☐	Used some time ago	☐		
_____	☐	Between 3 and 6 months	☐	Injected	☐	Used a long time ago	☐		
_____	☐	More than 6 months	☐	Pour on	☐	Treated animals			
		Dosage:		Wipe cloth	☐	Only those affected	☐	All	☐
		Duration of Tto.		Withdrawal time (days)		Dry cows	☐	Calves	☐
		Efficacy (%)		Milk_____ Beef____		Lactating cows	☐	Bulls	☐

What do I use before this product?_____ How long did you use it?_____

Fipronil:

Trade name		Frequency of use		Application		This product was			
Iveryl	☐	Less than one month	☐	In immersion baths	☐	Lately used	☐		
Ectonil	☐	Between 1 and 2 months	☐	Bath spray	☐	Used some time ago	☐		
_____	☐	Between 3 and 6 months	☐	Injected	☐	Used a long time ago	☐		
_____	☐	More than 6 months	☐	Pour on	☐	Treated animals			
_____	☐	Dosage:		Wipe cloth	☐	Only those affected	☐	All	☐
		Duration of Tto.		Withdrawal time (days)		Dry cows	☐	Calves	☐
		Efficacy (%)		Milk_____ Beef____		Lactating cows	☐	Bulls	☐

What do I use before this product?_____ How long did you use it?_____

Amides+Pyretroides:

Trade name		Frequency of use		Application		This product was			
Ectogan	☐	Less than one month	☐	In immersion baths	☐	Lately used	☐		
Ectosul	☐	Between 1 and 2 months	☐	Bath spray	☐	Used some time ago	☐		
_____	☐	Between 3 and 6 months	☐	Injected	☐	Used a long time ago	☐		
_____	☐	More than 6 months	☐	Pour on	☐	Treated animals			
_____	☐	Dosage:		Wipe cloth	☐	Only those affected	☐	All	☐
		Duration of Tto.		Withdrawal time (days)		Dry cows	☐	Calves	☐
		Efficacy (%)		Milk_____ Beef____		Lactating cows	☐	Bulls	☐

What do I use before this product?_____ How long did you use it?_____

Organophosphates + Pyretroides:

Trade name		Frequency of use		Application		This product was			
Derribante	☐	Less than one month	☐	In immersion baths	☐	Lately used	☐		
Impacto	☐	Between 1 and 2 months	☐	Bath spray	☐	Used some time ago	☐		
_____	☐	Between 3 and 6 months	☐	Injected	☐	Used a long time ago	☐		
_____	☐	More than 6 months	☐	Pour on	☐	Treated animals			
_____	☐	Dosage:		Wipe cloth	☐	Only those affected	☐	All	☐
		Duration of Tto.		Withdrawal time (days)		Dry cows	☐	Calves	☐
		Efficacy (%)		Milk_____ Beef____		Lactating cows	☐	Bulls	☐

What do I use before this product?_____ How long did you use it?_____

Organophosphates + Pyretroides+ Fipronil:

Trade name		Frequency of use		Application		This product was	
Biorvoss	☐	Less than one month	☐	In immersion baths	☐	Lately used	☐

_____	☐	Between 1 and 2 months	☐	Bath spray	☐	Used some time ago	☐
_____	☐	Between 3 and 6 months	☐	Injected	☐	Used a long time ago	☐
_____	☐	More than 6 months	☐	Pour on	☐	**Treated animals**	
		Dosage:		Wipe cloth	☐	Only those affected ☐	All ☐
		Duration of Tto.		**Withdrawal time (days)**		Dry cows ☐	Calves ☐
		Efficacy (%)		Milk_____ Beef____		Lactating cows ☐	Bulls ☐

What do I use before this product?_____ **How long did you use it?**_____

Fipronil+Fluazuron:

Trade name		**Frequency of use**		**Application**		**This product was**		
Virkos	☐	Less than one month	☐	In immersion baths	☐	Lately used	☐	
Garrakill	☐	Between 1 and 2 months	☐	Bath spray	☐	Used some time ago	☐	
Abamectin	☐	Between 3 and 6 months	☐	Injected	☐	Used a long time ago	☐	
Garrapaticin	☐	More than 6 months	☐	Pour on	☐	**Treated animals**		
Cipermetrin	☐	**Dosage:**		Wipe cloth	☐	Only those affected ☐	All	☐
Bayticol	☐	**Duration of Tto.**		**Withdrawal time (days)**		Dry cows ☐	Calves	☐
Butox		**Efficacy (%)**		Milk_____ Beef____		Lactating cows ☐	Bulls	☐

What do I use before this product?_____ **How long did you use it?**_____

50. Where do you purchase used acaricides? Visitor ☐ Informal market ☐ Agricultural warehouse ☐

51. Products used have sanitary registration (Verification) No ☐ Yes ☐

52. Do you use any alternative methods for tick control? No ☐ Yes ☐

Use of birds, insects and/or fungi	☐	Flooding of pastures	☐
Pasture rotation	☐	Burning of pastures	☐
Baths with medicinal plants	☐	Transhumance	☐
Planting repellent plants	☐	Spraying of acaricides on pastures	☐
Use of burned oil (Kerosene)	☐	Vaccination	☐
Crossbreeding of breeds	☐	Baths with Sulphur	☐

Products used: Brief description and frequency of the method used:

SECTION C: INPUTS, OUTPUTS, AND LABOR FORCE USED IN THE FARM

53. What is your farm's cattle production system? Milk ☐ Meat ☐ Mixed or Double Purpose ☐

54. Destination of milk production

Destination	Quantity (lt)	Frequency of sale	Price ($/lt)
Regional dairy plant			
Local dairy plant			
Cheese factories			
Farm industrialization			
Calf rearing			NOT APPLICABLE
Human consumption			NOT APPLICABLE
Direct sale			
Other destination:			

In case of industrialization on the property:

Product	Quantity of milk used (lt)	Nº Product obtained	Frequency of production	Sale price ($/Unit)

55. Sale of Beef Cattle

Cattle	Nº of cattle sold	Frequency of sale	Average weight at sale (kg)	Destination	Price ($/Unit)
Calf for fattening					
Bullocks					
Steer for fattening					
Replacement cows/heifers					
Discard cows					
Discard bulls					

56. In case you have pigs/poultry/fish, answer:
Pigs

Category	Sale of live animals			Sale of live animals to a broker			Sale of slaughtered animals to the consumer			Sale of slaughtered animals to butcher shops		
	N°	Frequency	$/Unit	N°	Frequency	$/Unit	N°	Frequency	$/Unit	N°	Frequency	$/Unit
Piglets												
Sows												
Fattening pigs												
Boars												

Poultry

Category	Sale of live animals			Sale of live animals to a broker			Sale of slaughtered animals to the consumer			Sale of slaughtered animals to butcher shops		
	N°	Frequency	$/Unit	N°	Frequency	$/Unit	N°	Frequency	$/Unit	N°	Frequency	$/Unit
Layers												
Breeders												
Fattening												

Aquaculture

N° of fish harvested (monthly)	Self-consumption	Sale	
	lb	lb	($/lb)

57. Is there family labor for farm work? No ☐ Yes ☐

58. Family composition and occupation

Family relationship	Age (years)	Sex (F/M)	Main activity on the farm	Frequency		Payment for activities in the farm		Activities outside the farm	
				Hours/day	Days/week	Yes/No	($/Frequency)	Yes/No	($/Frequency)

59. Are the workers on your farm? Occasional ☐ Permanent ☐

60. Compensation of workers

N° of workers	Hours/Day	Days /Month	Pay ($/Frequency)	Extra bonus

61. Purchase of food supplement

Supplement	Quantity	Frequency	Cost ($/Unit)
Balanced cows			
Calf feed			
Overfeed			
Calf substitute			
Mineral salts			
Banana rejection			
Palm heart rejection			
Silo			
Hay			
Other.....			

62. Annual farm costs

Item	Quantity	Estimated Value ($/Unit)	Years of useful life	Years in use	Maintenance cost ($/Frequency)
Milking equipment					
Cooling tank					
Corrals and handling chutes					
Input storage					
Electric fence					

Tractor					
Irrigation equipment					
Fumigation pump					
Scythe					
Grinder					
Biodigester					
Water pump					
Others:					

63. Payments to third parties

Item	Cost	Frequency
Electricity		
Water		
Mobilization		
Laboratory services		
Land/pasture lease payment		
Payment of agricultural loans		

64. Have there been any cattle thefts on the farm this year? No ☐ Yes ☐ Heads per year:_____

65. What were the changes in your livestock and/or agricultural system due to the Covid-19 pandemic?

66. Based on the practices, this exploitation is: Extensive ☐ Intensive ☐ Semi intensive ☐

SECTION C: PHARMACOLOGICAL INPUTS AND FARMING PRACTICES

67. Farming Practices

Activity	Nº of animals	Frequency	Persons performing the activity			Time spent (Hours/Frequency)	Payment
			Sex	Who?	Nº of people		
LIVESTOCK MANAGEMENT							
Cattle branding/marking							
Dehorning							
Castration							
Weight registration							
Herding cattle							
Milking cows							
SANITARY CONTROL							
Acaricide control: bath spray							
Acaricide control: manual removal of ticks							
Acaricide control: wipe-cloth							
Acaricide control: inyection							
Deworming							
Vaccination							
Diagnosis/treatment of disease (Vet. care)							
REPRODUCTIVE ACTIVITIES							
Insemination							
Synchronize heats							
Pregnancy test (palpation)							
Pregnancy test (ultrasound)							
Other							
INSTALLATION MAINTENANCE							
Raising fences	NA						
Cleaning of corrals	NA						
Cleaning of paddocks	NA						
Cleaning of milking parlor	NA						
FEEDING	NA						
Silage	NA						
Haymaking	NA						
Cut and carry pasture	NA						
Offer feed/by-products							

Feeding calves							
Other:							

68. Purchase of inputs.

Input	Trade name	Quantity	Frequency	Cost ($/unit)
Detergents				
Sealants				
Mastitis Test				
Drying towels				
Semen				
Analgesic				
Antibiotics				
Sanitizers				
Healing products				
Hormones				
Minerals				
Vaccines				
Vitamins				
Internal dewormers				
External dewormers				
Restoratives (serums)				
Fertilizers				
Phytosanitary products				

Experimental section

Study 2

An economic evaluation of cattle tick acaricide-resistances and the financial losses in subtropical dairy farms of Ecuador: A farm system approach

PLOS ONE 2023 18(6): e0287104.

Valeria Paucar, Ximena Pérez-Otáñez, Richar Rodríguez-Hidalgo, Darío Cepeda-Bastidas, Cecilia Perez, Jorge Grijalva, Sandra Enríquez, Susana Arciniegas-Ortega, Luis Sandoval-Trávez, Bryan Benavides-Erazo, Sophie O. Vanwambeke, Claude Saegerman, Lenin Ron-Garrido

Preamble

Controlling tick infestations in cattle populations is crucial for managing livestock effectively, as it helps reduce negative impacts on animal health, productivity, and welfare. However, the indiscriminate and prolonged use of acaricides has led to resistance among tick populations. Treatment and control measures for tick infestations also incur additional costs, including the purchase of acaricides, labour force for application, and veterinary services. The objective of this study was to perform an economic analysis of the farms under study and determine what percentage of the costs incurred on the farms is dedicated to tick control.

PLOS ONE

RESEARCH ARTICLE

An economic evaluation of cattle tick acaricide-resistances and the financial lossesin subtropical dairy farms of Ecuador: A farmsystem approach

Valeria Paucar-Quishpe [1,2], Ximena Pérez-Otáñez [1,3], Richar Rodríguez-Hidalgo [1,4], Darío Cepeda-Bastidas [5],Cecilia Perez-Escalante [4], Jorge Grijalva-Olmedo [4], Sandra Enríquez [1], Susana Arciniegas-Ortega [6], Luis Sandoval-Trávez [4], Bryan Benavides-Erazo [5],Sophie O. Vanwambeke [3], Claude Saegerman [2,☯,*], Lenin Ron-Garrido [1,4,☯,*]

1 Instituto de Investigación en Zoonosis (CIZ), Universidad Central del Ecuador, Quito, Ecuador, **2** Research Unit of Epidemiology and Risk Analysis applied to Veterinary Science (UREAR-ULg)/Fundamental and Applied Research for Animals & Health (FARAH) Center/Faculty of Veterinary Medicine, University of Liege, Liège, Belgium, **3** Georges Lemaitre Centre for Earth and Climate Research, Earth & Life Institute, UCLouvain, Louvain-la-Neuve, Belgium, **4** Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, Quito, Ecuador, **5** Facultad de Ciencias Agrícolas, Universidad Central del Ecuador, Quito, Ecuador, **6** Facultad de Geología, Minas y Petróleo, Universidad Central del Ecuador, Quito, Ecuador

☯These authors contributed equally to this work.
* claude.saegerman@uliege.be (CS); ljron@uce.edu.ec (LRG)

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Abstract

Estimates of economic losses in cattle due to tick infestations in subtropical areas are limited, such as in Ecuador. Ticks affect animal production and health, but those direct effects are difficult to estimate since financial exercises carried out in farms consider both costs of the inputs and revenues. This study aims to quantify the costs of inputs involved in milk production and to know the role of acaricide treatment in the production costs on dairy farms in subtropical zones using a farming system approach. Regression and classification trees were used to study the relationship between tick control, acaricide resistance and the presence of high level of tick infestation in the farm system. Even though there was no significantdirect association between high levels of tick infestation and the presence of acaricide resistance in ticks, a more complex structure for resistances operates in the manifestation of high tick infestation involving levels of farm technology and no acaricide resistance. Farms with higher levels of technology allocate a lower percentage of sanitary expenses to control ticks (13.41%) in comparison to semi-technified (23.97%) and non-technified farms (32.49%). Likewise, more technified and bigger herds have a lower annual expenditure on acaricide treatment (1.30% of the production budget equivalent to 8.46 USD per animal) compared to non-technified farms where it can represent more than 2.74% of the productionbudget and where the absence of cypermethrin resistance increases the treatment cost to 19.50 USS per animal annually. These results can motivate the development of information campaigns and control programmes targeted to the reality of small and medium farms that are the most affected in terms of the money they invest in controlling ticks.

1. Introduction

Ticks affect almost 80% of the world's cattle population in tropical and subtropical areas. These ectoparasites cause impacts on animal production and health, either through the effect of their bites, which cause anaemia when high parasite loads are present or through the transmission of pathogens [1]. In addition, there are economic losses associated with control of tick infestations or tick-borne diseases' (TBDs) treatment; and the revenue not perceived due to reduced meat or milk production are part of the indirect costs [2, 3]. In Ecuador, approximately 6.15 million litres of raw milk are produced daily, according to the National Institute of Statistics (INEC) [4]. Of them, herds located in tropical and subtropical areas produce around 1.5 million litres of milk/day (25% of national production), and most of them belong to small size farms [4, 5]. Livestock production in those areas is very suitable for tick infestation because of the humid tropical climate, the use of specialised dairy breeds, and sowing of pastures of short-cycle as the primary feed source [6, 7].

On the other hand, nowadays, the main usual tool for tick control is the use of chemical acaricides. In Ecuador, the most commonly used acaricides for tick control are amitraz, ivermectin, and alpha-cypermethrin [8]. However, other acaricides based on organophosphates, fluazuron, fipronil, and other ingredients are also available in the market. There are also various forms of application and presentations of these products, such as fluid products or wetpowder that are diluted and sprayed on animals. Pour-on or injectable control methods are easy to use but are expensive or generate residues in milk and meat for several weeks after [9– 11]. Likewise, incorrect use of these pesticides, such as under dosing, inadequate preparations, and misapplications, can lead to treatment failure. Added to this, the exclusive strategy of chemical control may be inadequate due to the development of tick resistance [12, 13]. Various levels of presence of resistance in *Rhipicephalus microplus* were observed to amitraz [14, 15], alpha-cypermethrin, and ivermectin [15] in some producing areas of the Ecuadorian Coast.

Estimations of the economic losses due to tick infestation, especially in tropical endemic areas, are scarce. The main strategies used to find the effects are obtained from specific experiments designed to evaluate tick load versus reduced milk or meat production; or from studies that produce specific data on system components that are then used in simulation models to predict those losses. An example of this is the study by Jonsson (2006) [16], who estimated that; on average, each engorged female tick is responsible for the loss of 1.37 g of body weight in *Bos taurus* cattle. Similarly, the comparable value for *B. taurus* × *B. indicus* cattle is 1.18 g. Other studies have also observed that in tick-infested animals, each engorged female tick was responsible for a decrease of 8.9 ml of daily milk production and 1.0 g of body weight [17].

Another approach to estimate losses is the economic systems approach, in which all production traits and their interactions are controlled simultaneously. The farming systems approach is useful for quantifying the effects of tick control, as the determinants of tick control effects are extremely complex. Indeed, Ocaido et al. (2009) [18] in Uganda estimated that costs for controlling ticks and TBDs constitute between 73.8% and 85.6% of total disease control costs, with tick control alone accounting for between 83.1% and 87.9% of those costs. In this sense, in general, production costs are directly influenced by treatment costs and by farm care on the health status of animals, and such parameters are related to farm profitability.

The present study focused on cattle milk production in subtropical areas of Ecuador. The aim of this study was to estimate the costs of inputs involved in cattle milk production in order to evaluate the influence of acaricide resistance in the components of the production costs on cattle farms. This information will support and allow the development of information campaigns that will help to raise awareness among farmers and target key messages on tick control guidance. It will also allow the creation of tick control programs focused on the reality of small producers in ecologically vulnerable subtropical areas since the main reasons for the failure or lack of sustainability of control programs have been the economic limitations of the farmers

[19]. Control program guidelines will help the producer optimise costs, maintaining a low tickinfestation level that does not decrease livestock production.

2. Materials and methods

Ethics statement

Due to the nature of the study and the low risk to participants, no formal Ethics Committee approval was required. All animals were treated with care, and the usual farm management of tick collection was followed without mistreatment and ensuring animal welfare. The farmers were properly informed and gave their written consent before starting tick collection from their animals. The survey collected on each farm was coded with numbers and letters according to the farm and area visited.

Study area and sampling design

This study is part of the project "Socio-eco-epidemiology of ticks, tick-borne parasites, acaricide resistance and residual effects of acaricides in Ecuadorian tropical livestock: environmental, animal and public health impacts"; and focuses on the estimation of economic losses caused by tick infestation. Between November 2020 and March 2021, 139 dairy cattle farms were visited in two sub-tropical areas of Ecuador located in the occidental and oriental foothills in the Ecuadorian Andes, one is in the Quijos River Valley, and the other is in the northwest of Pichincha province. Both places are zones of dairy production and are near to rainforest biodiversity reserves. Due to the absence of a sampling frame, the farms were selected using the snowball sampling technique [20]. However, only 105 farms were considered for this study because they had the necessary information to estimate all the production costs.

Economic analysis

To analyse the information and identify the revenues and expenses of the cattle farms, the data of an entire year was collected through a previously validated survey using the Epicollect-5 [21] mobile application. The survey was previously validated in the field and by national and international experts. In addition to this, on each farm, the storage place of veterinary drugs was visited. A record of the drugs used, presentation, price, and quantity used for one year was filled out on each farm. The economic data are expressed in US dollars (USD) and correspond to the 2020–2021 fiscal period.

Expenses

The costs used to determine the total cost of livestock production are described in Fig 1.

Costs were classified as variable (VC) and fixed (FC). In addition to the costs described in Fig 1, information on farming loans was available. However, this information was not taken into account since this cost was included in economic costs E9 and E17.

$$VC = E_1 + E_2 + E_3 + E_4 + E_5 + E_6 + E_7 + E_8 + E_{10} + E_{11} + E_{12} + E_{17} \quad \text{(Eq1)}$$

$$FC = E_9 + E_{13} + E_{14} + E_{15} + E_{16} + E_{18} \quad \text{(Eq2)}$$

The total livestock production cost (TC) corresponds to fixed plus variable costs. Two scenarios were considered, one without the cost of subsidised veterinary services (TCA) and one with subsidized veterinary services (TCB). It was necessary to consider this division given the fact that local autonomous governments use to support farmers with veterinarians or veterinary technicians. These services are free of cost and are available to farmers who wish to use them. There are several methodologies to calculate the cost of production of a litre of raw milk.

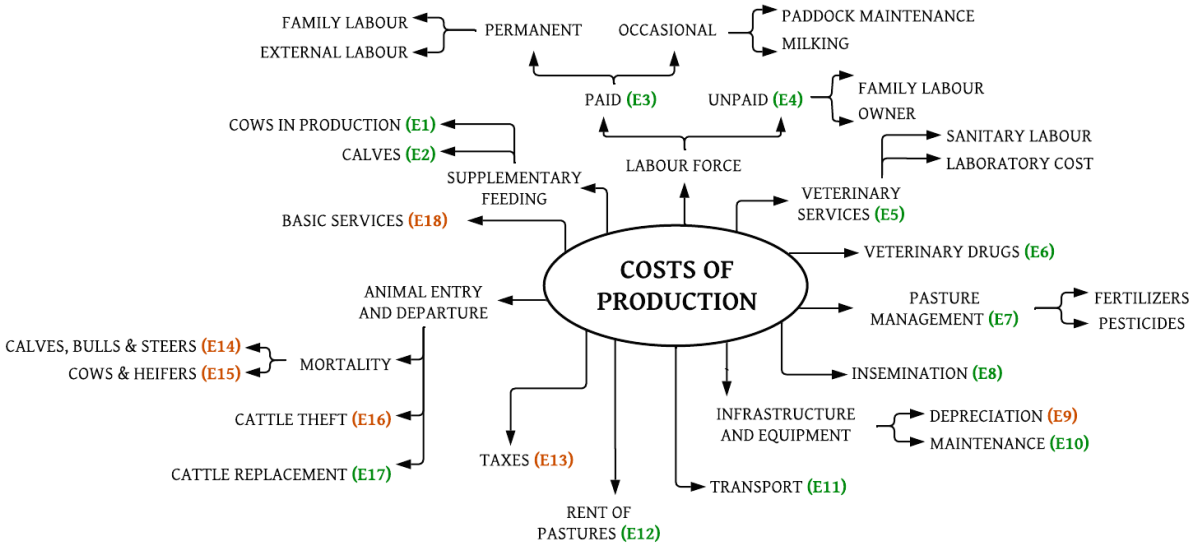

Fig 1. Costs of production associated with milk production in the study area. E1 to E18 are a codification of costs used in the different equations (seeafterward). The green coding corresponds to variable costs, and the red coding to fixed costs.

https://doi.org/10.1371/journal.pone.0287104.g001

However, this study is based on the actual costs directly related to milk production throughout the year (costs of cows and heifers) [22, 23]. For the calculation of the total cost of milk production (TCM), calf feeding costs (E2), mortality (E14, E15), and cattle theft (E16) were excluded (red coded in Eqs 1 and 2), as data on the purchase of replacement animals were used instead. The lack of animals due to mortality is reflected in the decrease in litres of milk delivered and animals sold. Scenario A was used for this calculation.

Supplementary feeding (E1 and E2). Livestock feed includes all costs related to supplementation by concentrate, silage, hay, and by-products (beer bran, molasses); also, mineral salts received by cows in production at milking time. In calves, supplementary feeding includes the cost of concentrate and milk replacer if purchased.

Labour force (E3 and E4). The cost of the labour force includes both paid (E3) and unpaid (E4) labour. Paid labour can be permanent or occasional. Permanent paid labour included workers who perform daily activities during a specific number of hours and receive a monthly salary, and family members receiving compensation.

Occasionally, the paid labour force performs certain activities such as milking or paddock maintenance. Occasional personnel employed for milking are also called "permanent occasional" since they only come daily for milking. They are paid daily, weekly, biweekly or monthly. The occasional labour force is employed for paddock maintenance operations such as equalisation cuts or paddock cleaning. This service is performed for one week or one month per year, and they are generally paid just for their service, regardless of the time used. Then, based on the number of days and hours of work, the payment for one-day work (8 hours per day) was determined.

In the case of the unpaid owner and family labour, the opportunity cost of a permanent day's work in the study areas was used. The number of family workers employed was estimated based on the literature. One person is required to milk 18 animals (manual milking) or 25 animals in the case of mechanical milking [24].

Veterinary services (E5). Both real and state-subsidized costs were considered. In the case of state-subsidized veterinarians, the average price of visiting private veterinarians, as reported by respondents, was considered as the opportunity cost assigned.

Veterinary drugs (inputs) (E6). The cost of veterinary supplies includes antibiotics, anti-inflammatories, antiseptics, sanitisers, hormones, vaccines, repellent spray, hemo-parasiticides, vitamins, and minerals (application parenteral), and inputs used in milking (teat sealants, detergents for milking equipment, among others), internal parasiticides and acaricides. The cost for acaricides includes acaricides used parenterally or topically (spray or pour-on).

The price of each drug was collected and consolidated in three different ways. 1. Hard paper survey carried out during fieldwork. 2. Retail price obtained from the catalogue of each distribution company. 3. Interviews at agricultural warehouses in the study zones (8 agricultural warehouses were visited in each zone). The interviews in agricultural warehouses and catalogues consultations were carried out to complete missing prices and corroborate the information obtained in the field. Since the visit to agricultural warehouses took place one year after the field visit, the 2.56% corresponding to annual price inflation in January 2022 was reduced concerning January 2021 [25].

Pasture management (E7). It includes all pesticides, organic fertilisers, and chemicals fertilisers used to maintain the paddocks. The costs of equalisation cut and maintenance of paddock fences were placed in occasional labour. It is usually done in the areas once or twice a year.

Insemination (E8). These are the costs for purchasing straws and filling the nitrogen tank if the farm implements this. This cost is included in veterinary services (E5) if a veterinarian or technician performs insemination.

Infrastructure and equipment (E9 and E10). Those values correspond to the cost of depreciation (E9) and annual maintenance (E10). Infrastructure includes stables, corrals, cattle handling systems, and storage warehouses. The equipment consists of milking equipment, cooling tanks, electric fences, irrigation equipment, fumigation pumps, scythes, grass shredders, biodigesters, and other equipment used for livestock farming. Depreciation was calculated by using a straight-line method, according to the years of the useful life of each asset reported by farmers during the survey. For infrastructure, the average time of useful life was 20 years (infrastructure) and for equipment 11 years.

Transport (E11). These are the costs destined to cover vehicle rental expenses or fuel, as long as they are used in the entry or exit of animal feed, animals, or any input related to livestock raising.

Rent of pastures (E12). This cost was included because it is a common practice in the study areas. On farms where there are not enough paddocks, farmers rent paddocks from neighbouring properties.

Taxes (E13). Corresponds to the payment of the land tax.

Animal entry and departure (E14-E17). The entry and departure of animals from the farm corresponds to the entry of replacement animals, and the departure is due to mortality on the farm and cattle theft.

Information reported by the farmer on sick or dead animals was used to determine the cost of mortality (E14 and E15). The principal diseases present in the study areas and reported by local veterinarians were used. For each disease, information was available on the number of animals affected, age, and mortality. The cost of sale of the animals corresponds to the average reported in each zone. The mortality costs per farm were limited to 3.00%, corresponding to the average mortality in the study area. Cattle theft (E16) was also considered, as it is usually experienced by cattle farmers in the area. The annual depreciation (straight-line) value of the animals was taken into account in the departure of the animals. In the case of cows, the lost remuneration for the litres of milk they stopped producing per year and the cost of a newborn calf was added.

For the calculation of animal replacement (E17), the proportion of the cost of the animal about the average useful life of the animal was used. The valuable life corresponds to the average reported in the study areas. Indeed, a life expectancy of 9 years was considered for cows and 5 years for bulls.

Basic services (E18). Corresponds to the amount paid for water and electricity.

Revenues

Revenues correspond mainly to milk produced and complementarily to cattle sales (Fig 2).

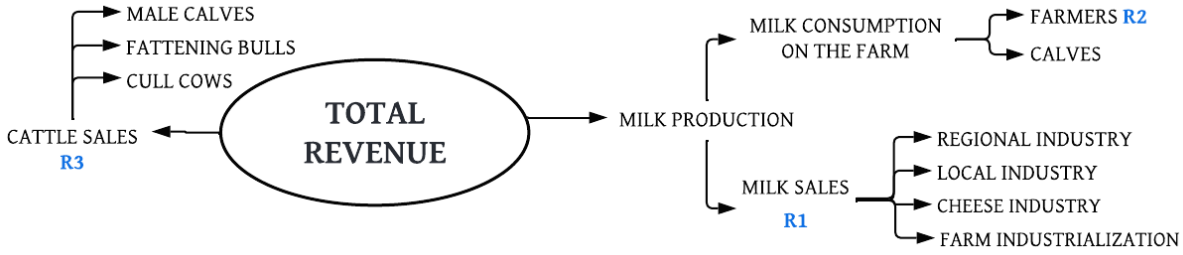

Fig 2. Revenues from a dairy farm in the study area. R1 to R3 are codifications of revenues used in the different equations (see after).

https://doi.org/10.1371/journal.pone.0287104.g002

For the calculation of total revenue (TR) from the sales of milk, the average number of cows in production, the average milk production per day (litre/cow/day), and the price received for the sales of milk were used. We averaged cow/day production in the dry and rainy seasons.

Revenue from milk consumed on the farm in the form of raw milk or milk by-products was based on the opportunity cost in the area. The consumption of milk per calf was not used to calculate total revenue because if it is not used; a milk substitute is purchased, an expense that was considered on farms where it is used.

For the calculation of cattle sales, new-born male calves, on-farm born bulls for fattening, or the sale of old cull animals were included. Similarly, the market value for calves and cull cows was determined using the opportunity cost for each zone.

$$TR = R_1 + R_2 + R_3 \quad \text{(Eq3)}$$

The Annual Profit (P) is the subtraction between the total revenue and total costs of livestock production.

$$P = TR - TC_L \quad \text{(Eq4)}$$

The production costs of a litre of milk (CPM) were calculated dividing TCM to the total amount of litres produced per year.

Acaricide resistance test

On each farm visited, a minimum of 40 engorged female ticks were collected from five randomly selected animals. The collected samples were transported to the laboratory of applied entomology at the Research Institute of Zoonosis (CIZ) in Central University of Ecuador in Quito, where resistance testing was performed on three acaricides: amitraz, ivermectin, and alpha-cypermethrin, using the larval package test. Samples were tested at the farm level and consisted of two replicates per acaricide plus a control group. Approximately 100 larvae were placed in each package. Mortality readings were taken 24 hours after larval seeding. The average of the two replicates was used to determine the percentage of resistance to the acaricide [26]. Concentrations used for the resistance response were: for alpha-cypermethrin 0.02%, for amitraz 0.1% and for ivermectin at 0.1% [15]. Resistance results were classified into four levels: susceptible, low resistance, medium resistance, and high resistance, as described before [15, 27]. The farms were classified as with and without resistance. Farms considered with resistance have medium and high resistance (Table 1).

Table 1. Interpretation of acaricide resistance.

Category	Larvae (Survival %)
Susceptible	<10%
Susceptible with Restriction	10-20%
Emerging Resistant	20-50%
Resistant	>50%

Adapted form Rodríguez-Hidalgo et al., 2017; Junte, 2008 [15, 27]

https://doi.org/10.1371/journal.pone.0287104.t001

Level of tick infestation

The level of infestation at the farm level was determined according to the level of infestation in the animals sampled, and they were classified according to the methodology explained by Paucar et al. (2022) [28]. This variable was expressed as farms with or without high level of tick infestation.

Statistical analysis

Farm typology. The k-means [29] algorithm was used to obtain a typology of the farms using the following variables: level of technology, veterinary control, farm size (ha), use of external paddocks, owner's level of education, owner's time dedicated to livestock, the destination of milk delivery, milk delivery price (USD), presence of permanent paid labour force, presence of crops for sale, feed supplementation to cattle, and grass cut. The level of technology was determined by the presence of installations, use of artificial insemination, and type of milking [28]. The presence of veterinary control was determined according to the frequency of visits (never, rarely, sometimes, and permanent) regardless of whether it was subsidised or not. Permanent and frequent visits were considered as the presence of veterinary control. The presence of crops on the farm was only considered for sale; crops for self-consumption were not considered. The analysis was performed using R [30]. Elbow Method [31] was used to determine the optimal number of clusters (groups). The packages FactoMineR [32] and Factoextra [33] were used. Both numerical and categorical variables were recategorised into ordinal variables for use in the k-means algorithm.

Decision tree analysis. A decision tree was used to determine the impact of management variables on economic variables. Decision trees have been widely used in animal health economic analyses [34–37]. Herd management variables used for the analyses were: technology level, herd size, use of paddocks outside the farm boundaries (external paddocks), the use of manual removal of ticks, the study area, presence of high level of tick infestation (farm level), and presence of tick resistance to three acaricides (Table 2). These variables were selected because of their importance in a previous study [28] or because their common use in livestock management.

The cost of acaricides, hemo-parasiticides, and repellents was used to calculate the cost of drugs used for the presence of ticks. The labour force cost was determined by the time taken to apply the acaricide treatments and the labour force cost in each study area. The sanitary cost includes the cost of drugs and inputs, veterinary services, and the cost of the labour force used for sanitary work (vaccination, deworming, bathing, castration, identification, dehorning, and insemination). To calculate the annual cost per animal, the total costs of acaricide treatment was divided by the number of adult cattle (female or male animals over 1 year old) present on each farm.

In addition, a decision tree was constructed between the presence of high level of tick infestation (response variable), resistance to three acaricides, and level of technology, as these are essential variables to understand this relationship.

Table 2. Variables used to construct decision trees.

Explanatory variables	Categories
Technology level	Non-technified Semi-technified Technified
Herd size	1-20 animals 21-70 animals >71 animals
External paddocks	Yes No
Manual removal of ticks	Yes No
Study area	Quijos river valley (Area 1) Northwest of Pichincha (Area 2)
Presence of high level of tick infestation	Yes No
Amitraz resistance	Yes No
Ivermectin resistance	Yes No
Alpha-cypermethrin resistance	Yes No

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Decision trees were built using the rpart and rpart.plot packages [38, 39] packages in R. Validation was performed using a split of the database for training the model (75% of random data) and the rest for testing the model, which is a procedure that reveals the performance of the model with new data [40]. For model prediction, we ran the decision trees procedure several times (one hundred times) and chose only the models in which the prediction response and observation were in concordance. It means models in which the slope of the line formed between observed vs. prediction response was close enough to one and the intercept close enough to zero, and where the differences between prediction and observation did not differ significantly according to Bland-Altmant agreement between two quantitative measurements (package BlandAltmanLeh) [41]. We also chose the models also with minimum mean square errors.

3. Results

Tick infestation and acaricide resistance

Of the 105 farms analysed, 45.71% (48 farms) were had high tick infestation. In general, in farms with and without high level of infestation, the resistance to alpha-cypermethrin was about 53.33%, and it was the highest level of resistance compared to the other acaricides (Table 3). The levels for amitraz and ivermectin resistances were 49% and 37%, respectively. Table 4 shows the results of combined resistances. Thirty-two percent of the farms with or without high level of tick infestation are resistant to both amitraz and alpha-cypermethrin. There was no significant difference between the presence of high level of infestation and resistance in single and combined resistances.

A decision tree was constructed for the presence of high level of tick infestation in relation to the presence of tick resistance to three acaricides, and also with the level of technology. It presented an area under the receiver operator characteristic curve (AUC-ROC) of 0.64 (95% CI: 0.54–0.74) (Fig 3), showing that those results are not random. In this model (Fig 4), it was observed that when a farm is sensitive (farm without resistance) to the acaricides, there were higher levels of tick infestation in the farm, but low level of technology.

Table 3. Contingency table between the high level of infestation and resistance to acaricides.

High level of tick infestation	AM resistance		IV resistance		CY resistance	
	p-value = 0.85		*p*-value = 1.00		*p*-value = 0.33	
	No	Yes	No	Yes	No	Yes
No (N = 57)	26.67% (N = 28)	27.62% (N = 29)	34.29% (N = 36)	20.00% (N = 21)	22.86% (N = 24)	31.43% (N = 33)
Yes (N = 48)	23.81% (N = 25)	21.90% (N = 23)	28.57% (N = 30)	17.14% (N = 18)	23.81% (N = 25)	21.90% (N = 23)
Total	50.48%	49.52%	62.86%	37.14%	46.67%	53.33%

AM = Amitraz; IV = Ivermectin; CY = Alpha-cypermethrin; N = number of farms; *p*-value was obtained using Fisher's exact test.

https://doi.org/10.1371/journal.pone.0287104.t003

Table 4. Contingency table with combined resistance to acaricides.

High level of tick infestation	AM & CY resistance		AM & IV resistance		CY & IV resistance		AM -CY & IV resistance	
	p-value = 0.54		*p*-value = 1.00		*p*-value = 0.82		*p*-value = 1.00	
	No	Yes	No	Yes	No	Yes	No	Yes
No (N = 57)	35.24% (N = 37)	19.05% (N = 20)	41.90% (N = 44)	12.38% (N = 13)	40.00% (N = 42)	14.29% (N = 15)	42.86% (N = 45)	11.43% (N = 12)
Yes (N = 48)	32.38% (N = 34)	13.33% (N = 14)	35.24% (N = 37)	10.48% (N = 11)	35.24% (N = 37)	10.48% (N = 11)	39.05% (N = 41)	6.67% (N = 7)
TOTAL	67.62%	32.38%	77.14%	22.86%	75.24%	24.77%	81.90%	18.90%

AM & CY = Farms with resistance to amitraz and alpha-cypermethrin; AM & IV = Farms with resistance to amitraz and ivermectin; CY & IV = Farms with resistance toalpha-cypermethrin and ivermectin; AM, CY & IV = Farms with resistance to amitraz, ivermectin, and alpha-cypermethrin; *p*-value was obtained using Fisher's exact test.

https://doi.org/10.1371/journal.pone.0287104.t004

Farm typology

The five groups or types of farmers were formed using means of the variables described in Table 5. Group 1 has 21 of 105 farms (20.00%), group 2 has 17 of 105 farms (16.19%), group 3 has 27 of 105 farms (25.71%), group 4 has 23 of 105 farms (21.90%), and group 5 has 17 of 105 farms (16.19%). The groups were divided into small, medium, and large farms according to farm size. The medium farms were classified into three groups according to their level of technology and whether they cultivate and sell crops. Concentrate supplementation to cattle is done in four out of five groups, in contrast to supplementation with cutting pastures, which is only done in the group of large farms. Small farms delivered milk to the local industry, medium farms to the cheese industry, and large farms to the milk regional industry. Milk prices were similar in four out of five groups (local industry and cheese factories). However, the large farms obtained better remuneration per litre of raw milk due to their main delivery destination is the regional industries.

The five groups are described below.

Group 1—Small, non-technified cattle farms. Farms in this group have an area of 1- 20ha, requiring the rental of external paddocks to feed their cattle, especially for dry cows. Their level of technology is low, but they have frequent veterinary controls. Since they are small farms, do not require permanent labour, but 95.23% of these farms use occasional personnel for specific tasks such as milking or pasture maintenance. In addition, 80.95% of these farms use unpaid family labour. The owner has an elementary school education and is involved in animal management, spending an average of 3 hours per day on it. Almost equal numbers of men (52.38%) and women (47.52%) are managers of livestock.

Group 2—Medium, non-technified cattle and agricultural farms. Farms in this group have an area of 21-45ha, requiring the rental of external paddocks to feed their cattle. The animals do not receive

supplementary feed. Their level of technology is low, and they do not have veterinary control. They do not have permanent labour. However, 64.70% of these farms use occasional labour for pasture maintenance. In addition, 88.24% of these farms use unpaid family labour. The owner has a high school education and is involved in animal management.

These farms, apart from being livestock farms, are dedicated to the cultivation and sale of palm hearts, bananas, coffee, and cocoa, among others. The farmer his time spending, an average of 4.38 hours per day. Almost equal numbers of men (52.94%) and women (47.06%) are managers of livestock.

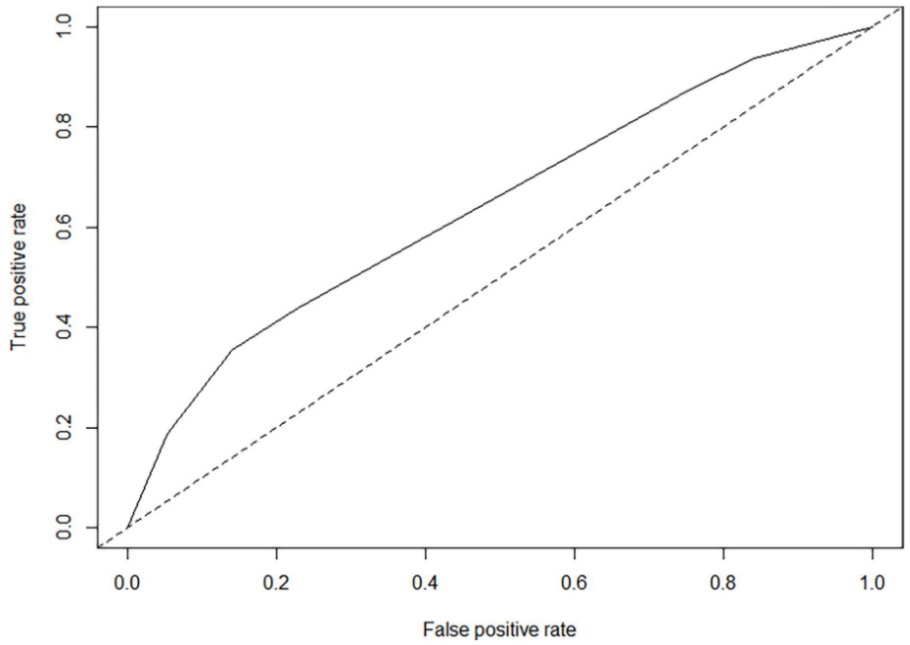

Fig 3. Receiver operating characteristic (ROC) curve of acaricide resistance, presence of high level of tick infestation, and level of technology.

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Group 3—Medium, non-technified cattle farms. Farms in this group have an area of 21- 45ha and have the necessary pastures to feed their cattle. Their level of technology is low, and they do not use veterinary control. They have a permanent labour force; in addition, 70.37% of the farms have an occasional labour force for pasture maintenance in general. Unpaid family labour is only used on 22.22% of farms. The owner has a high school education and generally participates only in milking activities, dedicating an average of 1.75 hours per day to the herd. The managers of the livestock on most of the farms are men (62.96%).

Group 4—Medium, semi-technified cattle farms. Farms in this group have an area of 21-45ha and have the necessary paddocks to feed their cattle. Their level of technology is medium, and they use veterinary control. They do not have a permanent labour force; however, 73.91% of these farms use occasional personnel for pasture maintenance. In addition, 82.61% of these farms use unpaid family labour. The owner has a high school education and is involved in animal management, dedicating an average of 5 hours per day to these tasks. The managers of the livestock on most of the farms are men (78.26%).

Group 5—Large, technified cattle farms. Farms in this group have an area of over 46ha and the necessary paddocks to feed their cattle. They are technified, have veterinary control, and the animals receive concentrated feed and cut pasture, and mineralised salt. They have a permanent labour force, and, in addition, 76.47% of the farms have occasional labour for pasture maintenance in general. Unpaid family labour is used on 29.41% of farms. The owner has a university education and is not involved in animal management; they spend an average of 1.16 hours per day on these administrative activities. The managers of the livestock on most of the farms are men (70.59%).

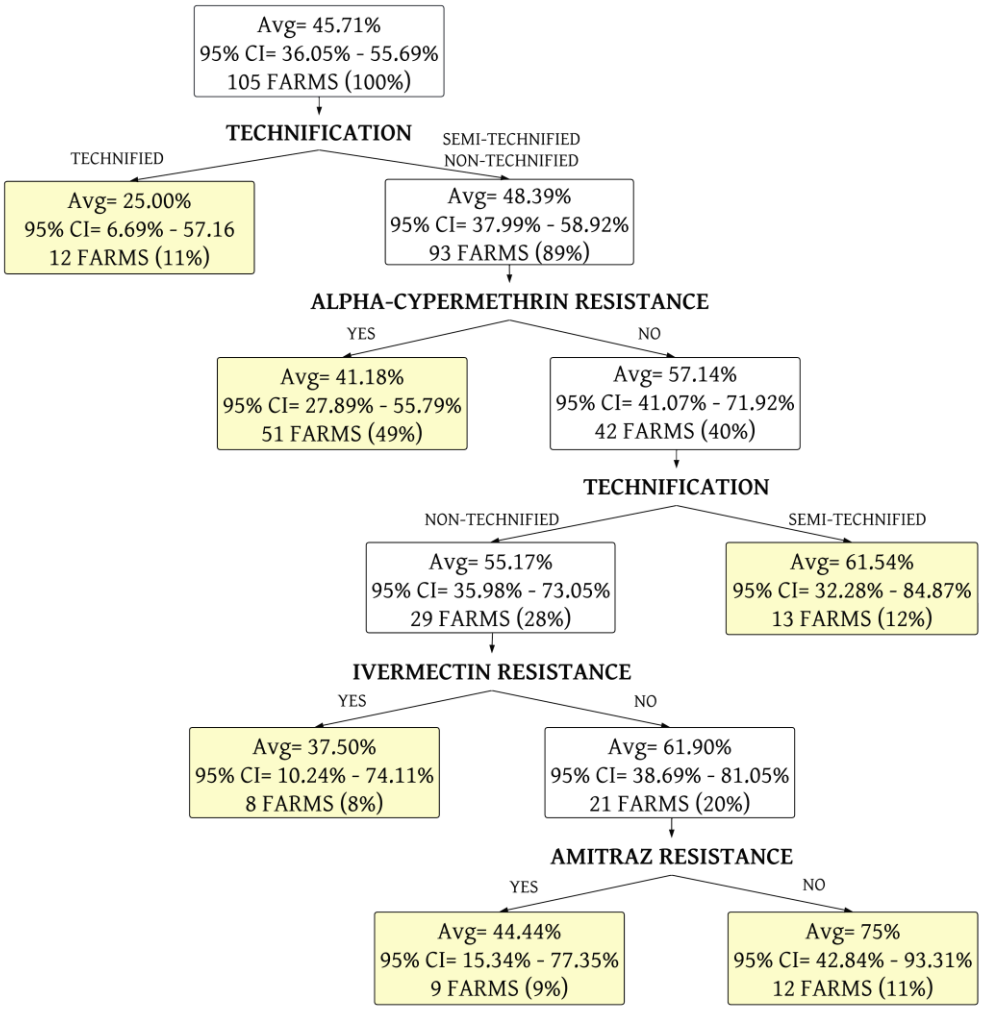

Fig 4. Decision tree for high level of tick infestation on animals using the levels of acaricide resistance, and level of farm technology. The yellow boxesrepresent the terminal nodes of the decision tree. The average (Avg) corresponds to the % of infested farms. CI is the confidence interval.

https://doi.org/10.1371/journal.pone.0287104.g004

Economic analysis

The main expenses in livestock farming are labour and supplementary livestock feed (Table 6). In the groups of small and medium farms (groups 1 to 4), the main costs correspond to the labour force, while in large farms (group 5), it is supplementary feeding. In general, within labour, 54.18% of the expenses correspond to paid labour and 45.82% to unpaid family labour (those activities were set as part of the opportunity costs). However, when analysed by groups, the cost of paid labour in groups 5 and 3 corresponds to 89.56% and 80.17%, respectively. In contrast, groups 1 (74.02%), 2 (88.54%), and 4 (92.91%) correspond to unpaid labour, either from the owner or the family.

Veterinary services were evaluated in two scenarios, without (Scenario A) and with subsidy (Scenario B). The subsidised veterinary technician is in charge of insemination, dehorning, castration, oestrus synchronisation, diagnosis, and treatment of diseases, although they are not always licensed professionals. In addition, to perform these activities, the private veterinarian focused on reproductive check-ups (gynaecological-obstetrical). The cost of veterinary services in the overall analysis ranged from 1.25% (Scenario A) to 1.99% (Scenario B) of the total cost of livestock production. Nevertheless, when analysed by groups, it represents an economic advantage for groups 1, 2, and 4. In groups 3 and 5, costs with or without veterinary services varied very little.

Table 5. Farm typology in the study area.

Variable	Group of farm				
	1	**2**	**3**	**4**	**5**
Farm size	1-20ha	21-45ha	21-45ha	21-45ha	>46ha
External paddocks	Yes	Yes	No	No	No
Level of technification	Non- technified	Non- technified	Non- technified	Semi- technified	Technified
Veterinary control	Yes	No	No	Yes	Yes
Permanent paid labour force	No	No	Yes	No	Yes
Crops for sale	No	Yes	No	No	No
Concentrate supplementation	Yes	No	Yes	Yes	Yes
Grass cut	No	No	No	No	Yes
Milk delivery price (USD)	0.37	0.37	0.38	0.37	0.47
Destination of milk delivery	Local industry	Cheese industry	Cheese industry	Cheese industry	Regional industry
Owner's level of education	Primary school	Primary school	High school	High school	University
Owner's time dedicated to livestock	3.00 hrs.	4.38 hrs.	1.75 hrs.	5.00 hrs.	1.16 hrs.

Primary school, including farmers without formal education; High school, including farmers with unfinished university education.

https://doi.org/10.1371/journal.pone.0287104.t005

The average annual profit varies little between the two scenarios. The average production price per litre of milk is 0.30 USD. The cost of production is highest in the small farms (0.34 USD) and lowest in the medium, non-technified cattle farms (0.25 USD).

Table 6. Fixed and variable costs in livestock farming.

Group		E_1-E_2	E_3-E_4	E_5(A)	E_5(B)	E_6	E_7	E_8	E_9,E_{10}	E_{11}	E_{12}	E_{13}	E_{14}-E_{17}	E_{18}
1	AVG	2205.11	4754.72	47.80	153.60	706.07	54.55	32.38	409.71	410.00	293.33	36.24	494.10	94.29
	SD	1696.56	2528.09	51.14	159.66	693.86	141.85	72.17	404.60	731.17	474.22	42.62	532.55	139.18
	%A	23.12	49.85	0.50	-	7.40	0.57	0.34	4.30	4.30	3.08	0.38	5.18	0.99
	%B	22.86	49.30	-	1.59	7.32	0.57	0.34	4.25	4.25	3.04	0.38	5.12	0.98
2	AVG	756.38	4186.77	36.35	104.45	645.24	7.97	36.47	330.69	599.65	156.47	96.35	557.51	289.41
	SD	449.89	865.90	67.61	168.40	431.34	20.99	119.21	349.26	1285.45	307.75	104.69	992.15	372.72
	%A	9.82	54.38	0.47	-	8.38	0.10	0.47	4.30	7.79	2.03	1.25	7.24	3.76
	%B	9.74	53.90	-	1.34	8.31	0.10	0.47	4.26	7.72	2.01	1.24	7.18	3.73
3	AVG	2126.13	6854.49	147.68	227.92	1147.76	54.95	33.70	613.11	460.74	50.00	113.67	750.23	241.78
	SD	1475.81	3907.01	290.61	325.31	929.57	148.40	132.46	437.34	277.32	196.61	140.16	752.33	238.07
	%A	16.88	54.43	1.17	-	9.11	0.44	0.27	4.87	3.66	0.40	0.90	5.96	1.92
	%B	16.77	54.08	-	1.80	9.06	0.43	0.27	4.84	3.64	0.39	0.90	5.92	1.91
4	AVG	1682.27	4732.99	54.30	269.87	621.69	42.05	19.78	406.54	555.30	26.09	73.00	600.53	122.61
	SD	1613.55	1189.27	90.66	856.30	372.70	138.07	67.09	377.06	731.79	125.11	67.79	906.67	137.11
	%A	18.82	52.96	0.61	-	6.96	0.47	0.22	4.55	6.21	0.29	0.82	6.72	1.37
	%B	18.38	51.71	-	2.95	6.79	0.46	0.22	4.44	6.07	0.29	0.80	6.56	1.34
5	AVG	13409.98	10261.82	683.12	716.16	2787.32	94.97	506.21	3579.03	963.82	0.00	366.24	1130.27	1256.00
	SD	1613.55	1189.27	90.66	856.30	372.70	138.07	67.09	377.06	731.79	125.11	67.79	906.67	137.11
	%A	38.27	29.29	1.95	-	7.95	0.27	1.44	10.21	2.75	0.00	1.05	3.23	3.58
	%B	38.24	29.26	-	2.04	7.95	0.27	1.44	10.20	2.75	0.00	1.04	3.22	3.58
Total	AVG	3649.84	6089.57	175.92	281.30	1128.28	50.92	107.34	961.65	575.25	102.57	127.36	696.54	358.10
	SD	10998.55	5585.50	950.68	934.58	2464.00	258.29	612.57	1940.44	903.72	0.00	506.22	1579.82	1220.47
	%A	26.03	43.42	1.25	-	8.05	0.36	0.77	6.86	4.10	0.73	0.91	4.97	2.55
	%B	25.83	43.10	-	1.99	7.99	0.36	0.76	6.81	4.07	0.73	0.90	4.93	2.53

%A = Percentage that corresponds to each expenditure, with respect to the total cost of livestock production, within Scenario A (cost of veterinary services without state subsidies); %B = Percentage that corresponds to each expenditure, with respect to the total cost of livestock production, within Scenario B (cost of veterinary services with state subsidies); AVG, average; SD, standard deviation.

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In general, the cost of milk production is 0.30 USD (95% CI: 0.17–0.43). In farms from groups 1 and 2, the production cost per litre of milk in higher, in contrast to farms in groups 3,4, and 5 (Table 7). However, the difference was only significant between groups 1 and 3 (p-value = 0.03).

Table 7. Economic evaluation according to farm typology.

		Group 1	Group 2	Group 3	Group 4	Group 5	Total
TC_A	AVG	9538.31	7699.26	12594.24	8937.16	35038.78	14023.34
	SD	5279.02	2706.80	5529.43	3117.79	3117.79	21272.13
TC_B	AVG	9644.11	7767.36	12674.48	9152.73	35071.82	14128.73
	SD	5287.09	2669.62	5656.09	3216.60	3216.60	21255.63
AMP	AVG	31641.61	26908.78	51767.24	37490.71	120349.55	51693.97
	SD	21392.84	15004.43	23953.17	30143.71	71379.06	47316.52
TR	AVG	13704.66	11950.53	21476.78	16107.37	64103.67	24105.35
	SD	9429.39	7067.14	8905.42	14570.78	48902.19	27944.26
P_A	AVG	4166.35	4251.26	8882.54	7170.21	29064.89	10082.01
	SD	4166.35	4251.26	8882.54	7170.21	29064.89	10082.01
P_B	AVG	4060.55	4183.16	8802.30	6954.64	29031.85	9976.63
	SD	4980.53	6645.17	7805.33	13023.12	32107.77	17191.80
CPM	AVG	0.34	0.32	0.25	0.30	0.30	0.30
	SD	0.15	0.14	0.09	0.14	0.11	0.13

TC_A = Total livestock production cost in Scenario A; TC_B = Total livestock production cost in Scenario B; AMP = Annual Milk Production; TR = Total revenue; P_A =Annual Profit in Scenario A; P_B = Annual Profit in Scenario B; CPM = Cost of production of a litre of milk; AVG, average; SD, standard deviation.

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The survey also revealed the repercussions suffered by farmers due to the COVID-19 pandemic. A total of 69.52% (73 farms) of the farmers stated that their income from milk sales decreased due to the limitations caused by the quarantine in the country, which was more evident at the beginning of it (March 2020 to August 2020). During this period, some milk collection companies did not pay on time or did not collect milk every day. Hence, several farms looked for another company to deliver milk or implement homemade cheese production to sell it. The decrease in the payment per litre of milk and the limitation of the volume of milk received by the milk collection companies was also still occurring at the time of the data collection for this study (Table 8). All these consequences are even more evident in small (Group 1) and medium farms (Group 2, 3, and 4).

Table 8. Farms that experienced economic repercussions due to the COVID-19 pandemic.

Effect	Group 1		Group 2		Group 3		Group 4		Group 5	
	Farms	%	Farms	%	Farms	%	Farms	%	Farms	%
Decrease in the price per litre of milk	13	86.67%	12	92.31%	19	90.48%	18	100.00%	3	50.00%
Limited the volume of milk to be commercialised	0	0.00%	0	0.00%	0	0.00%	1	5.56%	2	33.33%
The company did not collect the milk	6	40.00%	3	23.08%	5	23.81%	5	27.78%	1	16.67%
Changed milk collection company	3	20.00%	1	7.69%	0	0.00%	2	11.11%	0	0.00%
Made cheeses for marketing	3	20.00%	1	7.69%	4	19.05%	3	16.67%	0	0.00%
Milk sales payments not on time	0	0.00%	0	0.00%	1	4.76%	0	0.00%	1	16.67%
Sold cattle	2	13.33%	1	7.69%	3	14.29%	1	5.56%	1	16.67%
Dry Cow Therapy	2	13.33%	1	7.69%	0	0.00%	1	5.56%	0	0.00%

Group 1 = 71.43% (15 of 21 farms); Group 2 = 76.47% (13 of 17 farms); Group 3 = 77.78% (21 of 27 farms; Group 4 = 78.26% (18 of 23 farms); Group 5 = 35.29% (6 of 17 farms).

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Impact of acaricide resistance and tick infestation on total costs. The percentage of acaricide treatment costs to management variables was grouped into six homogeneous terminal nodes using a decision

tree analysis (Fig 5 and S1 Table).This model presented a determination coefficient (R2) of 0.26 and a mean squared error (MSE) of 2.74% in training data. The R2 was 0.21, and MSE was 3.70% in testing data.

The six terminal nodes are described below.

Terminal node A1. This group corresponds to technified farms (100.00%), with veterinary control in all farms; with high level of tick infestation in 25.00% of farms. The main problem is resistance to amitraz in 50.00% of the farms, followed by alpha-cypermethrin (37.50%) and ivermectin (12.50%). Regarding combined resistance, 37.50% of the farms showed resistance to both amitraz and alpha-cypermethrin. Their cost of acaricide treatment is 1.30% of the total annual cost (Average = 30031.33 USD). Most of the farms in this terminal node belong to Group 5.

Terminal node B1. This group corresponds to semi-technified farms (100.00%), with veterinary control in 85.71% of cases; with high level of tick infestation in 71.43% of farms. These farms are sensitive to alpha-cypermethrin; its main problem is resistance to ivermectin (42.86%) and amitraz (14.29%). Regarding combined resistance, 14.29% of the farms showed resistance to both amitraz and ivermectin. Their cost of acaricide treatment is 3.43% of the total annual cost (Average = 14804.77 USD).

Terminal node C1. This group corresponds to non-technified farms (100.00%), with veterinary control in 57.14% of cases; and with high level of tick infestation in 50.00% of farms.

These farms are sensitive to alpha-cypermethrin, and its main problem is resistance to amitraz (42.86%) and ivermectin (35.71%). Regarding combined resistance, 14.29% of the farms showed resistance to both amitraz and ivermectin. Their cost of acaricide treatment is 6.24% of the total annual total cost (Average = 9646.38 USD). Most of the farms in this terminal node belong to Group 3.

Terminal node D1. This group corresponds to non-technified farms (68.42%), with veterinary control in 47.37% of cases; and without high levels of tick infestation on the farms. The main problem is resistance to alpha-cypermethrin in all farms, followed by amitraz (57.89%) and ivermectin (47.37%). Regarding combined resistance, 57.89% of the farms showed resistance to both amitraz and alpha-cypermethrin. Their cost of acaricide treatment is 2.74% of the total annual costs (Average = 12004.77 USD). Most of the farms in this terminal node belong to Groups 3 and 4.

Terminal node E1. This group corresponds to non-technified farms (72.73%), with veterinary control in 54.55% of cases; and high level of tick infestation in all farms. The main problem is resistance to alpha-cypermethrin in all farms, followed by amitraz (54.55%) and ivermectin (45.45%). Regarding combined resistance, 54.55% of the farms showed resistance to both amitraz and alpha-cypermethrin. Their cost of acaricide treatment is 4.28% of the total annual costs (Average = 9566.59 USD). Most of the farms in this terminal node belong to Group 1 and 2.

Terminal node F1. This group corresponds to non-technified farms (68.42%), with veterinary control in 84.21% of cases; and high level of tick infestation in 42.11% of farms. The main problem is resistance to amitraz in 52.63% of the farms, followed by alpha-cypermethrin (42.11%) and ivermectin (31.58%). Regarding combined resistance, 26.32% of the farms showed resistance to both amitraz and alpha-cypermethrin. Their cost of acaricide treatment is 5.75% of the total annual costs (Average = 11499.42 USD). Most of the farms in this terminal node belong to Group 1.

Annual cost of acaricide treatment per animal. Annual cost in USD of acaricide treatment per animal to management variables was grouped into five homogeneous terminal nodes using a decision tree analysis (Fig 6 and S2 Table). This model presented a R2 of 0.16, a MSE of 16.23 USD in training data. The R2 was 0.35 and MSE of 10.08 USD in testing data. The five terminal nodes are described below.

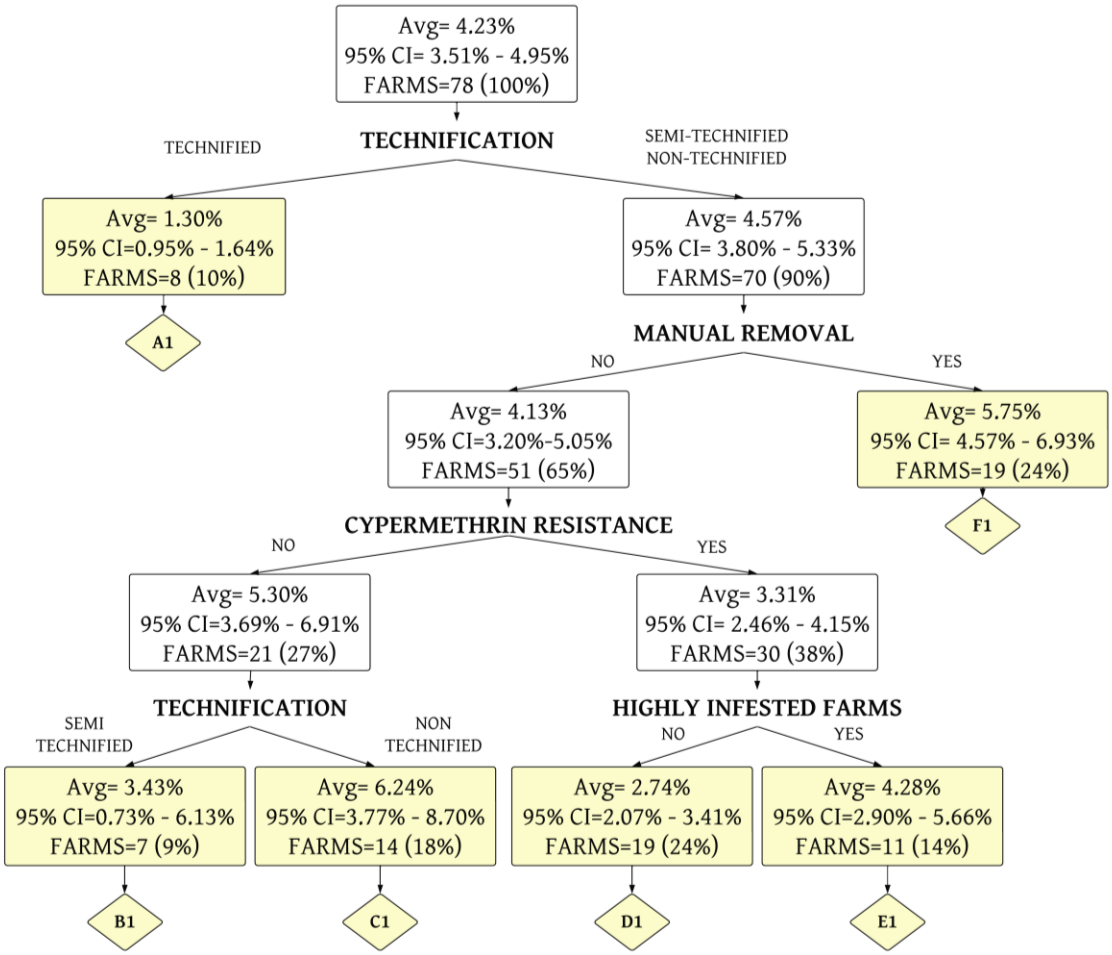

Fig 5. Decision tree analysis of the percentage of total costs allocated to acaricide treatment (Model 1). A1 until F1 are the terminal nodes of the tree(yellow boxes). The average (Avg) corresponds to the % of total costs destined to the acaricide treatment. CI is the confidence interval.

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Terminal node A2. This group corresponds to technified farms (100.00%), with high level of tick infestation in 37.50% of farms. The main problem is resistance to amitraz in 75.00% of the farms, followed by alpha-cypermethrin (62.50%) and ivermectin (25.00%). The 50.00% of these farms perform manual removal of ticks during milking activities. Pour-on treatments are used on 50.00% of the farms. The 87.50% of the farms use acaricides correctly. Farmers refrain from mixing commercial presentations, overdose or under-dose. On average, acaricide treatment is given every 53.42 days. Their cost of acaricide treatment per animal is 8.46 USD. Most of the farms in this terminal node belong to Group 5.

Terminal node B2. This group corresponds to non-technified farms (92.86%), with high level of tick infestation in 57.14% of farms. These farms are sensitive to alpha-cypermethrin; its main problem is resistance to amitraz and ivermectin, with 35.71% in both cases. These farms do not manually remove ticks during milking activities. Pour-on treatments are used on 64.29% of the farms. The 71.43% of farms use acaricides incorrectly. Farmers are mixing commercial presentations (35.71%), overdosing (50.00%) or underdosing (14.29%) acaricides. On average, acaricide treatment is given every 28.52 days. Their cost of acaricide treatment per animal is 13.00 USD. Most of the farms in this terminal node belong to Group 3.

Terminal node C2. This group corresponds to non-technified farms (60.87%), with high level of tick infestation in 52.17% of farms. All farms are resistant to alpha-cypermethrin, followed by amitraz and ivermectin, with 47.83% in both cases. These farms do not manually remove ticks during milking activities. Pour-on treatments are used on 26.09% of the farms. The 65.22% of farms use acaricides incorrectly. Farmers

are mixing commercial presentations (17.35%), overdosing (56.52%) or underdosing (8.70%) acaricides. On average, acaricide treatment is given every 26.74 days. Their cost of acaricide treatment per animal is 19.50 USD. Most of the farms in this terminal node are medium cattle farms (Groups 2, 3 and 4).

Terminal node D2. This group corresponds to non-technified farms (78.57%), with high level of tick infestation in 35.71% of farms. The main problem is resistance to alpha-cypermethrin in 57.14% of the farms, followed by amitraz (50.00%) and ivermectin (42.86%). All farms perform manual removal of ticks during milking activities. Pour-on treatments are used on 35.71% of the farms. The 42.86% of farms use acaricides incorrectly. Farmers are mixing commercial presentations (21.43%), overdosing (35.71%) or underdosing (7.40%) acaricides. On average, acaricide treatment is given every 15.79 days. Their cost of acaricide treatment per animal is 23.85 USD. Most of the farms in this terminal node belong to Group 1.

Terminal node E2. This group corresponds to non-technified farms (57.89%), with high level of tick infestation in 52.63% of farms. The main problem is resistance to amitraz in 57.89% of the farms, followed by alpha-cypermethrin and ivermectin with 47.37% in both cases. The 36.84% of these farms perform manual removal of ticks during milking activities. Pour-on treatments are used on 26.32% of the farms. The 42.11% of farms use acaricides incorrectly. Farmers are mixing commercial presentations (42.11%), overdosing (36.84%) or underdosing (5.26%) acaricides. On average, acaricide treatment is given every 19.63 days. Their cost of acaricide treatment per animal is 28.44 USD. Most of the farms in this terminal node belong to Group 1.

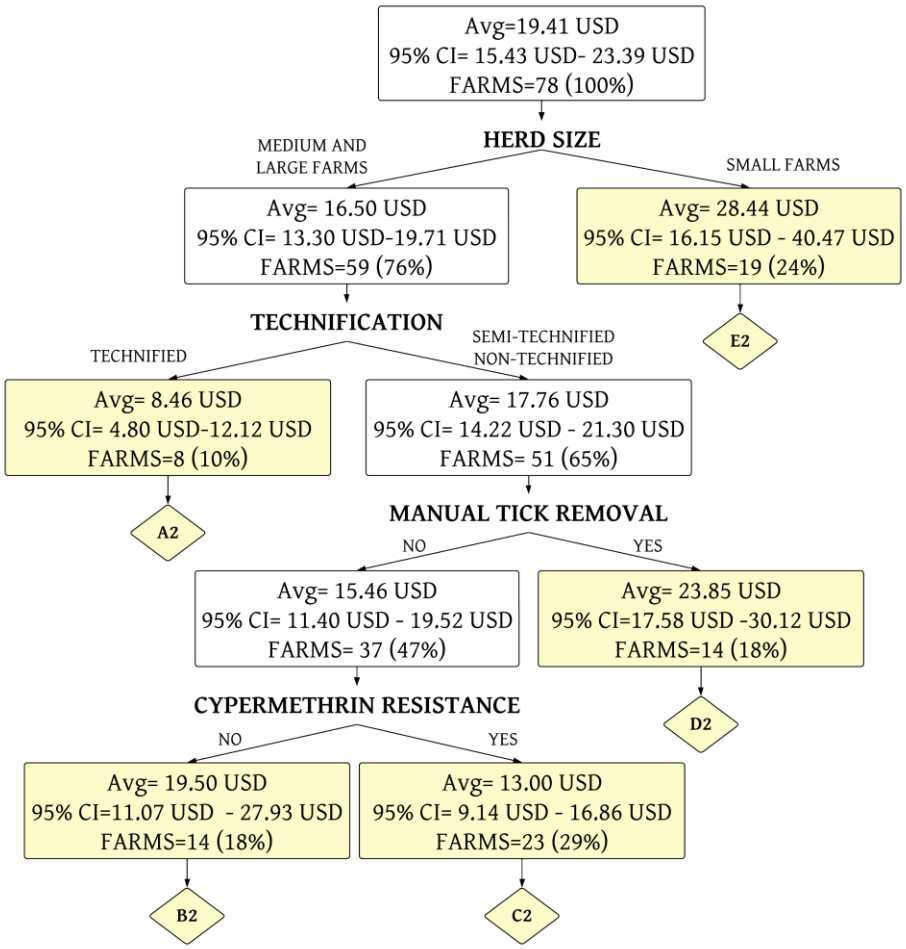

Fig 6. Decision tree analysis of the annual cost of acaricide treatment per animal (Model 2). A2 until E2 are the terminal nodes of the tree (yellowboxes). The average (Avg) corresponds to the annual cost (USD) of acaricide treatment per animal. CI is the confidence interval.

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Impact of acaricide resistance and tick infestation on drug costs (E6). The percentage of acaricide drug costs to management variables was grouped into seven homogeneous terminal nodes using a decision tree analysis (Fig 7 and S3 Table). This model presented a R2 of 0.26 and a MSE of 19.54% in training data. The R2 was 0.20 and MSE was 20.42% in testing data.

The seven terminal nodes are described below.

Terminal node A3. This group corresponds to technified farms (100%), with veterinary control in 90.00% of cases; high level of tick infestation in 20.00% of farms. The main problem is resistance to amitraz in 70.00% of the farms, followed by alpha-cypermethrin (50.00%) and ivermectin (20.00%). Regarding combined resistance, 50.00% of the farms showed resistance to both amitraz and alpha-cypermethrin. Their cost for acaricide drugs is 12.29% with respect to the costs of all veterinary inputs (this annual average cost is 1628.19 USD). Most of the farms in this terminal node belong to Group 5 (S1 Table).

Terminal node B3. This group corresponds to semi-technified farms (100.00%), with veterinary control in 72.73% of cases, and they are not highly infested by ticks. Its main problem is resistance to alpha-cypermethrin in 63.64% of the farms, followed by amitraz and ivermectin, with 36.36% in both cases. Regarding combined resistance, 36.36% of the farms showed resistance to amitraz and alpha-cypermethrin. Their cost for acaricide drugs is 25.34% with respect to the costs of all veterinary inputs with an average 969.86 USD per year. The farms belonging to this node are part of small (Group 1) and medium cattle farms (Groups 3 and 4).

Terminal node C3. This group corresponds to non-technified farms (100.00%), with veterinary control in 41.67% of cases and are not highly infested by ticks. These farms are sensitive to amitraz. Their main problem is resistance to alpha-cypermethrin in 66.67%, followed by ivermectin with 50.00%. Regarding combined resistance, 25.00% of the farms showed resistance to both alpha-cypermethrin and ivermectin. Their cost for acaricide drugs is 44.13% compared to the costs of all veterinary inputs with average 691.19 USD per year. Most of the farms in this terminal node belong to Group 3.

Terminal node D3. This group corresponds to non-technified farms (100.00%), with veterinary control in 54.55% of cases, and are not infested by ticks. All farms are resistant to amitraz, followed by alpha-cypermethrin in 63.64% of farms and ivermectin (36.36%). Regarding combined resistance, 63.64% of the farms showed resistance to both amitraz and alpha-cypermethrin. Their cost for acaricide drugs is 30.95% of the costs of veterinary inputs (691.19 USD per year). Most of the farms in this terminal node belong to Group 4.

Terminal node E3. This group corresponds to non-technified farms (81.82%), with veterinary control in 81.82% of cases, and with high levels of tick infestation (100.00%). Its main problem is resistance to amitraz in 54.55% of the farms, followed by alpha-cypermethrin (45.45%) and ivermectin (36.36%). Regarding combined resistance, 36.36% of the farms showed resistance to both amitraz and alpha-cypermethrin. Their cost for acaricide drugs is 48.60% of the costs of veterinary inputs, which were 568.37 USD on average per year. Most of the farms in this terminal node belong to Group 1.

Terminal node F3. This group corresponds to non-technified farms (64.29%), with veterinary control in 92.86% of cases, and with high levels of tick infestation (100.00%). The farms are resistant to amitraz in 35.71%, followed by ivermectin (28.57%). In addition, they are sensitive to alpha-cypermethrin. Regarding combined resistance, 21.43% of the farms showed resistance to both amitraz and ivermectin. Their cost for acaricide drugs is 30.74% with respect to the costs of veterinary inputs, on average 1286.04 USD per year. The farms belonging to this node are part of small (Group 1) and medium non-technified cattle farms (Group 3).

Terminal node G3. This group corresponds to non-technified farms (70.00%), with veterinary control in 60.00% of cases, and with high levels of tick infestation (100.00%). Their main problem is resistance to

alpha-cypermethrin in all farms, followed by amitraz (60.00%) and ivermectin (40.00%). Regarding combined resistance, 60.00% of the farms showed resistance to both amitraz and alpha-cypermethrin. Their cost for acaricide drugs is 44.28% of the costs of veterinary inputs, which was on average 736.66 USD per year. Most of the farms in this terminal node belong to Group 2.

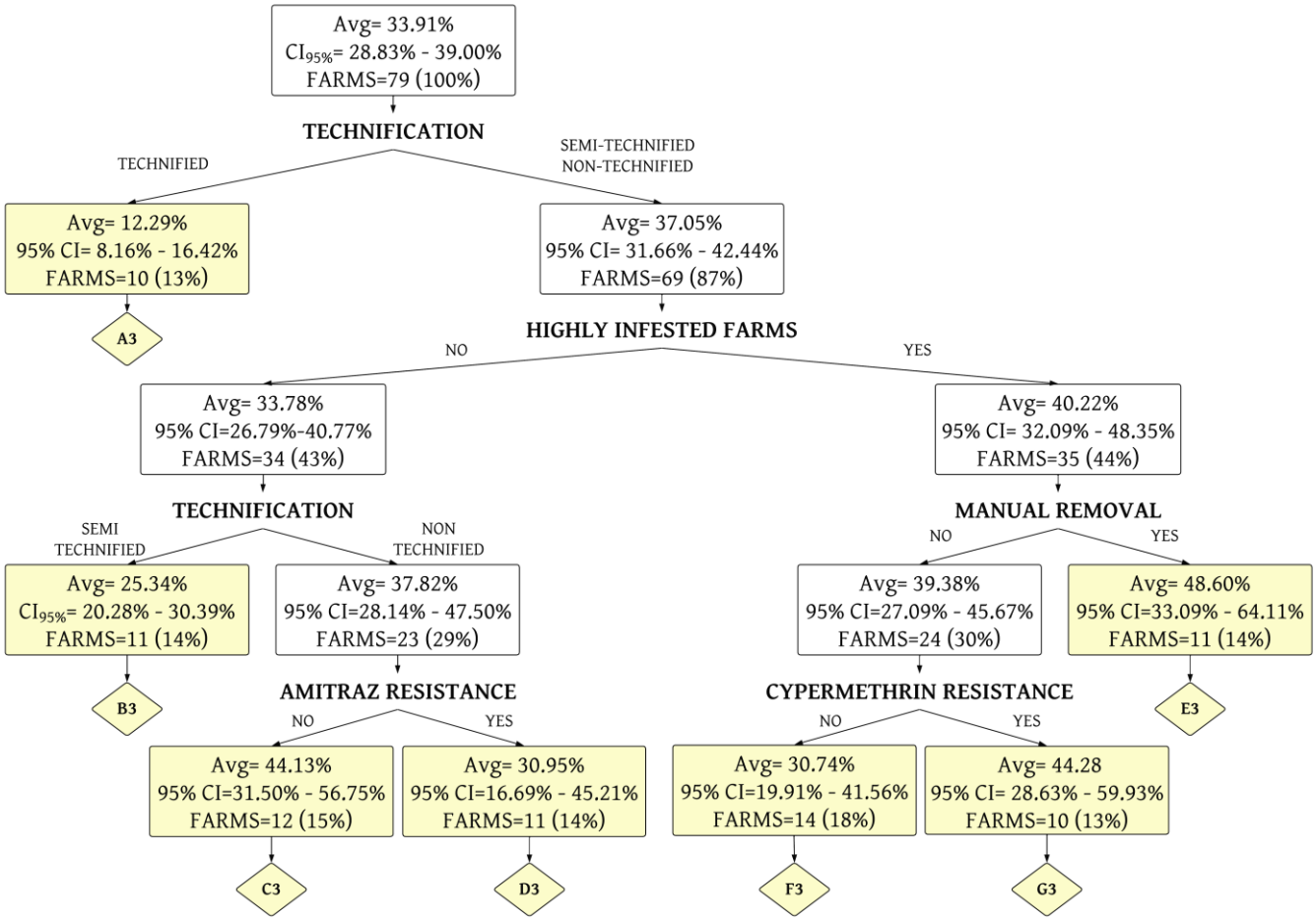

Fig 7. Decision tree analysis of the percentage of drug costs (E6) allocated to tick and TBDs control. A3 until G3 are the terminal nodes of the tree (yellowboxes). The average (Avg) corresponds to the % of E6 used for tick and TBDs control. CI is the confidence interval.

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Impact of acaricide resistance and tick infestation on sanitary costs. The percentage of acaricide treatment costs to management variables was grouped into seven homogeneous terminal nodes using a decision tree analysis (Fig 8 and S4 Table). This model presented a R2 of 0.37 and MSE of 15.91% in training data. The R2 was 0.38 and MSE was 16.80% in testing data. The seven terminal nodes are described below.

Terminal node A4. This group corresponds to technified farms (100.00%), with veterinary control in 87.50% of cases; high level of tick infestation in 25.00% of farms. The main problem is resistance to amitraz in 75.00% of the farms, followed by alpha-cypermethrin (50.00%) and ivermectin (25.00%). Regarding combined resistance, 50.00% of the farms showed resistance to both amitraz and alpha-cypermethrin. Their cost of acaricide treatment is 13.41% of the 2975.83 USD on the average annual cost of veterinary inputs. Most of the farms in this terminal node belong to Group 5.

Terminal node B4. This group corresponds to semi-technified farms (100.00%), with veterinary control in all cases; a high level of tick infestation in 40.00% of farms. Its main problem is resistance to alpha-cypermethrin and ivermectin, with 70.00% in both cases, followed by amitraz with 60.00%. Regarding combined resistance, 60.00% of the farms showed resistance to amitraz, alpha-cypermethrin, and ivermectin.

Their cost of acaricide treatment is 23.97% of the 934.49 USD on average annual of veterinary inputs. The farms belonging to this node are part of small (Group 1) and medium semi-technified cattle farms (Group 4).

Terminal node C4. This group corresponds to non-technified farms (100.00%), with veterinary control in 86.67% of cases; a high level of tick infestation in 66.67% of farms. Its main problem is resistance to alpha-cypermethrin with 53.33%, followed by amitraz (33.33%) and ivermectin (13.33%). Regarding combined resistance, 20.00% of the farms showed resistance to both amitraz and alpha-cypermethrin. Their cost of acaricide treatment is 32.49% of the 1251.32 USD on average annual of veterinary input costs. Most of the farms in this terminal node belong to Group 3.

Terminal node D4. This group corresponds to non-technified farms (61.54%), with veterinary control in 38.46% of cases; a high level of tick infestation in 46.15% of farms. These farms are sensitive to amitraz; its main problem is resistance to alpha-cypermethrin and ivermectin, with 38.46% in both cases. Regarding combined resistance, only 7.69% of the farms showed resistance to both alpha-cypermethrin and ivermectin. Their cost of acaricide treatment is 37.25% of the 2322.71 USD on average annual of veterinary input costs. Most of the farms in this terminal node belong to Group 3.

Terminal node E4. This group corresponds to non-technified farms (75.00%), with veterinary control in 25.00% of cases; a high level of tick infestation in 41.67% of farms. All farms are resistant to amitraz, followed by alpha-cypermethrin in 75.00% of farms and ivermectin (50.00%). Regarding combined resistance, 75.00% of the farms showed resistance to both amitraz and alpha-cypermethrin. Their cost of acaricide treatment is 49.17% of the 1331.13 USD on average annual of veterinary inputs. The farms belonging to this node are part of medium cattle farms (Groups 2, 3 and 4).

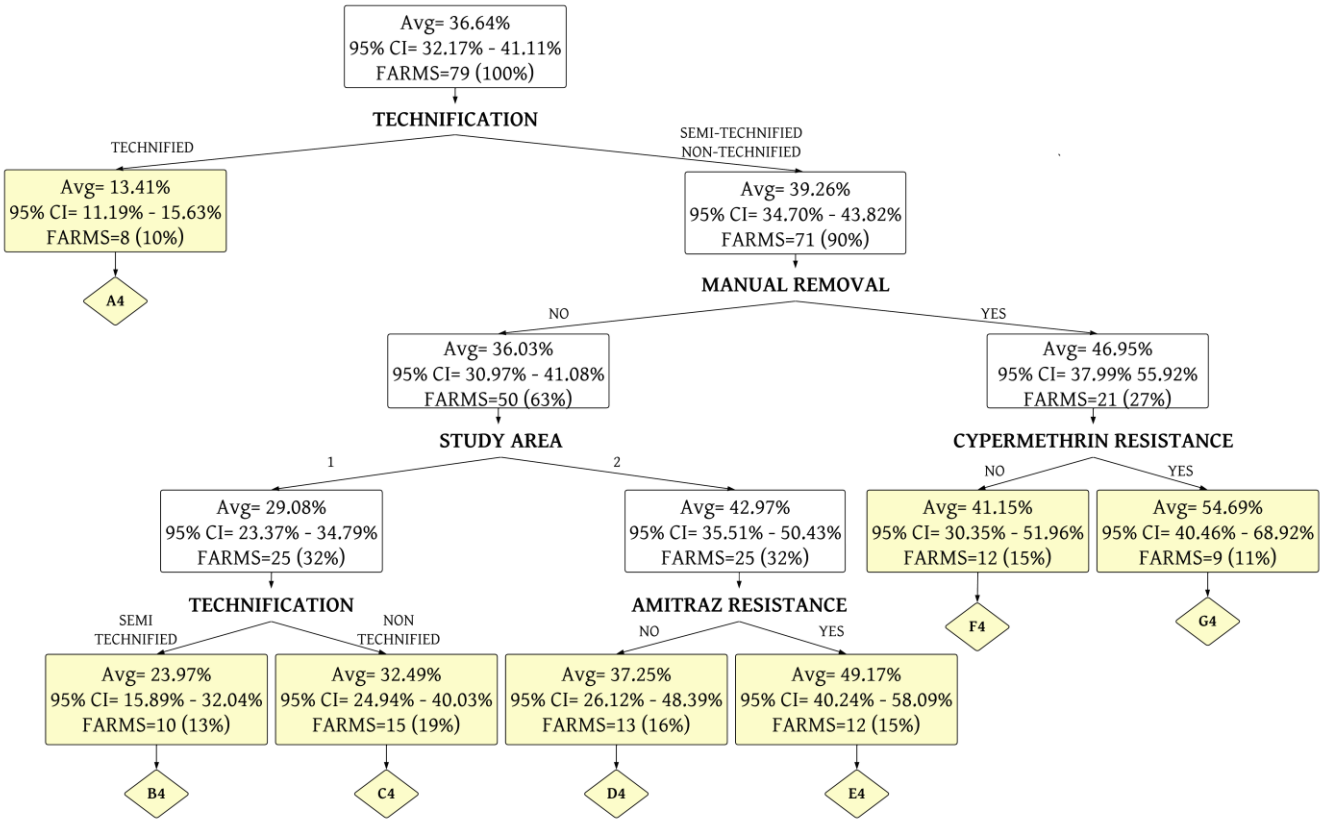

Fig 8. Decision tree analysis of the percentage of sanitary costs allocated to acaricide treatment (Model 4). A4 until G4 are the terminal nodes of the tree (yellow boxes). The average (Avg) corresponds to the % of sanitary costs destined for the acaricide treatment. CI is the confidence interval.

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Terminal node F4. This group corresponds to non-technified farms (75.00%), with veterinary control in 91.67% of cases; a high level of ticks infestation in 50.00% of farms. The farms are resistant to amitraz in 41.67%, followed by ivermectin (3.33%). In addition, they are sensitive to alpha-cypermethrin. Regarding combined resistance only, 8.33% of the farms showed resistance to amitraz, alpha-cypermethrin, and ivermectin. Their cost of acaricide treatment is 41.15% of the 1563.37 USD on average annual of veterinary inputs. Most of the farms in this terminal node belong to Group 1.

Terminal node G4. This group corresponds to non-technified farms (77.78%), with veterinary control in 55.56% of cases; a high level of ticks infestation in 44.44% of farms. Its main problem is resistance to alpha-cypermethrin in all farms, followed by amitraz (55.56%) and ivermectin (33.33%). Regarding combined resistance, 55.56% of the farms showed resistance to both amitraz and alpha-cypermethrin. Their cost of acaricide treatment is 54.69% of the 917.42 USD on average annual of veterinary inputs. The farms belonging to this node are part of small (Group 1) and medium cattle farms (Groups 3 and 4).

4. Discussion

Tick infestation and acaricide resistance

Although there was no statistically significant difference between the presence or absence of tick infestation and acaricide resistance (Table 3 and Table 4), the decision trees indicated that farms without acaricide resistance had higher levels of tick infestation, showing that this effect is more complex than the direct effect. This effect can be associated with the fact that for successful tick control requires, both the parasitic and non-parasitic phases must be controlled. The lack of control of the non-parasitic phase results in a high population of tick larvae remaining in the paddocks and rapidly reinfesting animals after chemical control [41, 42], regardless of whether the farm is resistant to acaricides or not. The classification model (Fig 2) presented an AUC = 0.64.

The AUC is an important metric for classification, and it is often used as a measure of model performance. In our tree model, the level of technology had an important inverse relationship with the presence of high infestation and resistance to acaricides. Farms with higher technification are less infested (25.00%) than semi-technified and non-technified farms, as reported previously [28].

Resistance to acaricides results showed that 49.52% of farms had resistance to amitraz, 53.33% to alpha-cypermethrin, and 37.14% to ivermectin, respectively. Rodríguez-Hidalgo et al. (2017) [15] already reported resistance to these acaricides in 67.00 (amitraz), 50.00% (cypermethrin), and 25.00% (ivermectin) of the farms, using a similar methodology in another subtropical zone. The resistance levels are similar to those reported in this study. The amitraz resistance allele in *R. microplus* was also found in 62% of farms of other Ecuadorian zone (Santo Domingo de los Tsáchilas) indicating its widespread character [14].

Amitraz is considered the first line of action for tick control [43]. However, observing that its inefficacy, farmers opt for other acaricides applied in bath spray, such as alpha-cypermethrin or organophosphates. In Ecuador, alpha-cypermethrin and amitraz are marketed under different trade names and active ingredients (single or combined with other acaricides). This situation makes the farmer believe that he is rotating the active ingredient but, he is using the same one, which encourages increasing resistance. Also, it is well known that pyrethroids insecticides create a rapid evolution towards their [44, 45]. Similar situations occur with the use of ivermectin. Ivermectin and doramectin are the only injectable antiparasitic products marketed for the control of endo- and ectoparasites in cattle [46].

Farm typology and economic analysis

Five groups of farms were formed, and they were classified as small farms (one group), medium farms (three groups), and large farms (one group). Similarly to other studies [47–49], labour force and

supplementary feeding are the most important items used for the classification of farmers, representing 43.42% and 26.03% of the total costs, respectively.

While on large farms, the main item for the classification is the cost of supplemental feed, on small and medium farms, it is the labour force. In both groups, grazing is the main source of animal feed [50, 51]. In groups 1, 2, and 4, unpaid family labour accounts for more than 70% of the labour force, consistent with previous studies realised by Paez (2001) [52] and Posadas et al. (2014) [53], where family labour predominates in farms with low productive intensity. The use of family labour on these farms represents a strategy to take advantage of the human capital of the family nucleus [53], and this characteristic has been observed in groups outside the labour market and where it is difficult to find a paid job, either because of their age or because they could not be employed full-time [54].

As for the administration by women on the farms studied, their presence was determined, but there was no majority in any group. Female management occupies values close to 50% in Group 1 (47.52%) and Group 2 (47.06%) farms. However, it corresponds to small and medium farms that are not technified.

The costs of feeding are the main costs of livestock farming and are associated with farmers trying to make up for the nutrient deficiency of the pastures or low paddock availability, by using concentrates, silage, hay, and by-products (brewer's bran, molasses). Moreover, farmers spend less than 1% of the costs in purchasing fertilisers or herbicides for the paddocks, so that they prefer to invest directly in the animals instead of the pastures. In addition, tropical and subtropical livestock farming in developing countries, and in Ecuador in particular, usually is developed in vulnerable areas, where soils have few nutrients and where sustaining pastures for a long time seems difficult [55].

The impact of veterinary inputs (E6) on these dairy production systems is approximately 8%, in agreement with Carmona and Vindas (2007) [56], where this cost ranged between 5–8%. This cost ranks third from the total costs, behind the cost of labour and supplementary feed. Rees et al. [57] and Tang et al. [58] mentioned that reducing the use of drugs in livestock contributes to reducing antimicrobial resistance in both human and animal populations. On the other hand, the cost of veterinary services is among what the farmer spends the least on (1.25%). If the opportunity cost (subsidised veterinary services) would be considered, it will increase to 1.99%. These two values show farmers' low use of veterinary services, regardless of whether they pay for them or not. Romero and Villamil [59], found that in developing countries, most farmers rarely use veterinary services and rely first on their experience, followed by the farm worker or neighbours.

The cost of production per litre of milk (CPM) is 0.30 USD (95% CI: 0.17–0.43), similar to Hoyos et al. [49], where the weighted average was 0.27 USD. It should be emphasised that in our study, the CPM was determined by limiting the family labour force to the number of people needed to carry out livestock work. Other current studies conducted in the country indicate that the CPM is between 0.35 USD [60] and 0.43 USD [47]. However, these data vary widely depending on the area where the study was conducted and the size of the producers [49]. Within the groups, group 3 stands out as the most efficient. In this group, the average CPM of 0.25 USD is lower than the average cost of groups 4 and 5, which are more technified groups. In addition to this, group 3 produces 14,000 litres of milk more per year compared to group 4, which has a similar amount of land. We relate a higher CPM of group 4 to the fact that this group uses family labour in the management, which is not efficient and increases production costs, as labour is one of the main elements of livestock production. Although group 4 is semi-technified, most farms belong to the Quijos Valley Zone, where the parish government provides veterinary technical advice, free insemination, and in many cases, provides infrastructure; these parameters mean that this group is classified as semi-technified despite not having mechanical management in most cases. In addition, the location of most of the farms in group 3 in the northwest of Pichincha, where there is relatively better pasture quality due to the climate and soil quality, means that this group has a lower CPM and produces a higher quantity of milk than group 4.

On the other hand, in group 5, despite having more land and technology, the average production cost is higher than in group 3, which was associated with the fact that although there is a large extension of land, farmers are not able to give good maintenance to the pastures [61], which decreases the efficiency in milk production.

Most farmers deliver their production to the local milk industry or to local cheese industry and receive on average 0.37 USD per litre for the raw milk. According to Ecuador's Ministerial Resolution 394, the price per litre of milk corresponds to 52.4% of the retail price of UHT milk (0.80 USD) plus quality bonuses, which represents a remuneration near to 0.42 USD per litre of raw milk [62]. As observed in this and other studies [49, 60, 63], this base price is not met in most cases. Only group 5, large farms that deliver milk to the regional industry receive on average of 0.47 USD per litre of milk usually because of the farm location and better milk quality. In addition, the regional industries recognise and pay the bonuses established by the state for being certified brucellosis and tuberculosis free farms (0.01 USD per litre of raw milk) and certified farms with good livestock practices (0.02 USD per litre of raw milk) [62]. From the other hand, the low remuneration registered in this study may also be associated with the fact that the data was collected in 2020–2021, during the COVID-19 pandemic. Furthermore, according to our reports, 69.5% of farmers reported decreased income from milk sales due to the pandemic. As a result, milk collectors decreased the payment per litre of milk (indirect sales) or limited the volume of milk to be commercialised.

Impact of acaricide resistance and tick infestation on acaricide drug and treatment costs.

When determining the percentage of veterinary inputs used for acaricide control (Model 3), it was observed that the semi-technified farms, despite not having animals highly infested animals by ticks, spent more than twice (25.34%) the costs of acaricide products compared to the technified farms, which spend less for this purpose (12.29%). Similar results are obtained in model 4, which analyses the acaricide drugs and labour used for acaricide treatment. Here, technified farms allocate a lower percentage of sanitary expenses (13.41%) to this activity compared to semi-technified (23.97%) and non-technified farms with (32.49%). In non-technified farms when observing the results of farms with and without resistance to amitraz in Model 3, farms without resistance (C3) spend a higher percentage of veterinary inputs (44.13%) in comparison with farms with resistance to amitraz (D3), which spend 30.95% of this cost. In could be due to the use of more expensive acaricides in those groups. When labour costs were included in Model 4 (as part of model prediction) farms with resistance (E4) spent a higher percentage 49.17% of the sanitary cost to control ticks in relation to farms without resistance to amitraz (37.25%), belonging to terminal node D4. In both models, it is observed that the farms corresponding to terminal nodes C3, D3 (Model 3) and D4, E4 (Model 4) share similar characteristics in terms of the level of low levels of technology and low level of tick infestation in animals. However, they differ in terms of resistance to other acaricides. Farms with higher acaricide expenditure (G3 and E4) are farms that have higher resistance to alpha-cypermethrin and ivermectin. This suggests that lower costs in tick control with acaricide is not only prevented by having sensitivity to amitraz; it is also the sum of good management use of alpha-cypermethrin and ivermectin; those farms, in most cases, did not have high level of tick infestation.

On the other hand, when analysing the terminal nodes of the farms with and without resistance to cypermethrin, the nodes are made up of farms with low or medium technification, and also, it was found that those farms have the highest percent of expenditure are those with resistance to alpha-cypermethrin (G3 and G4) in relation to those without resistance (F3 and F4). When analysing the typology of these farms, they have similarities in terms of the presence of high level of tick infestation (F3 and G3 = 100.00%; F4 and G4 = 50.00% and 44.44%, respectively) but differ in terms of veterinary control. Therefore, in these nodes, the farms with a lower percentage of expenditures for tick control are the farms with higher veterinary control. In the terminal nodes of Model 3, formed based on resistance or non-resistance to

amitraz, farms with resistance to amitraz (C3) did not generate higher expenses than those without resistance. This did occur in farms with resistance to alpha-cypermethrin (F3). We associate this with the price difference of these acaricides. The use of alpha-cypermethrin accounts for the majority of expenditures on veterinary inputs, regardless of whether the farms are sensitive or not to amitraz. Acaricides, whose main active ingredient is alpha-cypermethrin, are generally marketed in association with other acaricides and also are applied as pour on animals and have a higher cost than amitraz-based drugs, which usually are not marketed in association with other ingredients. Cypermethrin's are also seen by farmers as a rapid action product [64].

Model 1 shows that the cost of acaricide treatment varies according to the technification of the farms. Technified farms have a lower expenditure on acaricide treatment (1.30%) compared to semi-technified farms (3.43%) and non-technified farms (6.24%). Although resistance to alpha-cypermethrin plays a role in the formation of the decision tree, it does not affect the cost of the acaricide treatment if the farm has resistance. In addition to having or not resistance to alpha-cypermethrin, the cost of acaricide treatment varies according to the presence of high infestation and the degree of technification. Farms with high infestation spend more (4.28%) than farms without high infestation (2.74%). In addition, the labour invested to remove ticks manually increases the cost of on-farm acaricide treatment to 5.75%.

When analysing model 2, the average annual cost of acaricide treatment is 19.41 USD per adult animal per year. However, this cost increases or decreases like the other models depending on the size and level of technification. Large and technified farms (terminal node A2) have a treatment cost per animal of 8.46 USD, much lower than the general average.

These farms have better access to the market and can negotiate prices by buying in volume, and are more efficient at applying acaricide treatments than the other groups. They use pour-on treatments (Fipronil and Fluazuron) and combination acaricides, which are more expensive. However, having fewer treatments per year does not increase the cost of treatment per year. At the other extreme, most small and non-technified farms are distributed in terminal nodes D2 and E2. These farms have a treatment cost per animal of 23.85 USD and 28.44 USD, respectively. About 42% of these farms have bad management of acaricides, mix different commercial presentations and overdose the recommended dose; they are also the groups with the highest number of spray baths per year. The higher cost of treatment on farms in terminal node E2 is associated with a higher percentage of farms with high infestation. In addition, many farms in this group (42.11%) mix commercial presentations, and the majority (89.00%) of the farms are located in the Valle de Los Quijos area, where they do not have the same access to agricultural warehouses where they can buy inputs. The medium-sized farms are distributed in terminal nodes B2 and C2 according to the presence or absence of resistance to alpha-cypermethrin. It is striking that this does not matter at the time of acaricide treatment, as the two groups use a similar percentage of alpha-cypermethrin-based acaricides, and the frequency of acaricide treatment is similar. Therefore, we associate the higher cost of animal treatment per year in terminal node B2 to the fact that there is greater use of treatments in the form of pour-on treatments and that these farms mix acaricides of different commercial presentations.

In general, the cost of annual acaricide treatment per animal varies according to the size and technology of the farms. Moreover, within groups with similar conditions, it varies according to the correct or incorrect use of acaricides (mixture of commercial presentations). Considering the treatment method, the cost of treatment increases on farms where pour-on or acaricides with a higher amount of active ingredients are used. There are several studies where the cost of acaricide treatment is calculated. For example, Frisch et al., in 2000 [65] determined that the cost per year of treating cattle on farms in northern Australia is 8.04 USD, which is close to that found on large, technified farms in our study. Other studies vary in price according to the type of acaricide used and the frequency of spray baths. For example, in Mexico [66],

acaricide treatment with Ivermectin costs 53.23 USD, Organophosphates 8.23 USD and Cypermethrin 5.55 USD.

The typology formed did not fit exactly with the terminal nodes formed. It could be observed that there are very solid groups, such as group 5 of technified farms. However, the rest of the group is distributed in the terminal nodes formed by semi and non-technified farms, but not in a distinctive way, such as group 5 in terminal node 1.

5. Conclusions

In conclusion, models 3 and 4 present similar results, in which we observe the importance of the level of farm technification, alpha-cypermethrin and amitraz resistance in terms of the percentage of money spent on acaricide control. In models 1 and 2, although alpha-cypermethrin resistance is part of the decision tree, when determining the annual cost of acaricide treatment at the farm and animal level, the presence of resistance did not increase the cost of treatment. It was found that in addition to having or not having resistance, the cost varies according to the level of infestation, technification and use of more expensive acaricides. In any model, did the presence or not of resistance to ivermectin play a major role, despite being an antiparasitic not only used to control ticks but also to control frequent internal parasites in the study areas such as Paramphistomum sp. and Fasciola hepatica [67, 68]. This observation is associated with the fact that dairy farmers are aware of the restriction of use in dairy cows and try not to use it or did not report its use in this study. Although the use of ivermectin did not have repercussions on the profitability of the farms, it is known that it can be public health from residues in milk and meat [69, 70]. The present study is one of the few studies describing the economic impact of diseases affecting production animals in Ecuador. Furthermore, it is the first to quantify the economic effects of tick presence. The data obtained in this research confirmed the impact of ticks on cattle farms in subtropical areas, especially on small and medium sized farms, which invested the most money in trying to control the presence of ticks. Therefore, in terms of research, it is recommended that studies be carried out to determine the extent of its use of ivermectin and to assess its putative health consequences on the population that consumes it. In term of decision-making, information campaigns using the present results should be developed in order to help farmers to improve the health status of their animals. Alternatively, the present results can contribute to the development of a control programme of tick infestation for small and medium-sized farms, which are the most affected in terms of the money they invest in controlling ticks.

Supporting information

S1 Table. Characterisation of the farms belonging to the terminal nodes of Model 1. The data correspond to the percentage of farms in each terminal node; Tech = Technified farms; semi = Semi-technified farms; non = Non technified farms; AM = farms with resistance to amitraz; CY = farms with resistance to alpha-cypermethrin; IV = farms with resistance to ivermectin; AM and CY = Farms with resistance to amitraz and alpha-cypermethrin; AM and IV = Farms with resistance to amitraz and ivermectin; CY and IV = Farms with resistance to alpha-cypermethrin and ivermectin; AM, CY and IV = Farms with resistance to amitraz, ivermectin, and alpha-cypermethrin.
(DOCX)

S2 Table. Characterisation of the farms belonging to the terminal nodes of Model 2. The data correspond to the percentage of farms in each terminal node; Tech = Technified farms;semi = Semi-technified farms; non = Non technified farms; AM = amitraz; CY = alpha-cypermethrin; IV = ivermectin; ORG = Farms with resistance to amitraz and alpha-cypermethrin; FI = Fipronil (Fenilpirazoles); FLU = Fluazuron (Benzoylphenyl urea). * Buying commercial presentations with more than 2 active ingredients. ** Mixing of 2 or more commercial presentations with different or the same active ingredient.
(DOCX)

S3 Table. Characterisation of the farms belonging to the terminal nodes of Model 3. Thedata correspond to the percentage of farms in each terminal node; Tech = Technified farms; semi = Semi-technified farms; non = Non technified farms; AM = farms with resistance to amitraz; CY = farms with resistance to alpha-cypermethrin; IV = farms with resistance to ivermectin; AM and CY = Farms with resistance to amitraz and alpha-cypermethrin; AM and IV = Farms with resistance to amitraz and ivermectin; CY and IV = Farms with resistance to alpha-cypermethrin and ivermectin; AM, CY and IV = Farms with resistance to amitraz, ivermectin, and alpha-cypermethrin.
(DOCX)

S4 Table. Characterisation of the farms belonging to the terminal nodes of Model 4. The data correspond to the percentage of farms in each terminal node; Tech = Technified farms; semi = Semi-technified farms; non = Non technified farms; AM = farms with resistance to amitraz; CY = farms with resistance to alpha-cypermethrin; IV = farms with resistance to ivermectin; AM and CY = Farms with resistance to amitraz and alpha-cypermethrin; AM and IV = Farms with resistance to amitraz and ivermectin; CY and IV = Farms with resistance to alpha-cypermethrin and ivermectin; AM, CY and IV = Farms with resistance to amitraz, ivermectin, and alpha-cypermethrin.
(DOCX)

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Author Contributions

Conceptualization: Valeria Paucar-Quishpe, Ximena Pérez-Otáñez, Richar Rodríguez- Hidalgo, Darío Cepeda-Bastidas, Cecilia Pérez-Escalante, Jorge Grijalva-Olmedo, Sophie O. Vanwambeke, Claude Saegerman, Lenin Ron-Garrido.

Data curation: Valeria Paucar-Quishpe, Ximena Pérez-Otáñez, Claude Saegerman, Lenin Ron-Garrido.

Formal analysis: Valeria Paucar-Quishpe, Claude Saegerman.

Funding acquisition: Richar Rodríguez-Hidalgo, Sophie O. Vanwambeke, Claude Saegerman, Lenin Ron-Garrido.

Investigation: Valeria Paucar-Quishpe, Ximena Pérez-Otáñez, Richar Rodríguez-Hidalgo.

Methodology: Valeria Paucar-Quishpe, Lenin Ron-Garrido.

Project administration: Richar Rodríguez-Hidalgo, Sophie O. Vanwambeke, Claude Saegerman.

Resources: Richar Rodríguez-Hidalgo, Cecilia Pérez-Escalante, Jorge Grijalva-Olmedo, Sandra Enríquez, Susana Arciniegas-Ortega, Luis Sandoval-Trávez, Bryan Benavides-Erazo, Sophie O. Vanwambeke, Claude Saegerman, Lenin Ron-Garrido.

Software: Valeria Paucar-Quishpe, Claude Saegerman, Lenin Ron-Garrido.

Supervision: Darío Cepeda-Bastidas, Claude Saegerman, Lenin Ron-Garrido.

Validation: Valeria Paucar-Quishpe, Ximena Pérez-Otáñez, Darío Cepeda-Bastidas, Claude Saegerman, Lenin Ron-Garrido.

Visualization: Valeria Paucar-Quishpe, Darío Cepeda-Bastidas, Claude Saegerman, Lenin Ron-Garrido.

Writing – original draft: Valeria Paucar-Quishpe.

Writing – review & editing: Ximena Pérez - Otáñez, Richar Rodríguez -Hidalgo, Darío Cepeda-Bastidas, Cecilia Pérez -Escalante, Jorge Grijalva-Olmedo, Sandra Enríquez, Susana Arciniegas-Ortega, Luis Sandoval-Trávez, Bryan Benavides-Erazo, Sophie O. Vanwambeke, Claude Saegerman, Lenin Ron-Garrido.

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7. Supplementary File

TABLE S1. CHARACTERISATION OF THE FARMS BELONGING TO THE TERMINAL NODES OF MODEL 1.

Variable	Terminal nodes of model 1					
	A1	**B1**	**C1**	**D1**	**E1**	**F1**
Veterinary control presence	100.00	85.71	57.14	47.37	54.55	84.21
Highly Infested Farms	25.00	71.43	50.00	0.00	100.00	42.11
Manual tick removal	62.50	0.00	0.00	0.00	0.00	100.00
Presence of external paddocks	25.00	42.86	35.71	5.26	54.55	68.42
Level of technification	tech	semi	non	non	non	non
AM Resistance	50,00	14,29	42,86	57,89	54,55	52,63
IV Resistance	12,50	42,86	35,71	47,37	45,45	31,58
CY Resistance	37,50	0,00	0,00	100,00	100,00	42,11
AM and CY Resistance	37,50	0,00	0,00	57,89	54,55	26,32
AM and IV Resistance	12,50	14,29	14,29	36,84	18,18	21,05
CY and IV Resistance	12,50	0,00	0,00	47,37	45,45	15,79
AM, CY and IV Resistance	12,50	0.00	0,00	36,84	18.18	15.79
Study area 1	62,50	57,14	57,14	42,11	63,64	68,42
Study area 2	37,50	42,86	42,86	57,89	36,36	31,58
Typology Group 1	12,50	14,29	14,29	5,26	27,27	42,11
Typology Group 2	0,00	28,57	21,43	10,53	36,36	15,79
Typology Group 3	0,00	14,29	42,86	36,84	18,18	10,53
Typology Group 4	0,00	14,29	21,43	47,37	9,09	26,32
Typology Group 5	87,50	28,57	0,00	0,00	9,09	5,26

The data correspond to the percentage of farms in each terminal node; Tech=Technified farms; semi=Semi-technified farms; non=Non technified farms; AM = farms with resistance to amitraz; CY = farms with resistance to alpha-cypermethrin; IV = farms with resistance to ivermectin; AM and CY= Farms with resistance to amitraz and alpha-cypermethrin; AM and IV= Farms with resistance to amitraz and ivermectin; CY and IV= Farms with resistance to alpha-cypermethrin and ivermectin; AM, CY and IV= Farms with resistance to amitraz, ivermectin, and alpha-cypermethrin.

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TABLE S2. CHARACTERISATION OF THE FARMS BELONGING TO THE TERMINAL NODES OF MODEL 2.

Variable	Terminal nodes of model 2				
	A2	B2	C2	D2	E2
Veterinary control presence	87.50	42.86	52.17	65.29	84.21
Highly Infested Farms	37.50	57.14	52.17	35.71	52.63
Manual tick removal	50.00	0.00	0.00	100.00	52.63
Presence of external paddocks	12.50	35.71	26.09	78.57	57.89
Level of technification	tech	non	non	non	non
AM Resistance	75.00	35.71	47.83	50.00	57.89
IV Resistance	25.00	35.71	47.83	42.86	47.37
CY Resistance	62.50	0.00	100.00	57.14	47.37
AM and CY Resistance	62.50	0.00	47.83	42.86	36.84
AM and IV Resistance	25.00	14.29	26.09	28.57	31.58
CY and IV Resistance	25.00	0.00	47.83	28.57	26.32
AM, CY and IV Resistance	25.00	0.00	26.09	28.57	21.05
Study area 1	25.00	35.71	39.13	42.86	89.47
Study area 2	75.00	64.29	60.87	57.14	10.53
Typology Group 1	0.00	14.29	8.70	42.86	42.11
Typology Group 2	0.00	14.29	17.39	21.43	31.58
Typology Group 3	0.00	50.00	39.13	21.43	0.00
Typology Group 4	0.00	14.29	26.09	14.29	26.32
Typology Group 5	100.00	7.14	8.70	0.00	0.00
Use of acaricide treatment with AM	25,00	42,86	52,17	78,57	63,16
Use of acaricide treatment with CY	50,00	64,29	60,87	64,29	89,47
Use of acaricide treatment with ORG	0,00	64,29	43,48	78,57	47,37
Use of acaricide treatment with IV	50,00	78,57	78,26	92,86	63,16
Use of acaricide treatment with FI	25,00	35,71	17,39	21,43	26,32
Use of acaricide treatment with FLU	25,00	35,71	8,70	14,29	5,26
Use of pour-on acaricide	50.00	64.29	26.09	35.71	26.32
Frequency of spraying baths (Days)	53.42	28.52	26.74	15.79	19.63
Number of active ingredients (acaricides) used annually	1.88	3.29	2.61	3.50	2.95
Use of acaricides with more than 2 active ingredients*	50.00	57.14	60.87	71.43	84.21
Incorrect dosage of acaricides	12.50	71.43	65.22	42.86	42.11
Subdoses	0.00	14.29	8.70	7.4	5.26
Overdose	0.00	50.00	56.52	35.71	36.84
Mixture of acaricides**	12.50	35.71	17.39	21.43	42.11

The data correspond to the percentage of farms in each terminal node; Tech=Technified farms; semi=Semi-technified farms; non=Non technified farms; AM =amitraz; CY = alpha-cypermethrin; IV = ivermectin; ORG= Farms with resistance to amitraz and alpha-cypermethrin; FI= Fipronil (Fenilpirazoles); FLU= Fluazuron (benzoylphenyl urea). * Buying commercial presentations with more than 2 active ingredients. ** Mixing of 2 or more commercial presentations with different or the same active ingredient.

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TABLE S3. CHARACTERISATION OF THE FARMS BELONGING TO THE TERMINAL NODES OF MODEL 3.

Variable	Terminal nodes of the model 3						
	A3	**B3**	**C3**	**D3**	**E3**	**F3**	**G3**
Veterinary control presence	90.00	72.73	41.67	54.55	81.82	92.86	60.00
Highly Infested Farms	20.00	0.00	0.00	0.00	100.00	100.00	100.00
Manual tick removal	70.00	27.27	25.00	45.45	100.00	0.00	0.00
Presence of external paddocks	30.00	36.36	16.67	9.09	72.73	57.14	60.00
Level of technification	tech	semi	non	non	non	non	non
AM Resistance	70.00	36.36	0.00	100.00	54.55	35.71	60.00
IV Resistance	20.00	36.36	50.00	36.36	36.36	28.57	40.00
CY Resistance	50.00	63.64	66.67	63.64	45.45	0.00	100.00
AM and CY Resistance	50.00	36.36	0.00	63.64	36.36	0.00	60.00
AM and IV Resistance	20.00	27.27	0.00	36.36	18.18	21.43	20.00
CY and IV Resistance	20.00	27.27	25.00	27.27	18.18	0.00	40.00
AM, CY and IV Resistance	20.00	27.27	0.00	27.27	18.18	0.00	20.00
Study area 1	50.00	72.73	41.67	45.45	81.82	71.43	80.00
Study area 2	50.00	27.27	58.33	54.55	18.18	28.57	20.00
Typology Group 1	10.00	27.27	8.33	9.09	45.45	35.71	20.00
Typology Group 2	0.00	9.09	25.00	18.18	18.18	14.29	50.00
Typology Group 3	0.00	27.27	41.67	9.09	18.18	28.57	20.00
Typology Group 4	0.00	36.36	25.00	63.64	18.18	14.29	10.00
Typology Group 5	90	0.00	0.00	0.00	0.00	7.14	0.00

The data correspond to the percentage of farms in each terminal node; Tech=Technified farms; semi=Semi-technified farms; non=Non technified farms; AM = farms with resistance to amitraz; CY = farms with resistance to alpha-cypermethrin; IV = farms with resistance to ivermectin; AM and CY= Farms with resistance to amitraz and alpha-cypermethrin; AM and IV= Farms with resistance to amitraz and ivermectin; CY and IV= Farms with resistance to alpha-cypermethrin and ivermectin; AM, CY and IV= Farms with resistance to amitraz, ivermectin, and alpha-cypermethrin.

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TABLE S4. CHARACTERISATION OF THE FARMS BELONGING TO THE TERMINAL NODES OF MODEL 4.

Variable	Terminal nodes of model 4						
	A4	**B4**	**C4**	**D4**	**E4**	**F4**	**G4**
Veterinary control presence	87.50	100.00	86.67	38.46	25.00	91.67	55.56
Highly Infested Farms	25.00	40.00	66.67	46.15	41.67	50.00	44.44
Manual tick removal	75.00	0.00	0.00	0.00	0.00	100.00	100.00
Presence of external paddocks	37.50	50.00	46.67	0.00	25.00	58.33	55.56
Level of technification	tech	semi	non	non	non	non	non
AM Resistance	75.00	60.00	33.33	0.00	100.00	41.67	55.56
IV Resistance	25.00	70.00	13.33	38.46	50.00	3.33	33.33
CY Resistance	50.00	70.00	53.33	38.46	75.00	0.00	100.00
AM and CY Resistance	50.00	50.00	20.00	0.00	75.00	0.00	55.56
AM and IV Resistance	25.00	50.00	6.67	0.00	50.00	8.33	33.33
CY and IV Resistance	25.00	50.00	6.67	7.69	50.00	0.00	33.33
AM, CY and IV Resistance	25.00	40.00	0.00	0.00	50.00	0.00	33.33
Study area 1	37.50	100.00	100.00	0.00	0.00	83.33	55.56
Study area 2	62.50	0.00	0.00	100.00	100.00	16.67	44.44
Typology Group 1	12.50	27.27	20.00	0.00	8.33	41.67	33.33
Typology Group 2	0.00	18.18	20.00	15.38	25.00	16.67	11.11
Typology Group 3	0.00	9.09	40.00	46.15	33.33	8.33	22.22
Typology Group 4	0.00	36.36	20.00	15.38	33.33	33.33	33.33
Typology Group 5	87.50	0.00	0.00	23.08	0.00	0.00	0.00

The data correspond to the percentage of farms in each terminal node; Tech=Technified farms; semi=Semi-technified farms; non=Non technified farms; AM = farms with resistance to amitraz; CY = farms with resistance to alpha-cypermethrin; IV = farms with resistance to ivermectin; AM and CY= Farms with resistance to amitraz and alpha-cypermethrin; AM and IV= Farms with resistance to amitraz and ivermectin; CY and IV= Farms with resistance to alpha-cypermethrin and ivermectin; AM, CY and IV= Farms with resistance to amitraz, ivermectin, and alpha-cypermethrin.

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Experimental section

Study 3

Farmers' adoption, knowledge, and perceptions of tick control measures on dairy farms in subtropical areas of continental Ecuador

Transboundary and Emerging Diseases 2024, 50232404.

Valeria Paucar-Quishpe, Ximena Pérez-Otáñez, Richar Rodríguez-Hidalgo, Cecilia Pérez-Escalante, Darío Cepeda-Bastidas, Jorge Grijalva, Sandra Enríquez, Susana Arciniegas-Ortega, Sophie O. Vanwambeke, Lenin Ron-Garrido, Claude Saegerman

Preamble

In the field of tropical dairy cattle management, the effectiveness of tick control strategies depends largely on the adoption, understanding and perception of these measures by farmers. Using surveys and meetings, we have explored correlations between management practices and tick-related outcomes. The present study aimed to describe tick control practices prevalent among dairy farmers and to discern their correlation with tick infestation and acaricide resistance.

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Research Article

Farmers' adoption, knowledge, and perceptions of tick control measures on dairy farms in subtropical areas of continental Ecuador

Valeria Paucar[1,2] **Ximena Pérez-Otáñez**[1,3] **Richar Rodríguez-Hidalgo**[1,4] **Cecilia Perez**[4] **Darío Cepeda-Bastidas**[5] **Jorge Grijalva**[4] **Sandra Enríquez**[1] **Susana Arciniegas-Ortega**[6] **Sophie O. Vanwambeke**[3] **Lenin Ron-Garrido**[1,4,†] **and Claude Saegerman**[2]

[1]*Zoonosis Research Institute (CIZ), Central University of Ecuador, Quito 170521, Ecuador*
[2]*Research Unit of Epidemiology and Risk Analysis Applied to Veterinary Science (UREAR-ULiège), Fundamental and Applied Research for Animals and Health (FARAH) Center, Faculty of Veterinary Medicine, University of Liege, Liège 4000, Belgium*
[3]*Georges Lemaitre Centre for Earth and Climate Research, Earth and Life Institute, UCLouvain, Louvain-la-Neuve 1348, Belgium*
[4]*Faculty of Veterinary Medicine and Zootechnics, Central University of Ecuador, Quito 170521, Ecuador*
[5]*Faculty of Agricultural Sciences, Central University of Ecuador, Quito 170521, Ecuador*
[5]*Faculty of Geological, Mining, Petroleum and Environmental Engineering, Central University of Ecuador, Quito 170521, Ecuador*

Correspondence should be addressed to Lenin Ron-Garrido; ljron@uce.edu.ec and Claude Saegerman; claude.saegerman@uliege.be

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The application of tick control strategies on tropical dairy cattle strongly relies on farmers' uptake, knowledge, and perceptions of the efficacy of control measures. This study aims to identify common and uncommon tick control practices employed by dairy farmers in subtropical areas of Ecuador and associate them with the presence of infestation and acaricide resistance. Data were collected through a cross-sectional survey and participatory meetings. Multiple correspondence analysis was used to explore the association between management variables and the level of tick infestation and resistance. It was determined that the main method of acaricide control is still chemical, mainly using spray baths. Generally, when this form of application is used, acaricides are overdosed, in contrast to the pour-on method with underdosage. Among the measures farmers adopt when chemical treatment has failed is to use overdoses of products, mix different acaricides, and use focused treatments (wipe cloth) with irritant substances. The absence of a high level of infestation was related to acaricide dips every 3–4 weeks and the use of intensive grazing. On the other hand, the high infestation was related to the use of organophosphates, wipe cloth application, and the report of tick-borne diseases (TBDs). A small group of farmers have good knowledge and seek alternatives to chemical control, experimenting with biological controls, herbal extracts, manual tick removal, and paddock control. Additionally, farmers reported the presence of TBDs (47%) and the presence of animals poisoned by acaricides (6%), which died in 75% of those cases. Farmers frequently mentioned that tick infestation induces milk drop production and weight loss and is associated with the presence of TBDs. This information is crucial to improve tick control management in Ecuador, particularly through implementing practices that mitigate resistance to acaricides and ensure long-term solutions that help maintain the efficacy of tick control treatments.

1. Introduction

The cattle tick *Rhipicephalus microplus* is a major cause of concern for cattle breeding in the tropical and subtropical areas of the world [1]. Although there are different methods of tick control in cattle, such as immunological control through vaccines, selection of tick-resistant cattle breeds, grazing management, manual removal of ticks, biological control, and the use of ethno-veterinary practices (herbal extracts), chemical control remains the primary method for tick control [2, 3].

In Ecuador, *R. microplus* is the main cattle tick [4, 5, 6, 7], and several acaricides are available for its control, with different active compounds, modes of action (Table 1), and application forms. A wide range of active compounds exerts their action at multiple points in the nervous system of ticks [17]. The first acaricides to be introduced in Ecuador were organophosphates, amides, and synthetic pyrethroids. By the end of the 1990s, macrocyclic lactones and phenylpyrazolones were already available on the market [18], and in the 2000s, the acaricide fluazuron (benzoylphenyl urea), belonging to a new category called insect growth regulators, was introduced to the market in Ecuador [18]. Unlike the acaricides mentioned above, fluazuron inhibits the molting process of tick larvae to nymphs and nymphs to adults [19, 20]. Although the choice of acaricide treatment to be used on the farm will depend mainly on the farmers, they usually receive advice from public and private veterinarians and commercial representatives of products. In addition to chemical control, the Gavac vaccine entered the market in 2022. However, farmers still lack confidence in its effectiveness as a control method due to the investment involved in its application, its unknown efficacy, and the fact that it must be used with a chemical treatment [21].

Although the chemical method was initially considered the primary strategy to control tick infestations, its inadequate management has led to the emergence of acaricide resistance and incurred additional costs associated with reported cases of resistance in the country [6, 7, 22, 23]. Furthermore, there is a concern about environmental contamination and the potential presence of acaricide residues in dairy and meat products, despite no reported cases in Ecuador, as their existence is known [24, 25, 26].

These problems highlight the need for refining the practices and open the window for alternative approaches to control tick infestations. Integrated tick management (ITM) consists of a combination of tools and strategies to manage tick infestations while maintaining adequate levels of animal production [27, 28, 29]. Implementing these strategies requires the appropriate acaricide management to ensure effective and sustainable control practices [30]. The ITM's success depends on individual actions, specifically the acceptance and application of recommendations provided by technicians, as well as on government policies that implement extension programs in the livestock industry, which are currently limited or nonexistent for small farmers [31, 32, 33].

Jack et al. [30] mentioned that several fundamental factors are involved in the process of implementing new control methods, such as farmer characteristics (knowledge, motivations, economics), local support organization (resources, priorities), and the interventions employed (training, leadership). Likewise, this study was built upon two previous studies that aimed to understand livestock practices and examine the level of tick infestation and the development of acaricide resistance in two subtropical areas in Ecuador with the dairy industry. Considering the challenges faced by the livestock industry in these study areas, this study tries to integrate various aspects, including perceptions, knowledge, infestation levels, and acaricide resistance found in these areas [7, 23]. Thus, the objective of this study was to qualitatively evaluate the perceptions, knowledge, and common tick control practices used by dairy farmers in subtropical areas of continental Ecuador and to associate them with the presence of infestation and acaricide resistance.

2. Materials and Methods

2.1. Participant Selection. Two main methods were employed in this study (Figure S1). First, a cross-sectional survey was carried out in two subtropical dairy production areas of Ecuador: Area 1, located in the Northwest of Pichincha Province in the Western Andean foothills, and Area 2, situated in the Quijos river valley in the Eastern Andean foothills. The participants were selected by snowball sampling [34] irrespective of their age, sex, and educational background. The only requirement to participate was to be the most knowledgeable person on the farm Special emphasis was placed on including farmers from small and medium-sized cattle ranches. Verbal informed consent was obtained from all participants.

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TABLE 1: Active components used to control ticks on cattle in Ecuador.

Acaricide (approximate date introduced*)	Active compounds**	Site of action	Mode of action	Reference
Organophospates (1950)	Ethion, chlorpyrifos, coumaphos, dichlorvos and trichlorfon	Nervous system	Acetylcholine esterase inhibitors	[8]
Amidines (1970)	Amitraz	Nervous system	Octopamine agonists	[9,10]
Synthetic pyrethroids (1970)	Alpha-cypermethrin, cypermethrin, deltamethrin, flumethrin and permethrin	Nervous system	Sodium channel modulators	[11, 12, 13]
Macrocyclic lactones (1981)	Doramectin, ivermectin and eprinomectin	Nervous system	Glutamate controlled chloride channel activator	[14]
Phenylpyrazoles (1990)	Fipronil	Nervous system	Blocking GABA mediated chloride channels	[15]
Benzoylphenyl ureas (1994)	Fluazuron	Exoskeleton	Inhibiting chitin incorporation into the tick's cuticle	[16]

*Approximate date of Introduction of acaricides into the global market; **Active ingredients have been registered until 2023 by the Phytosanitary and Zoo sanitary Regulation and Control Agency (Ecuador); GABA, gamma-aminobutyric acid.

The second part of the involved study was conducted in each study area using participatory methodology [35]. The attendees of these meetings were cattle ranchers of any age, sex, and education level. Participants were identified and invited either by phone or in person with the assistance of local government officials. Transportation was provided for the workshops to facilitate access.

2.2. Data Collection

2.2.1. Cross-Sectional Survey. The survey was part of the project "Socio-eco-epidemiology of ticks, tick-borne parasites, acaricide resistance and residual effects of acaricides in Ecuadorian tropical livestock: environmental, animal and public health impacts" [7]. In total, 138 farmers were interviewed, 71 from the Northwest of Pichincha province and 67 from the Quijos River valley in Napo province. This face-to-face survey contained questions on herd management, livestock diseases, ticks, and acaricide-related information such as perceptions, knowledge, and acaricide control methods. Commercial acaricides were grouped according to their active ingredient into amides, pyrethroids, macrocyclic lactones, organophosphates, phenylpyrazolones (fipronil), benzoylphenyl ureas (fluazuron), and combined products. In addition, information about the doses used, perceived efficacy, application method, number and kind of animals treated, acaricide rotation, and prices per product were recorded. The rotation of acaricides was considered incorrect if either different acaricide brands were within the same acaricide group or if the farmer was not sure of the brand name of the acaricide used previously. The efficacy evaluation was expressed in percentages according to the farmer's perception (1–100). Price information was confirmed by an additional interview conducted in the livestock warehouses in the study areas [23]. Local veterinarians were asked to mention the main diseases or events affecting animals in the area, and this list was used in the farmers' survey to assess general prevalence and mortality.

Farmers were asked to visually identify the species of cattle ticks they encountered based on illustrations of com- mon species found in the study areas [36, 37, 38]: *R. microplus*, *Amblyomma cajennense* and *Ixodes boliviensis*. In addition, all farmers were asked to indicate the season (dry season, rainy season, all over year) and the months during which tick infestation increases. Farmers' knowledge was evaluated with six elements: the biology of ticks, breed predisposition, tick-borne diseases (TBDs), knowledge of economic losses caused by ticks, and correct acaricide treatment. Knowledge of biology consisted of the correct identification of the tick species present on the farm and its life cycle (presence of larvae in paddocks). Morphological identification was carried out on tick samples [7]. Knowledge of TBDs was assessed as correct if the farmer mentioned anaplasmosis, babesiosis, or tick fever (the colloquial name for TBDs).

2.2.2. Participatory Meeting. Participatory methods are methods to collect data in participatory epidemiology, engaging com- munities in the surveillance, control, and prevention of animal diseases [35, 39].

Those observations play a crucial role during early detection and community response to diseases' effects. Perceptions of the effects caused by ticks and the economic losses they cause were evaluated in this part according to the opinion of the farmers. The methodologies used for this involved proportional piling and brainstorming (Figure 1).

The second part of the study was conducted in April 2022. Forty farmers participated, 13 from the Northwest of Pichincha and 27 from the Quijos River valley. We used proportional piling, which allows to collect the results numerically [35]. First, farmers were classified based on their perception of the level of tick infestation present in their animals. At the beginning of the participatory meetings, the participants were grouped in function of the response given to an illustration of a laterally viewed cow divided into three zones (Figure S2). Farmers indicated which parts of the animals were infested and considered one-third as infested when there were 20 or more ingrown ticks. The possible options were to have one-third to three-thirds infested. It was considered a low level of infestation if it was one-third infested, a medium level of infestation two- thirds, and a high level of infestation three-thirds infested [7]. Participants in the last two groups were grouped in the high level of tick infestation category.

Second, for brainstorming, each participant had four cards to write down their ideas about the effects of ticks. Only one idea was recorded per card. Using all cards was optional, and additional cards were provided upon request. An assistant helped to assist illiterate and older participants. Finally, the economic losses resulting from decreasing milk production, weight loss, and the devaluation of hides were weighted by proportional piling. During the exercise, meeting attendees engaged in a proportional piling activity, using balls as counters. A cloth with pockets was used for the proportional measurement to prevent the first participant's response from influencing subsequent responses. Fifteen counters were employed to weight these apparent economic losses.

2.3. Tick Infestation and Acaricide Resistance. The levels of infestation and the presence of acaricide resistance were determined in previous published studies [7, 23]. The level of infestation at the farm level (low or high) was determined according to the tick load observed per animal. Resistance testing was performed on three acaricides (i.e., amitraz, ivermectin, and alpha-cypermethrin) using the larval package test. The farms were classified as with and without acaricide resistance.

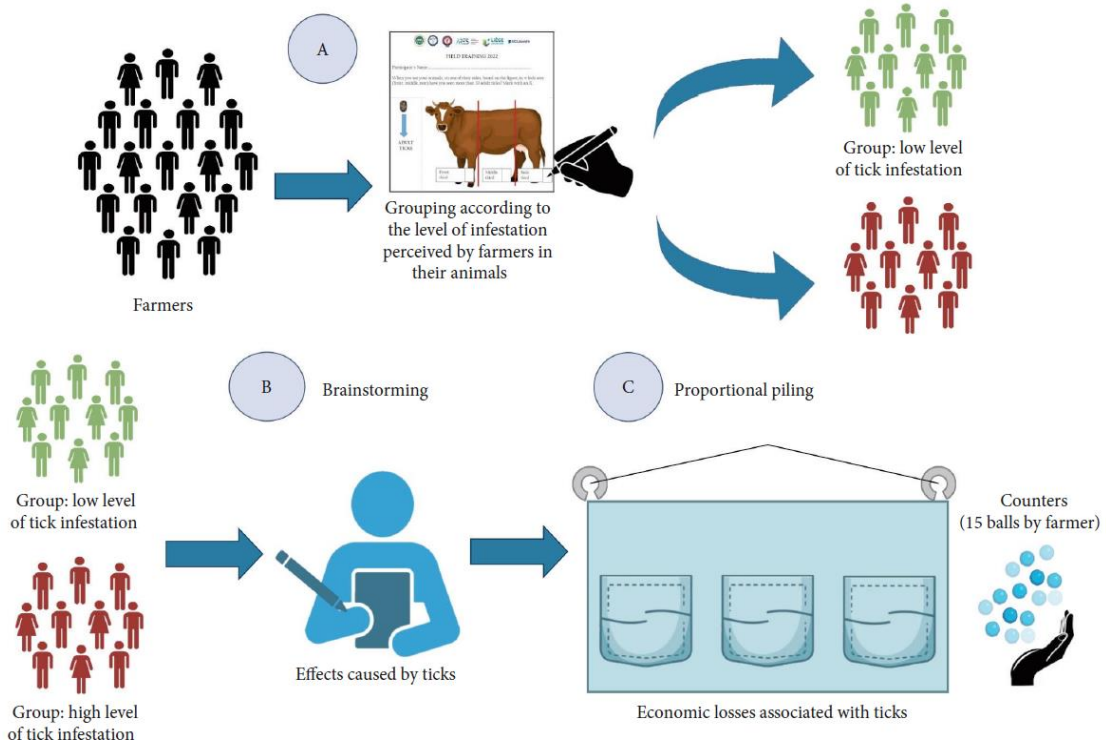

FIGURE 1: Methodologies used in the participatory meeting.

In the study, the variables considered to estimate the tick control practices and the economic losses are presented in Table 2.

2.4. Data Analysis. All the information collected in the cross- sectional survey and participatory meetings was entered into a Microsoft Excel® database. In order to preserve the anonymity of the study participants, the surveys were coded with numbers (participating farmer numbers) and letters (study area). The average price per milliliter (ml) or gram (g) of the active component of the acaricide was obtained by dividing the price of the commercial presentation by the number of ml or g. All data obtained for the different commercial presentations were averaged, and a price per ml or g of active ingredient was determined.

The prescribed dose, route of application, and composition of each commercial brand of acaricide were obtained from the package inserts. For injectable acaricides, the prescribed dose was 1 ml per 50 kg of body weight, and for pour- on acaricides, 1 ml per 10 kg of body weight. In the case of acaricides applied in spray baths, the dose was expressed in milligrams or grams of acaricide dissolved per liter of water, and 1 l of solution covers 100 kg of body weight. To calculate the cost per acaricide treatment, an adult animal weighing 400 kg was considered. To determine if there was a significant difference between the dose and the prescribed dose, a *t*-test was used. The prescribed dose was used as the real value of the mean. Statistical significance was set at 0.05. Statistical analyses were performed using the statistical package stats in R (R Core Team) [40], version 4.2.0. The level of agreement between the working groups was evaluated using Kendall's coefficient of concordance (*W*). The agreement was termed "weak agreement" if *W* values were less than 0.26, "moderate agreement" if they were between 0.26 and 0.38, and "strong agreement" if *W* values were greater than 0.38 [41]. Mann–Whitney test was used to analyze differences between infestation level and study areas.

Multiple correspondence analysis (MCA) was used to summarize associations between the risk practices in tick control, knowledge regarding ticks and TBDs, level of tick infestation, and acaricide resistance. The MCA is a statistical method to analyze patterns in the relationships between a set of qualitative variables, and its interpretation is based on proximities between points in a low-dimensional map [42]. The hierarchical classification on the principal components (hierarchical clustering on principal components (HCPC)) of the MCA was used for the clustering process. We used the FactoMineR package [43] in R to perform MCA and HCPC analyses. The functions fviz_mca_var and fviz_cluster (factoextra package) were used to visualize the results [44]. This study used 20 variables (Table 3) to establish the relationship and grouping farmers according to control practices and perceptions. Covariates with little or no variability were discarded from the analysis. Twelve farms were discarded from the MCA because acaricide resistance tests could not be

TABLE 2: Control and perception variables used in the study.

Topic	Variable	Source of information
Tick control practices	Percentage efficacy of acaricides Chemical control: frequency, acaricide dynamics used dosage, route of administration, who prepares the acaricide solution and treated animals Alternative control practices used Type of grazing implemented	Cross-sectional survey
Farmers' perception	Effect of tick infestation Seasonality of tick infestation	Cross-sectional survey and Participatory meeting
Farmers' knowledge	Biology of ticks Breed predisposition Tick-borne diseases Knowledge of economic losses caused by ticks Correct acaricide treatment	Cross-sectional survey
Direct and indirect economic losses	Price (USD) per milligram or gram of acaricide (active component) Cost (USD) treatment by animal Economic losses in milk, beef, and hide	Agro warehouses interview and cross-sectional survey Participatory meeting
Tick infestation	Level of tick infestation at the farm level	Paucar et al., [7]
Acaricide resistance	Presence or absence of resistance in three acaricides	Larval package test

performed there.The knowledge was judged as good, fair, or poor based on the number of correct answers. Poor knowledge meant zero to <35% of correct answers, fair knowledge was 35%–65% of correct answers, and good knowledge was >65% or all correct answers [45]. The reported efficacy of the acaricide treatment was grouped into three categories: low, medium, and high. Medium efficacy grouped reports of efficacy from 51% to 80%. Alternative control included acaricide control with entomopathogenic fungi, medicinal plants, or paddock control (equalization cuts).

TABLE 3: Variables used for the multicomponent analysis (N = 126 farmers considered).

Variable	Categories	Farms	Codification
Presence of high level of tick infestation	No	70	low infestation
	Yes	56	high infestation
Amitraz resistance	No	61	am res no
	Yes	65	am res yes
Ivermectin resistance	No	74	iv res no
	Yes	52	iv res yes
Alpha-cypermethrin resistance	No	58	cy res no
	Yes	68	cy res yes
Multiresistance: amitraz and ivermectin	No	94	am iv res no
	Yes	32	am iv res yes
Multiresistance: amitraz and alpha-cypermethrin	No	83	am cy res no
	Yes	43	am cy res yes
Multiresistance: alpha-cypermethrin and ivermectin	No	91	cy iv res no
	Yes	35	cy iv res yes
Multiresistance: amitraz, alpha-cypermethrin and ivermectin	No	101	X3res no
	Yes	25	X3res yes
Who preparated the acaricide treatment	Employed	27	Employed
	Owner	99	Owner
Mixture of different acaricides	No	95	acaricide mix no
	Yes	31	acaricide mix yes
Add additives	No	114	additives no
	Yes	12	additives yes
Acaricide application with a wipe cloth	No	113	appl wipe no
	Yes	13	appl wipe yes
Use of organophosphates	No	47	org use no
	Yes	79	org use yes
Alternative acaricide control	No	76	alt no
	Yes	50	alt yes
Manual removal of ticks	No	84	m removal no
	Yes	42	m removal yes
Frequency of bath sprays	1 or 2 weeks	55	bath 1-2
	3 or 4 weeks	42	bath 3-4
	5 weeks or more	21	bath >5
Reported efficacy: bath spray	High efficacy bath	23	high eff bath
	Low efficacy bath	29	low eff bath
	Medium efficacy bath	60	med eff bath
Reported efficacy: injection	High efficacy bath	50	high eff injection
	Low efficacy bath	22	low eff injection
	Medium efficacy bath	29	med eff injection

TABLE 3: *Cont.*

Variable	Categories	Farms	Codification
Tick-Borne Diseases Report	No	67	TBDs no
	Yes	59	TBDs yes
Knowledge level	Fair knowledge*	91	fair knowledge
	Good knowledge	35	good knowledge
Grazing system	Extensive**	30	ext graz
	Intensive**	96	int graz

*Due to the small amount of data from farmers with poor knowledge, they were grouped into the group with fair knowledge; **Intensive: grazing in small area enclosed paddocks, where the animals remain for short periods of occupation (1 day); Extensive: grazing in large areas paddocks where cattle remain for longer periods (more than 1 day).

3. Results

3.1. Visual Identification and Cattle Tick Seasonality. *R. microplus* was the main species recognized in the study areas. *R. microplus* was visually identified by 94% of the farmers in the Quijos River valley and 93% in Northwestern Pichincha as the species that attacks their animals. Ticks of *A. cajennense* were recognized by 29% and 24% of farmers in the Quijos River valley and Northwest Pichincha, respectively. Finally, *I. boliviensis* was recognized by 10% of farmers in the Quijos River valley and 12% of farmers in Northwestern Pichincha.

Figure 2 illustrates the perception of the seasons with the highest tick infestation. Farmers in the Quijos River valley reported experiencing infestations throughout the year. Most farmers in Northwestern Pichincha reported an upswing in infestation during the dry season, from July to September.

3.2. Farmers' Knowledge. Knowledge was evaluated according to the items shown in Figure 3. Seventy percent of participants had fair knowledge, and 27% had good knowledge. Three percent of the participants had poor knowledge. The farmers with "good knowledge" demonstrate excellent knowledge of the biology of ticks, the diseases they transmit, the economic losses they cause, and the breed predisposition. However, their understanding of the correct acaricide management is neither exceptionally excellent nor bad; it falls within an intermediate range. The "fair knowledge" group differs from the farmers with good knowledge because of a lack of understanding of TBDs and the correct use of acaricides. The results indicate that a significant number of farmers require further understanding of the appropriate acaricide dosage and rotation, with only 10% and 17% demonstrating correct management, respectively.

3.3. Chemical Acaricide Control and Perception of Its Efficacy. Of the 138 farms surveyed, 99% reported using chemical acaricide control. The most common form of application was spraying (95%, 131/138). Acaricides used as sprays can be mono-formulated with organophosphates, amides, and pyrethroids or coformulated with two (organophosphates and pyrethroids) or three active ingredients (organophosphates and pyrethroids and phenylpyrazolones) (Figure 4). Spraying baths are carried out using a 20-l knapsack sprayer. The most common equipment used for acaricide measurement were syringes (74%) and ungraduated acaricide bottle tops (20%). Only 6% of the participants stated that they did not measure the quantity used with any instrument. The most common water sources for mixing acaricides were tap water (11%) and water collected from natural reservoirs (89%) such as rivers, springs, wells, or drainage ditches. Injectable acaricides (Figure 4) were used by 86% of the respondents with macrocyclic lactones such as doramectin at 1% (10%) and ivermectin (90%), at concentrations ranging from 1% to 4%. Ivermectin 1% is the most commonly used (59% of the cases), followed by ivermectin 3.15% (25%) and ivermectin 4% (9%). Additionally, 52% of the respondents employed pour-on acaricides, a relatively new control method in our study areas. Pour-on acaricides can be composed of phenylpyrazolones or benzoylphenyl ureas or coformulated with two active compounds, such as the combination benzoylphenyl ureas (fluazuron) with phenylpyrazolones (fipronil), macrocyclic lactones (abamectin) or pyrethroids (flumethrin). In most farms, the weight of animals is unknown, with only 5% of the respondents utilizing weigh tape to determine the appropriate dose of acaricides. The rest of the farmers calculate the weight of the animals by visual assessment.

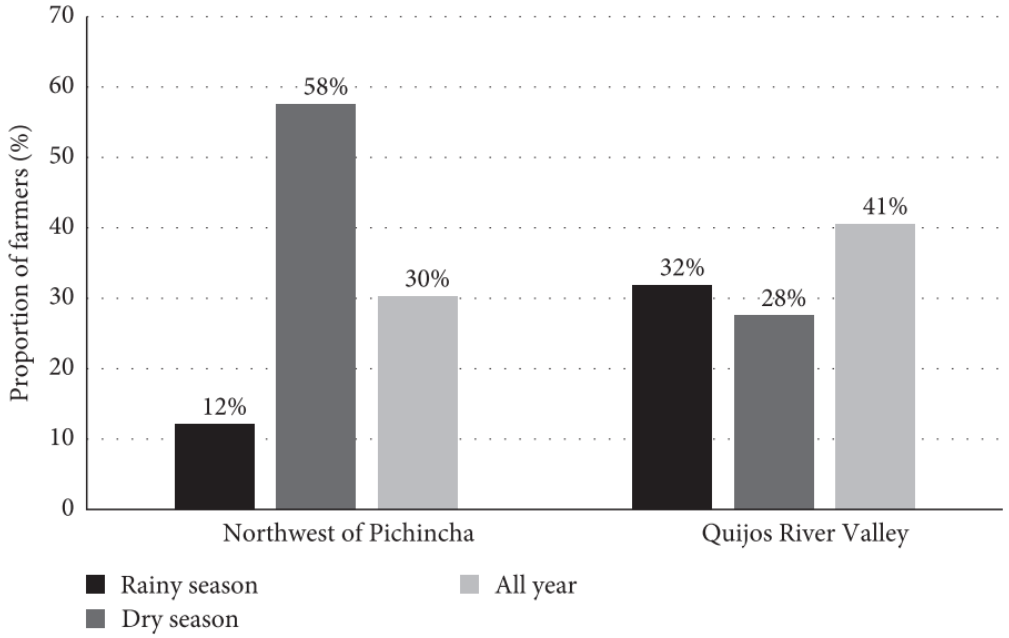

FIGURE 2: Perceptions of seasonal abundance of ticks ($N = 138$ farmers considered).

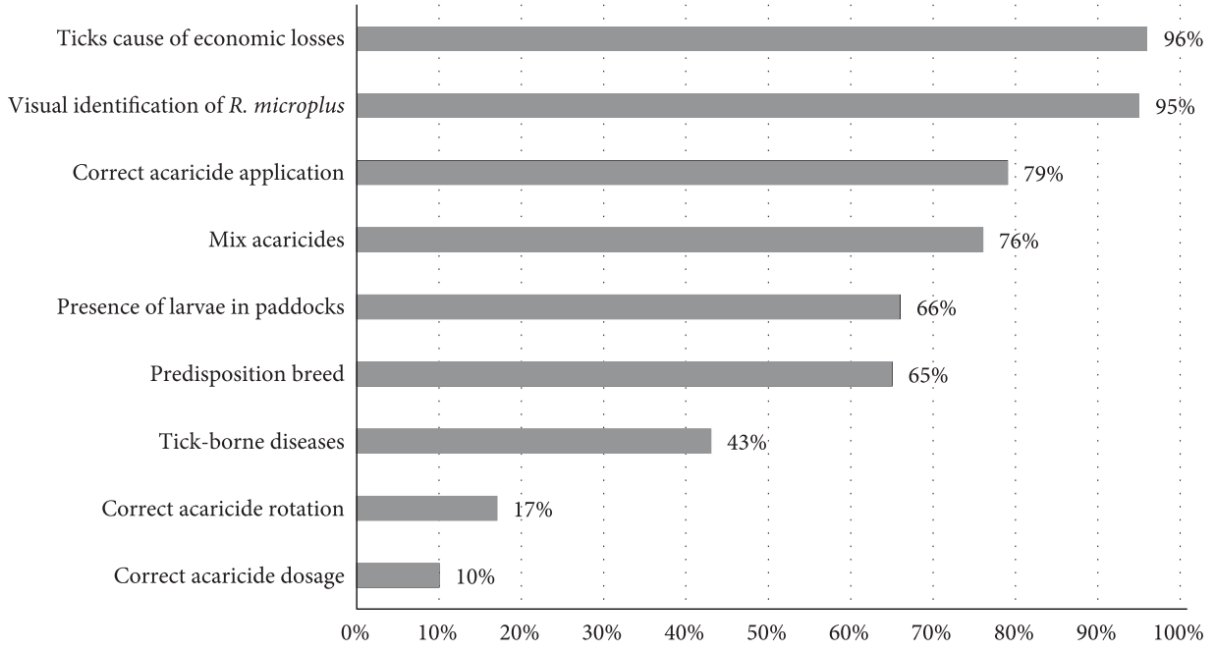

FIGURE 3: Farmers' knowledge about ticks and tick-borne diseases.

Only 17% of farms rotated acaricides correctly, 63% rotated incorrectly, and 19% cannot remember the previous acaricide used. Acaricide treatments in bath sprays and pour-on are generally applied to all animals on the farm. Farmers applied 1% macrocyclic lactones to cattle in production and calves and preferred ivermectin concentrations of 3.15% or 4% for dry cattle, bulls, and heifers. Only 4% of the respondents said they applied an acaricide treatment only to affected animals.

There were many acaricides with the same chemical composition but with different manufacturers and trade names. Approximately 67 different trade names are employed for the six active ingredients available on the market in the study areas. Ten trade names of amides, 13 of cypermethrin, eight for organophosphates, 26 of macrocyclic lactones, five of phenylpyrazolones, and five of benzoylphenyl ureas were mentioned.

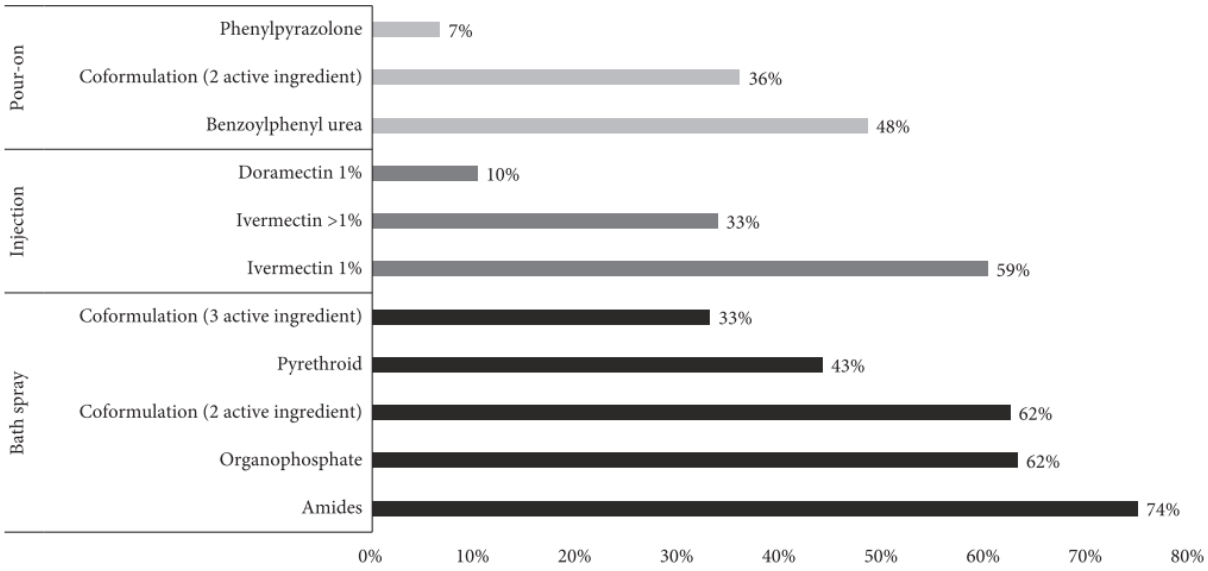

FIGURE 4: Acaricides used by farmers ($N = 136$) in the study areas, expressed in percent.

The reported efficacy of the chemical acaricides is summarized in Table 4. Respondents reported an efficacy of 59% for amides and pyrethroids; the acaricides were considered the least effective in acaricide control. Pour-on acaricides and macrocyclic lactones (3.15% and 4% concentration) were considered more effective; the farmers perceived the efficacy to be greater than 82%. Unfortunately, farmers do not respect the doses prescribed by the manufacturers (p-value < 0.05). Acaricides applied in spray baths were generally overdosed and cost much less than pour-on acaricides, which were generally used in underdoses. Acaricides applied by spraying are more economical compared to injectable and pour-on treatments.

3.4. Alternative Strategies Used by Farmers When Chemical Control Fails. Among the alternatives to chemical control, 36% of the farmers used equalization cuts in paddocks, and 5% of the respondents used baths with entomopathogenic fungi, herbal extracts (neem or garlic), or a mixture of sulfur and quicklime. Supplementation with sulfur in mineral mixtures or feedstuffs was also used as an alternative control method, and only two farms used these alternatives as the only control method. These alternative control methods are mostly used in the Northwest of Pichincha in addition to chemical control. The survey also reported that 11% of farmers use lemon, citric acid, or vinegar to acidify the bath solution because they perceived that it increases the efficacy of the acaricide. Thirty-three percent of respondents reported manually removing ticks from the animals. This is a laborious technique that farmers generally do not set aside a specific time for but do while milking.

The unusual forms of application were observed and 12% of respondents who use pour-on reported dissolving them in water and applying them with a spray pump. Eleven percent of farmers reported dissolving an overdose of the acaricide (up to five times the recommended dose) in water, cooking oil, or engine oil and applying it with a wipe cloth to the most affected areas. In addition, 25% of the farmers mixed different acaricides when preparing the solution for spraying. Generally, when using these techniques, the acaricides used are organophosphates.

3.5. Chemical Handling Safety. When applying the baths spray, none of the surveyed farmers used all individual protective equipment (coveralls, boots, masks, gloves, and goggles). Instead, they opted for various types of protective equipment, with boots (72%), followed by masks (45%), coveralls (24%), gloves (26%), and goggles (6%). Additionally, 22% of respondents mentioned not using any protective equipment. In addition, 48% of respondents reported taking a shower and changing clothes after spraying. Thirteen percent washed their hands and changed clothes. Seventeen percent only washed their hands, and 22% continued their work in the field without any posterior clean. Sixteen percent of farmers reported having at least one of the following signs: dizziness, vomiting, reddening of the skin, tearing, red eyes, and difficulty breathing after spraying animals.

TABLE 4: Chemical control of ticks: practices, perceived efficacy by farmers (*N* = 136) and cost of treatment.

Acaricide	*N*	Reported efficacy (%)	Doses (ml/g)			Cost treatment by animal (UDS)		
			Prescribed dose	Used dose	*p-value*	Prescribed dose	Used dose	*p-value*
a) Bath spray (ml or gr used by L)								
Amides	7	59	1.00	1.28 (1.17-1.40)	<0.01**	0.37	0.47 (0.43-0.51)	<0.01**
Pyrethroid	2	59	1.00	1.35 (1.10-1.59)	0.01**	0.36	0.49 (0.40-0.57)	0.01**
Pyrethroid + Organophosphate	1	67	1.00	1.30 (1.13-1.46)	<0.01**	0.37	0.48 (0.42-0.54)	<0.01**
Pyrethroid + Organophosphate+ Phenylpyrazolone	2	67	1.00	1.19 (1.00-1.38)	0.05*	0.36	0.43 (0.36-0.50)	0.05*
Organophosphate (liquid)	8	71	1.50	1.45 (1.00-1.90)	0.82	0.26	0.25 (0.17-0.32)	0.75
Organophosphates (powder)	3	67	1.50	0.98 (0.85-1.10)	<0.01**	0.55	0.36 (0.32-0.41)	<0.01**
b) Injection (ml used by 50kg weight)								
Macrocyclic lactone 1%	9	70	1.00	1.05 (0.94-1.17)	0.36	1.17	1.10 (1.02-1.18)	0.09
Macrocyclic lactone >1%	2	88	1.00	1.10 (0.94-1.26)	0.20	1.60	1.76 (1.51-2.00)	0.20
c) Pour-On (ml used by 10kg weight)								
Benzoylphenyl urea	9	83	1.00	0.86 (0.77-0.95)	<0.01**	3.62	2.96 (2.48-3.44)	0.01**
Benzoylphenyl urea + Phenylpyrazolone	4	91	1.00	0.69 (0.55-0.84)	<0.01**	2.14	1.45 (1.11-1.80)	<0.01**

The cost of acaricide treatment was calculated for a 400 kg adult animal. *Statistically significant (according to *t*-test, $p < 0.05$); **Statistically significant (according to *t*-test, $p < 0.01$). Acaricides with phenylpyrazolone or benzoylphenyl urea + macrocyclic lactone as active ingredients were discarded from this table due to insufficient data.

3.6. Risk Management Practices. The first two dimensions of 23 provided by the MCA were retained as they accounted for 19.1% and 8.9%, respectively (Figure 5). The first dimension can be interpreted as a gradient of "acaricide resistance" since the variables that contributed most were the variables of resistance and (multi)resistance to acaricides. The second dimension can be interpreted as a gradient of "acaricide control practices" since the variables that contributed most were level of knowledge, acaricide control practices (mixing acaricides and alternative control practices), and perception of the efficacy of acaricide control. In Figure 5, the first quadrant (I) shows the relationship between the presence of acaricide resistance and high infestation with good acaricide control practices. The second quadrant (II) shows the relationship between the absence of acaricide resistance and high infestation with good acaricide control practices. The third quadrant (III) shows the relationship between the absence of acaricide resistance and high tick infestation with poor acaricide control practices. The fourth quadrant (IV) shows the relationship between acaricide resistance and high infestation with poor acaricide control practices.

The results of the MCA analysis revealed several associations within the study. Acaricide resistance was associated with the use of a wipe cloth for acaricide application and if the employee was responsible for preparing and applying the acaricide treatments. Conversely, the absence of resistance was related to if the owner was responsible for preparing and applying the acaricide treatments and also the perception of high efficacy in acaricide treatments used in spray baths. Additionally, a high level of tick infestation was associated with factors such as the frequency of acaricide treatment, very frequent (1 or 2 weeks) or not regular (5 or more weeks) bath spray, the use of organophosphates, fair knowledge, low perception of injectable acaricide efficacy, reported TBD cases on the farm, and extensive grazing. A low tick infestation was related to not using a wipe cloth to apply the acaricide, using bath spray every 3–4 weeks, intensive grazing, the nonuse of organophosphates, and the absence of TBDs on the farm. The low perception of spray baths' efficacy was associated with the mixture of acaricides and the relative medium efficacy of the injectable treatment.

Furthermore, participants with good knowledge demonstrated a higher likelihood of adopting alternative methods to chemical control, such as equalization cuts, entomopathogenic fungal dips, herbal extracts, sulfur, or manual tick removal. In contrast, those with fair and poor knowledge displayed a lower tendency to implement alternative control methods.

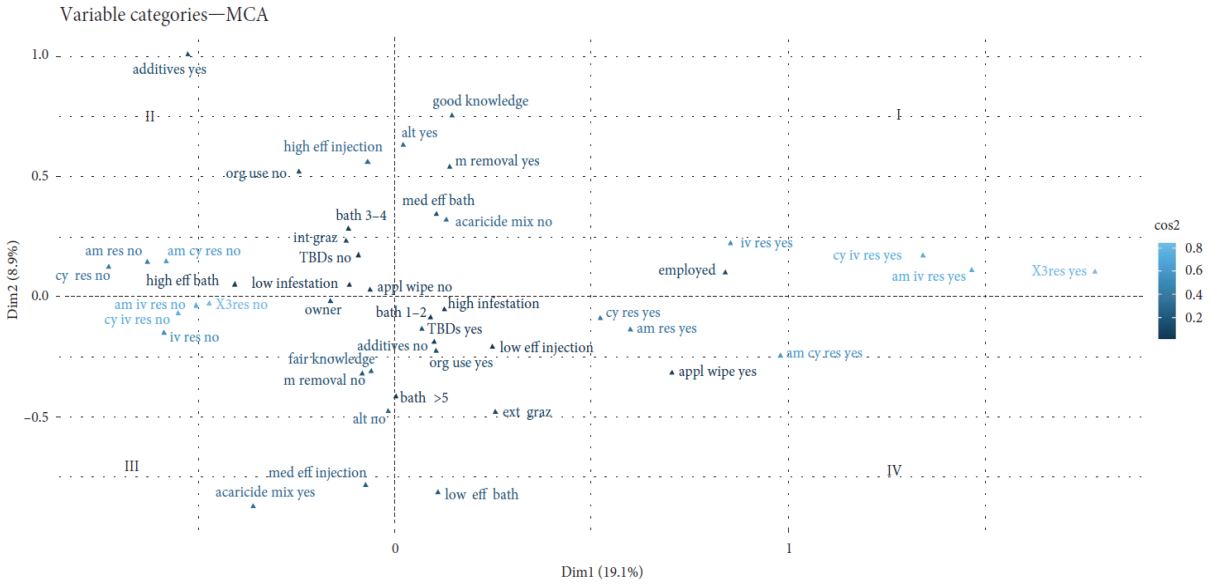

FIGURE 5: Multiple correspondence analysis map of risk and perceptions associated with the presence of infestation and acaricide resistance. I, first quadrant; II, second quadrant; III, third quadrant; IV, fourth quadrant; Dim1, first dimension; Dim2, second dimension; cos2, squared cosine. See Table 3 for the meaning of variable codes. To interpret the graph, the light blue-colored categories are considered to have the strongest contributions, whereas the dark blue ones are the least. Additionally, points located close together within the same quadrant and aligned in a similar direction from the centroid indicate potential associations.

Three farm clusters were identified through the MCA and subsequent hierarchical classification (Figure 6 and Table 5). The first cluster included 35% of farmers, and it was characterized as follows: 29% of farms with no resistance, 42% with mono-resistance, and 29% with multiresistance to two acaricides. This cluster reported a high infestation in 39% of cases. Generally, acaricides were not applied with a wipe cloth in this group, and the use of organophosphates (47%) was not common. Furthermore, only a few farms mixed different acaricides (13%). Notably, most farmers with good knowledge (12/25) and farms implementing alternative methods 32/55 of acaricide control or adding additives (8/10) to the acaricide solution were concentrated in this first cluster. The second cluster encompassed 44% of farmers and was characterized by farms with varying resistance profiles: 23% without resistance, 37% with mono-acaricide resistance, and 47% with multiresistance to two acaricides. In this cluster, the high infestation was prevalent in 57% of cases. Most farms in this group used organophosphates (93%) and mixed acaricides (53%). In addition, 10 of the 55 farms using alternative control methods were classified under Group 2. The third cluster included 21% of farmers and was distinguished by farms showing multiresistance to three acaricides, with high infestation reported in 50% of cases. Generally, farms in this group did not mix different acaricides but did use organophosphates (78%). Thirteen of the 55 farms using alternative control methods were classified within this group.

Farms that applied acaricides using a wipe cloth were distributed across Groups 2 and 3. Additionally, in these groups, the frequency of acaricide treatment by spray bath was generally every 2 weeks or less. Cases of tick fever were reported, and extensive grazing was employed in around 50% of the cases. In contrast, the frequency of bath spray occurred every 3 or 4 weeks, and a few farms experienced TBDs (34%). For Groups 1 and 2, the person in charge of preparing the acaricide solution and applying the treatment was typically the owner. In Group 3, the owner's participation was present in only 50% of the cases. Regarding the perception of efficacy, most farmers in all three groups rated the efficacy of the acaricide bath as "medium efficacy," while the injectable treatment was rated as "high efficacy" by farmers in Group 1. According to reports from farmers on 40 of 138 farms, there was mortality due to TBDs, accounting for 29% of the total deaths. In addition to the diseases described in Figure 7, the study areas also experienced incidents of animals falling down ravines (33%) and animals getting intoxicated by acaricides (6%), leading to mortality of 21% and 4%, respectively.

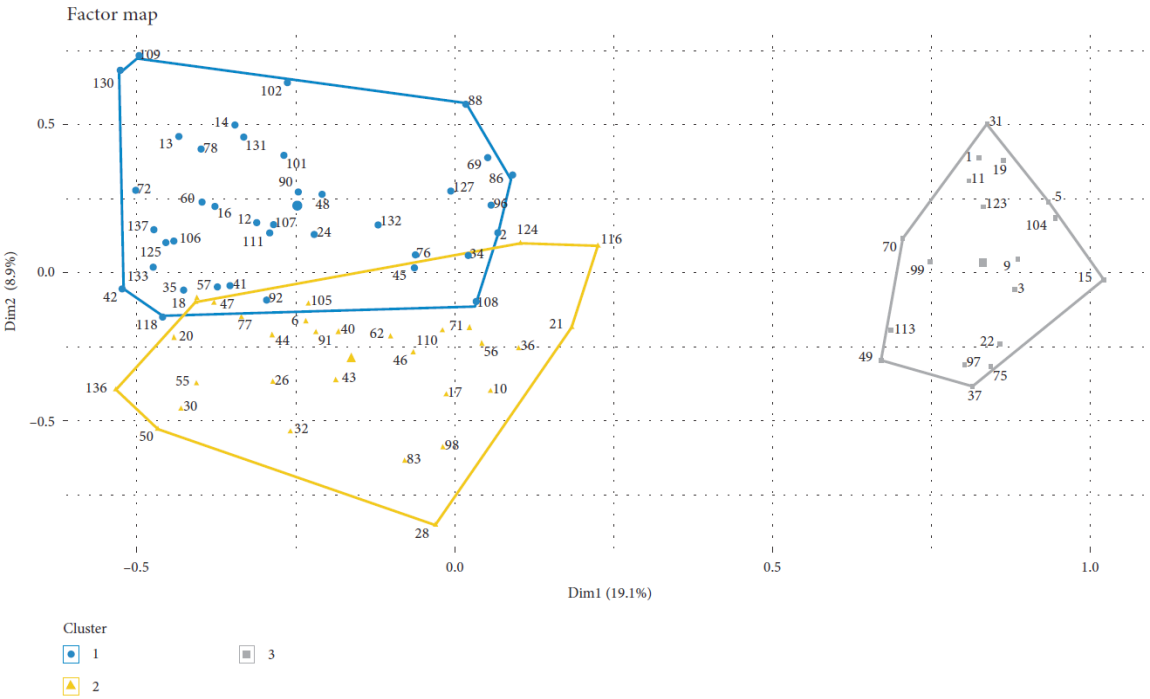

FIGURE 6: Graphical representation of farmers identifying three clusters (groups). Dim1, first dimension; Dim2, second dimension. Group 1 in blue color; Group 2 in yellow color; Group 3 in gray color.

TABLE 5: Characterization of groups formed in hierarchical classification.

Variable	Group 1		Group 2		Group 3		Total farms
	N	%	*N*	%	*N*	%	
Presence of high level of tick infestation	15	39	.17	57	9	50	41
Without Resistance	11	29	7	23	0	0	18
Mono acaricide resistance	16	42	11	37	0	0	27
Amitraz resistance	13	34	13	43	18	100	44
Ivermectin resistance	10	26	7	23	18	100	35
Alpha-cypermethrin resistance	15	39	17	57	18	100	50
Multiresistance to 2 acaricides	11	37	14	47	18	100	43
Multiresistance: Amitraz and ivermectin resistance	3	8	1	3	18	100	22
Multiresistance: Amitraz and alpha-cypermethrin resistance	5	13	9	30	18	100	32
Multiresistance: Alpha-cypermethrin and ivermectin resistance	3	8	4	13	18	100	25
Multiresistance to 3 acaricides	0	0	0	0	18	100	18
Frequency of acaricide bath spray: 1 or 2 weeks	15	39	17	57	9	50	41
Frequency of acaricide bath spray: 3 or 4 weeks.	18	47	8	27	6	33	32
Frequency of acaricide bath spray: 5 or more weeks.	5	13	5	17	3	17	13
Low efficacy in acaricide treatments applied in spray baths	3	8	12	40	6	33	21
Medium efficacy in acaricide treatments applied in spray baths	24	63	13	43	10	56	47
High efficacy in acaricide treatments applied in spray baths	11	29	5	17	2	11	18
Low efficacy in acaricide treatments applied in injection	5	13	10	33	4	22	19
Medium efficacy in acaricide treatments applied in injection	6	16	14	47	5	28	25
High efficacy in acaricide treatments applied in injection	27	71	6	20	9	50	42
Good level of knowledge	12	32	6	20	7	39	25
Aplicaction with wipe cloth	0	0	4	13	3	17	7
Use of organofosforados	18	47	28	93	14	78	60
Mixture of different acaricides	5	13	16	53	2	11	23
Intensive grazing	32	84	16	53	10	56	58
Add additives	8	21	1	3	1	6	10
Alternative acaricide control*	32	84	10	33	13	72	55
Tick-Borne Diseases Report	13	34	18	60	10	56	41
Owner was responsible for preparing and applying the acaricide treatments.	34	89	27	90	11	61	72

* Alternative acaricidal control includes equalization cuts, entomopathogenic fungal dips, herbal extracts, sulfur, or manual tick removal. Mono resistance refers to farms where there is resistance to a single acaricide.

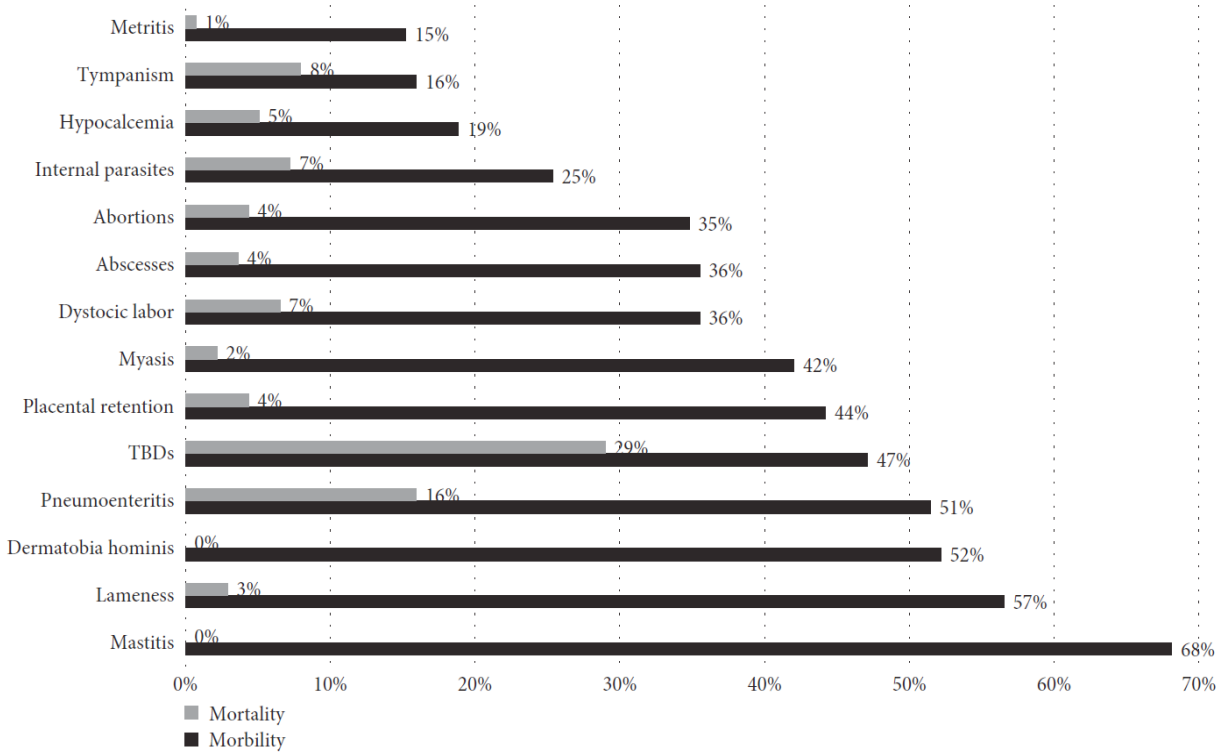

FIGURE 7: Percentage of farmers ($N = 138$) who cited the disease (morbidity and mortality). TBDs, tick-borne diseases.

3.7. Most Frequent Cattle Diseases Cited by Cattle Farmers. According to the farmers' reports in the study areas, health problems among livestock are primarily dominated by mastitis (68%) and lameness (56%). Myasis due to *Dermatobia hominis* (Linnaeus) was reported in 52% of the farms surveyed. TBDs were reported by 47% of farmers and they have the highest mortality on the farms studied.

3.8. Effect of Tick Infestation. The effects of infestation were investigated through both the survey and the participatory meetings. Among the farmers surveyed, 94% reported that cows in production were most affected by ticks, followed by calves (43%), dry cows (40%), and bulls (34%). Moreover, 90% of the surveyed indicated that ticks were most abundant on the front third of the animal, with 75% reporting their abundance on the back third and 34% on the middle third. Meeting participants listed several effects of tick infestation (Figure 8), the most common of which were decreased milk production (95%), weight loss (88%), and tick fever (83%).

Decreased fertility (13%), animal discomfort (10%), exposure of farmers to acaricides (8%), poisoned animals (5%), and environmental contamination (3%) was reported by a minority of participants. Animal death (38%) and skin and coat lesions (55%), including hair loss, scaling, and attack by other ectoparasites, were also mentioned. Higher economic investment (35%) encompasses the purchase of more expensive acaricides and treatment costs when animals become ill with tick fever.

The matrix scoring results of disease signs are shown in Table 6. The highest weighting was for economic losses caused by the decrease in milk production in farms with or without high infestation in both study zones. Economic losses caused by body weight loss obtained a higher weighting in the Northwest of the Pichincha zone. In contrast, the economic losses due to the decrease in the price of bad hides had a higher weighting in the Quijos River valley zone.

The results of Kendall's W are shown in Table 6. The level of agreement was weak for the high infestation group in the Quijos River valley ($W = 0.16$). On the other hand, the groups of low infestation in the two study zones ($W = 0.53$–0.84) and high infestation in the Northwest of Pichincha zone ($W = 0.74$) strongly agreed. In the Northwest Pichincha, the economic losses in milk, weight, and hide of the low infestation group were largely similar to the findings of the high infestation group.

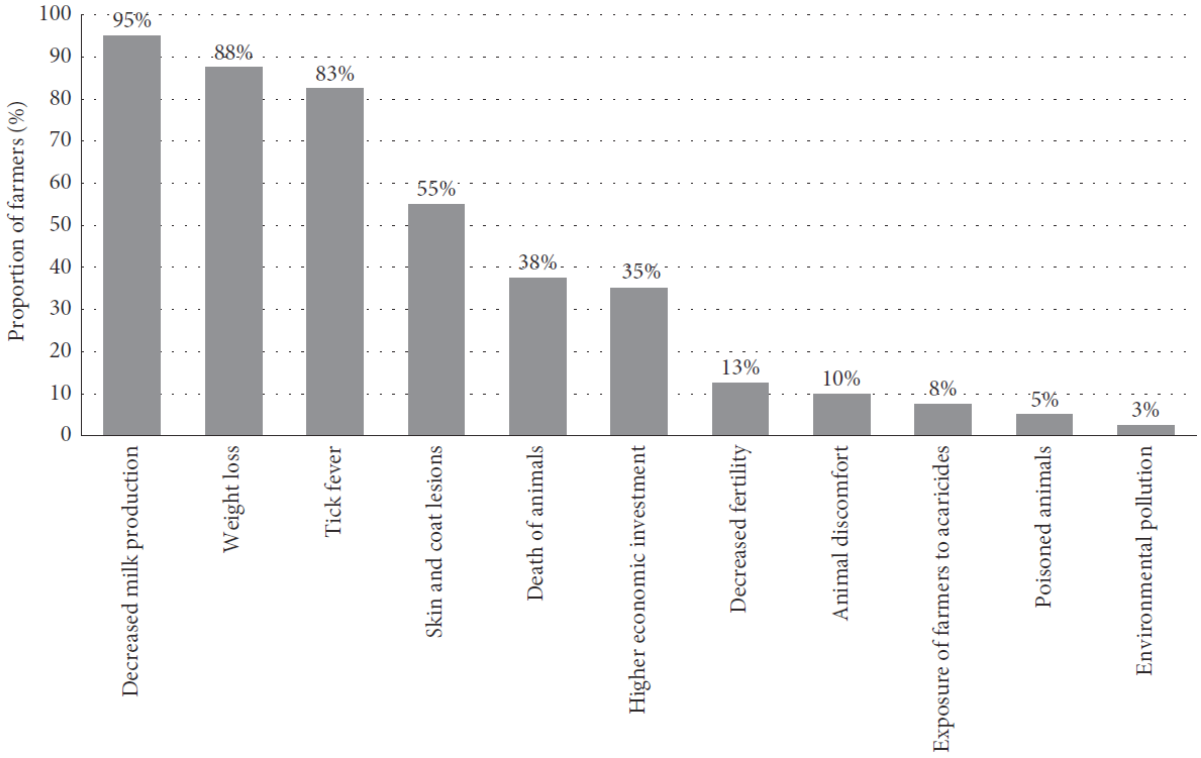

FIGURE 8: Perceptions of the effects caused by tick infestation according to farmers using participatory meetings, in decreasing order ($N = 40$ farmers considered).

The same occurred in the Quijos River valley area, between the high and low infestation groups for meat and hide losses. Only for the low infestation group in Quijos River valley, there was a significant difference between the opinions of these groups on economic losses caused by decreased milk production.

4. Discussion

4.1. Tick Control. The present study documents common and uncommon tick control practices in cattle in subtropical areas of Ecuador. It was determined that the main method for tick control is chemical control in bath spraying, which smallholder farmers consider a convenient and economical form of control [46, 47, 48]. The costs of acaricides applied in bath sprayings usually do not exceed USD 0.55 per animal per treatment, and farmers reported efficacy ranging from 59% to 71%. Although the treatment by bath spraying was assessed to be moderate, it is important to note that this evaluation does not necessarily indicate correct use. In fact, observations revealed that these acaricides are commonly overdosed. Treatments applied in pour-on form are the most expensive, costing up to USD 3.62 per animal per treatment. By contrast, these acaricides usually are underdosed, likely in relation to their high cost [49], which may contribute to resistance in the long term, previously described in other Latin American countries since 2007 for fipronil [50] and 2014 for fluazuron [1]. Although the price difference of acaricides can influence the farmer's decision on which product to buy and use since it is a more tangible cost, especially for the small farmer, relying solely on price differences does not provide a comprehensive assessment of a product's profitability over time. To accurately determine profitability, one must also evaluate the associated costs of labor, infrastructure, and treatment frequency in conjunction with the product's value. In addition, acaricides that are applied by pour-on cannot be administered to lactating dairy cattle, so they cannot be used as a unique control measure on dairy farms. A comprehensive approach that combines various control methods is necessary to ensure effective tick management.

Coformulated acaricides stands out as they are used by 83.33% of the surveyed farmers in spray baths or pour-on. While a reduction in the concentration of active ingredients in coformulation compared to their corresponding mono-formulations is justifiable under ideal conditions, their effectiveness may diminish in cases where resistance to one of the active ingredients has already developed.

TABLE 6: Summary scoring matrix of economic losses in milk, weight, and hide on dairy farms in tropical areas.

Study Area	N	High infestation level	Kendall's coefficient	Economic Losses		
				Milk	Weight	Hide
Quijos river valley	13	No	0.53**	•••• •• 6 (4 -8)	•••• 4 (2-5)	•••• • 5 (3-7)
				p-value 1.00	*p-value* 0.28	*p-value* 0.42
	14	Yes	0.16	•••• •••• 8 (6-9)	••• 3 (2-5)	•••• 4 (2-6)
Northwest of Pichincha	8	No	0.84**	•••• •••• 8 (7-9)	•••• •• 6 (4-7)	•• 2 (0-3)
				p-value 0.04*	*p-value* 0.76	*p-value* 0.06
	5	Yes	0.74**	•••• •••• 8 (6-9)	•••• ••• 7 (5-9)	 0 (0-2)

N = number of informant groups. *W* = Kendall's coefficient of concordance (**p* <0.05; ***p* <0.01). *W* values vary from 0 to 1.0; the higher the value, the higher the level of agreement between the informant groups. The black dots (•) represent the median scores (number of marble balls) used during the matrix scoring; 95% confidence limits are shown in parentheses. *Statistically significant (according to Mann–Whitney test, p <0.05).

"In such scenarios, exposure to suboptimal concentrations could accelerate the emergence of resistance against an otherwise effective molecule present in the coformulation due to inadequate exposure doses" [51]. Additionally, it was found that 25% of farmers make their own coformulated acaricides by blending different commercial acaricides, a practice reported in other studies, where farmers try to maximize and prolong the acaricidal effect [47]. While using coformulated acaricides purchased or modified by farmers may be perceived as an innovative and potentially more effective approach, it poses several concerns. It increases the costs associated with acaricide treatment and increases the risk of acaricide resistance [23, 46]. Moreover, the use of these mixtures raises the risk of poisoning in humans and animals (6% of animals getting intoxicated by acaricides in this study), as the combinations can significantly exceed the permitted dosage levels, particularly when the same active ingredient is used.

It was observed that farmers with "good knowledge," in addition to chemical control, have implemented other methods for acaricide control, such as manual tick removal, the addition of additives to regulate the pH of the water, use of entomopathogenic fungi, herbal extracts or grazing management. Although manual tick removal is only used by 33% of the surveyed farmers, as it is considered a tedious technique, it is known that if it is performed twice a week during milking, it reduces 21% of the parasite population [52, 53, 54]. Both the use of entomopathogenic fungi and herbal extracts are practiced at a very low rate by farmers in the study areas (5%). Although the use of entomopathogenic fungi has been a practice used in other countries for several years, in Ecuador, this alternative is relatively new and has been gaining importance since 2019 [55, 56, 57, 58], so we associate its low use to the lack of knowledge of the technique and/or its unknown effectiveness in the field. The same occurs with herbal extracts such as neem or garlic, used in a few farms in the study areas as an alternative to chemical control. Neem oil and garlic extracts have potential as acaricides and insect repellents. Neem plant contains azadirachtin, a chitin inhibitor, and garlic contains about 94% of volatile sulfur compounds acting as a repellent [3, 59, 60]. Following the line of acaricide control with sulfur, some farms have implemented control through baths with a mixture of sulfur and quicklime and/or the addition of sulfur to animal's diet, which, according to studies conducted in the study areas, seems to be a viable option [61]. In addition, its efficacy has already been studied in other mites, such as spider mites [62].

Another form of control alternative to chemical control used in the study areas is the implementation of equalization cuts in pasture management. In this method, grass residues left by animals after grazing are cut. This technique would help control acaricide infestation by creating open, unprotected spaces in the paddocks. This alteration of the ecological niches allows sunlight to enter directly, which increases mortality in both adult ticks and larvae due to the effect of the alteration of the electrolyte balance and the evapotranspiration gradient [63, 64, 65]. Additionally, this technique helps to expose larvae to biological controllers in the soil [66].

The MCA helped to better understand the interaction between management variables, acaricide resistance, and tick infestation; so, as mentioned earlier, the application of different tick control strategies relies on farmers' uptake, knowledge, and perception of their effects. This kind of information is crucial for improving tick control management, particularly those practices that mitigate acaricide resistance and ensure long-term solutions that help to sustain the efficacy of tick control measures [30]. The use of alternative control practices was found to be related to farmers' knowledge about ticks, with those with a better level of knowledge seeking alternatives to chemical control. Although most farmers practicing alternative control methods belonged to Group 1, some were also found in Groups 2 and 3, where acaricide resistance and high tick infestation exist. This underscores that effective and successful control requires a combination of tools and strategy [30]. The use of alternative control must be accompanied by proper acaricide management. In addition, regardless of the treatment method, producers must be aware of and correctly apply control methods to obtain maximum benefits [67]. This can be achieved through farmer education since many farmers only use chemical control because they are unaware of other control methods [30, 67].

In this study, it was observed that the management of acaricides and the management of grazing systems had an impact on the presence or absence of high tick burdens on the animals. On the one hand, the high level of infestation was related to the use of extensive grazing and extreme frequencies of spray baths (very frequent or very

infrequent treatments). We associate this with adequate tick control in animals, which requires the right combination of strategies [27–29]. Using an extensive grazing method causes animals to spend more time in contact with ticks, which contributes to increased infestation levels. As an effect of this, farmers, in their eagerness to combat high tick burdens, reduce the time between treatments (less than 15 days) [51, 68]. Although it could be seen that this was the main mode of action, there were also farmers that, despite having a high tick burden, applied acaricide baths infrequently (more than 5 weeks), which indicates that these farmers did not give importance to the presence of high tick loads, nor did they look for ways to control them.

On the other hand, it was determined that for the farms that managed a more intensive grazing system, the level of infestation was lower. Although this has already been reported in other studies [2, 69, 70], it is emphasized that this form of control must be accompanied by acaricide applications to reduce tick load from 77% to 89% [71]. Although this group of farmers used acaricide baths every 3 or 4 weeks, which is recommended to break the life cycle of these parasites [2], work should be done to educate farmers to treat only affected animals, since most of the farmers surveyed applied acaricide treatments to all animals, regardless of whether they required it or not.

Similarly, it was observed that farmers who tend to mix different acaricides also opt for other more extreme practices, including excessive and overdosage of acaricides (generally organophosphates) and the use of other irritant substances such as engine oil. These substances, in addition to irritating and causing skin lesions in animals, are toxic to livestock and humans if applied without adequate biosecurity measures [51, 72]. In addition, using engine oil is a risky practice that can cause food poisoning if it contaminates bovine products, which can affect human health long-term, even if consumed at very low doses [73, 74]. Pajurek et al. [74] presented a case of contamination of products of animal origin (eggs) with high levels of toxic substances (polychlorinated dibenzo-p-dioxin, dibenzofurans (PCDD/Fs)) due to the leakage of engine oil into the soil of the paddock where the animals were found.

4.2. Farmers' Knowledge. According to previous studies conducted as part of the project [7], it was determined that most of the respondents had at least 20 years of experience in cattle raising, so it is not surprising that the majority (93%) of the respondents were able to recognize the tick species found in the areas and perceived that its presence affected the farm's economy. However, not all farmers know the correct application of acaricides, the presence of larvae in the paddocks, and the predisposition to acaricide infestation in certain cattle breeds. This practical knowledge of ticks contrasted sharply with the lack of knowledge about TBDs and acaricide control regarding the correct dosage and rotation of acaricides. The lack of knowledge on these issues was already reported in studies by [75, 76], which further mentioned the existence of a directly proportional relationship between knowledge on TBDs and tick control with increasing levels of education and training courses for farmers. This makes sense since, in this study, most respondents have a basic or secondary level of education [7]. Although training is given at small rates, it is focused mainly on reproductive issues or good milking practices.

Given that farmers have limited access to formal education on ticks and TDB (training and university education), we can conclude that their knowledge is based on practical knowledge acquired through their farming experience and knowledge transmitted from generation to generation by their parents. Despite being informal, this knowledge can still be used to design and implement specific educational and training programs. These programs can help bridge the educational gap by incorporating traditional wisdom and introducing contemporary techniques to improve livestock management practices. This will empower livestock keepers with scientific knowledge and enhance their traditional techniques [77].

It is important to note that education programs should involve all decision-makers and stakeholders in acaricide control. Although the farmer–owner has the final decision on acaricide management, this decision may be influenced by suggestions or advice from livestock workers or neighboring farmers. Public veterinarians, while present in certain rural areas, usually provide technical advice on production and reproduction and leave sanitary control, including acaricide management, to private veterinarians, who may only be available to some farmers. Decision-making is also influenced by veterinary drug sellers, which in the case of large farms are veterinarians from pharmaceutical companies that offer their products from farm to farm; on the other hand, in the case of

small and medium producers, they accept advice from store sellers, who in most cases have no veterinary training.

Training stakeholders, (1) farmers, (2) public and private veterinarians, and (3) commercial representatives of veterinary products, is crucial in acaricide control. The education of farmers, including both employees and owners, should be done through practical illustrated manuals or education programs. Studies have revealed that education and training programs designed with more interactive, communicative, and participatory approaches significantly impact the assimilation of information and the effective implementation of acaricidal control strategies [76, 78, 79]. It is crucial to train public and private veterinarians in tick control, as their involvement in this field is limited. The creation of technical manuals and training will help private veterinarians, public veterinarians, and veterinary drug sellers provide accurate technical advice and transmit their solid and updated knowledge to farmers.

In addition, involving authorities in socioeconomic and political reforms is crucial for successful long-term tick population management [17, 78]. The regulation of acaricide sales by trained personnel, establishment of acaricide resistance diagnosis laboratories, and implementation of socioeconomic and political reforms all play significant roles in tick management. To stimulate the adoption of new policies, the authorities must understand that although farmers are the main social group affected both by the economic losses caused by ticks and by being constantly exposed to acaricides (occupational exposure), the misuse of these chemicals exposes the general population if they consume food or drinking water contaminated with acaricide residues [80, 81].

4.3. Perceptions of Cattle Tick Seasonality. The perceptions of livestock farmers on seasonality and tick infestation in animals differed in the study areas despite both areas being humid subtropical zones. On the one hand, rainfall in the Quijos River valley is constant throughout the year, but there is a dry season with less intense precipitation from July to September [82, 83]. On the other hand, the Northwestern Pichincha has a more defined dry season, characterized by little or no precipitation between June and November [84]. It is associated that ranchers in the Quijos River valley area perceive tick infestation to be relatively constant throughout the year, as there is no marked difference between the two seasons. However, in the Northwest of Pichincha area, having a more defined dry season, there is a difference in the perception of a greater infestation by ticks in the months of June to November, months corresponding to the dry season. Farmers' perception of a higher prevalence of ticks during the months corresponding to the summer season is consistent with other studies where increased tick infestation in summer is associated with elevated temperature and humidity that stimulate tick development, survival, and spread [83, 85, 86, 87]. This perception of a higher level of infestation in the dry season may also be associated with the fact that in season, as the availability and quality of pasture decreases, animals spend more time in the paddocks, which exposes them for a longer time to ticks [88]. Moreover, at this time, as a result of the lack of paddocks, farmers in the areas move the animals to external paddocks, which increases the risk of infestation [7]. In addition, animals with low nutrition are more attacked by ticks [89].

4.4. Effect of Tick Infestation. According to the farmers' reports, the diseases affecting these tropical areas were observed. While TBDs were not the most prevalent disease in the study areas, they were the deadliest cause. Myiasis due to *D. hominis* was also reported by several farmers (52%), which can be related to the presence of ticks and poor acaricide control practices. The lesions caused by ticks or bad control practices (overdose, motor oil) allow the entry of bacteria, fungi, and parasites [90]. In addition, it was observed that due to the improper use of acaricides, there were reports of animals poisoned by acaricides, which died in most cases. All farmers who participated in the participatory meetings reported that tick infestation negatively affected animal health and production. It was determined that decreased milk production, weight loss, and the presence of TBDs were the most frequent effects cited by farmers. Very few farmers mentioned exposure to acaricides and environmental pollution as potential effects, which is consistent with the low number of farmers with a good level of knowledge. By contrast, the nonuse of correct individual protective equipment and how farmers dispose of acaricide bottles are strong environmental concerns. When weighing the economic losses caused by ticks in three aspects: milk drop production, loss of weight, and skin damage. It was observed that the greatest weight in the

proportional piling fell on the economic losses caused by the decrease in milk, which we associate with the fact that the study areas are dairy areas, and their main income is the sale of milk. Although most of the participants talked about the skin lesions caused by the presence of ticks or larvae of *D. hominis*, at the time of weighing this aspect in the economic losses, it was the one that received the least weight in the proportional piling. This indicates that although it is a visual problem that bothers the farmers, at the moment of selling the animals, the good or bad condition of the skin does not interfere with the remuneration received for their sale.

5. Conclusions

The application of tick control strategies strongly relies on farmers' uptake, knowledge, and perceptions of the control effects [30]. This study identified tick control practices used by dairy farms in subtropical areas and described common and uncommon measures used. In our study, farmers with good knowledge had lower infestation rates and acaricide resistance. They were also most active in the use of alternative methods. While cross-sectional data cannot establish a causal relationship, our results strongly indicate that a good understanding of tick biology and well-informed use of tick control is key for tick management. The right combination of acaricidal control strategies can increase the efficacy of control methods, making them more cost-effective and ecologically sustainable [91]. Based on this and previous studies, the importance of working on three points is highlighted: (1) the implementation of an integrated tick-control program that allows evaluating the effectiveness of the strategies and the farmer uptake; (2) the education of farmers through the creation of extension programs with interactive, communicative, and participatory approaches and (3) the creation of working groups with authorities, national, and international experts. These three points together will help to promote the creation of sanitary control policies. Understanding the complex interplay between farmers' characteristics, practices, and perceptions is vital to developing well-informed strategies. By exploring these dynamics, farmers can tailor interventions to meet the specific needs and challenges faced by livestock in the country. This approach enables the development of more effective control measures that align with the reality of livestock management practices in Ecuador. The implementation of customized interventions not only mitigates economic losses associated with tick infestations but also enhances livestock health and productivity, benefiting both producers and consumers. This will help to control the advance of resistant tick populations, a global problem in which FAO [92] works in multidisciplinary and multisectoral programs to plan and collaborate in the sustainable management of cattle ticks. This kind of information is also crucial for improving tick control management in Ecuador, particularly to try to implement practices that mitigate acaricide resistance and ensure long-term solutions that help to sustain the efficacy of tick control treatments.

Data Availability

The data that support the findings of this study are available from the corresponding author upon request.

Ethical Approval

This study was approved by the Research Committee of the Faculty of Veterinary Medicine and Zootechnics (COIF- FMVZ), Central University of Ecuador (UCE-FMVZ-DEC- 2023-0631-O).

Consent

The farmers were properly informed and gave written informed consent before participating in the study. The survey collected on each farm was coded with numbers and letters according to the farm and area visited.

Confticts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Lenin Ron-Garrido and Claude Saegerman contributed equally to the work.

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Supplementary Materials

Supplementary 1. Figure S1: graphical abstract of the study.

Supplementary 2. Figure S2: image used to classify farms into no, low, medium, and high tick infestation.

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7. Supplementary File

FIGURE S1: GRAPHICAL ABSTRACT OF THE STUDY.

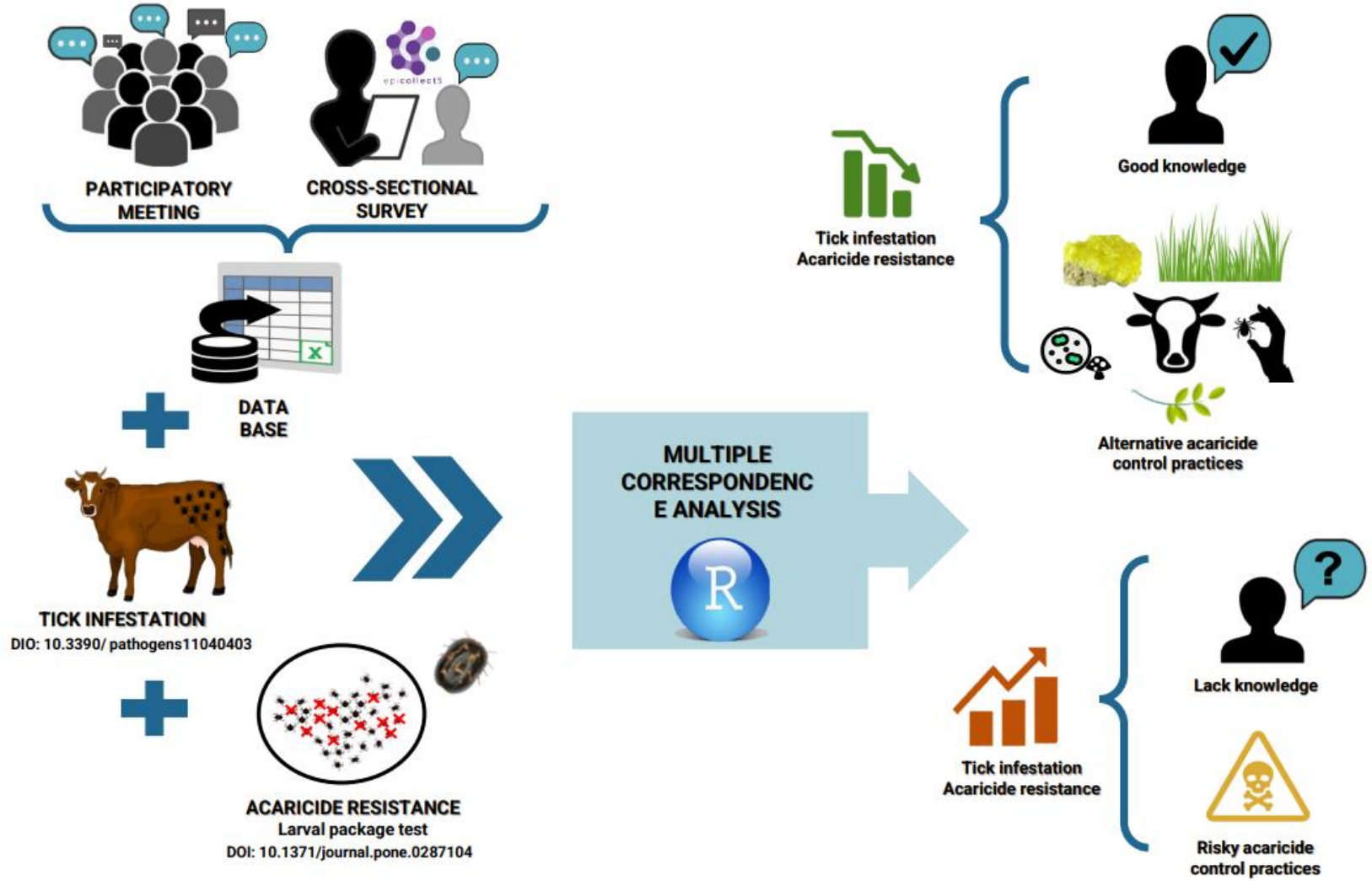
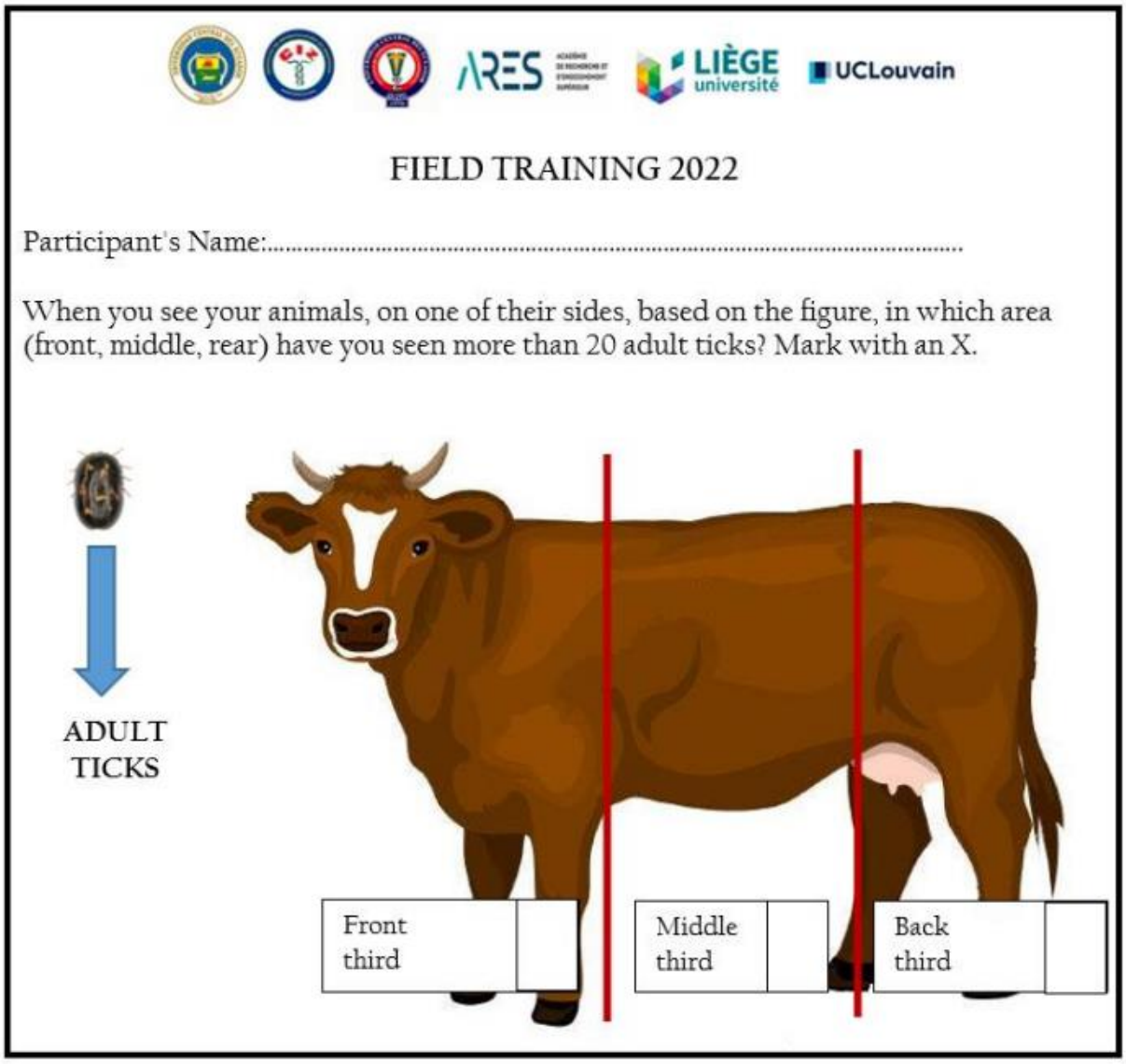


FIGURE S2: IMAGE USED TO CLASSIFY FARMS INTO NO, LOW, MEDIUM, AND HIGH TICK INFESTATION.



Experimental section

Study 4

What is the value of testing for tick-borne diseases in cattle in endemic areas? A case study of bovine anaplasmosis

Submitted in PLOS ONE

Valeria Paucar-Quishpe, Dirk Berkvens, Ximena Pérez-Otáñez, Richar Rodríguez-Hidalgo, Darío Cepeda-Bastidas, Cecilia Perez, Yadira Guasumba, Daniela Balseca, Kamilo Villareal, María A. Chávez-Larrea, Sandra Enríquez, Jorge Grijalva, Sophie O. Vanwambeke, Claude Saegerman, Lenin Ron-Garrido

Preamble

Ticks serve as vectors for a diverse array of pathogens, contributing significantly to the transmission of TBDs. In tropical regions where animals live constantly exposed to tick bites, understanding the immunological landscape of animal populations is paramount. Using a Bayesian approach, we assessed the true prevalence, the proportion of animals with protective antibodies against this TBD and test characteristics for the detection of Anaplasma spp. through multiplex polymerase chain reaction (mPCR), competitive-inhibition enzyme-linked immunosorbent assay (cELISA), and blood smear (BS). By contextualizing cELISA and BS results with mPCR, we aimed to provide comprehensive insights into anaplasmosis dynamics and endemic stability.

What is the value of testing for tick-borne diseases in cattle in endemic areas? A case study of bovine anaplasmosis

Valeria Paucar-Quishpe[1,2], Dirk Berkvens[3], Ximena Pérez-Otáñez[2,4], Richar Rodríguez-Hidalgo[2,5], Darío Cepeda-Bastidas[6], Cecilia Perez[5], Yadira Guasumba[2], Daniela Balseca[5,7], Kamilo Villareal[8], María-Augusta Chávez-Larrea[1,9], Sandra Enríquez[2], Jorge Grijalva[5], Sophie O. Vanwambeke[4], Claude Saegerman[1*], Lenin Ron-Garrido[2,5¶*]

[1] Research Unit of Epidemiology and Risk Analysis applied to Veterinary Science (UREAR-ULiège), Fundamental and Applied Research for Animals & Health (FARAH) Center/Faculty of Veterinary Medicine, University of Liege, Liège, Belgium. claude.saegerman@uliege.be

[2] Instituto de Investigación en Zoonosis (CIZ), Universidad Central del Ecuador, Quito, Ecuador. avpaucar@uce.edu.ec; xfperez@uce.edu.ec; ienriquez@uce.edu.ec

[3] Department of Biomedical Sciences, Institute of Tropical Medicine, 2180 Antwerp, Belgium. ddbdb@telenet.be

[4] Georges Lemaitre Centre for Earth and Climate Research, Earth & Life Institute, UCLouvain, Louvain-la-Neuve, Belgium. sophie.vanwambeke@uclouvain.be

[5] Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, Quito, Ecuador. rrodriguez@uce.edu.ec; bcperez@uce.edu.ec; jgrijalva@uce.edu.ec; ljron@uce.edu.ec

[6] Facultad de Ciencias Agrícolas, Universidad Central del Ecuador, Quito, Ecuador. dacepedab@uce.edu.ec, dcbalseca@uce.edu.ec

[7] Centro de Biología, Universidad Central del Ecuador. Quito, Ecuador. dcbalseca@uce.edu.ec

[8] Facultad de Ciencias Químicas, Universidad Central del Ecuador. Quito, Ecuador. ksvillarreal@uce.edu.ec

[9] Grupo de Investigación en Sanidad Animal y Humana (GISAH), Carrera de Ingeniería en Biotecnología, Departamento de Ciencias de la Vida y la Agricultura, Universidad de las Fuerzas Armadas ESPE, Sangolquí, Ecuador, machavezlarrea@student.uliege.be

* Correspondence author: e-mail: claude.saegerman@uliege.be; ljron@uce.edu.ec
¶ These authors contributed equally to this work.

Abstract: In areas where tick-borne diseases (TBD) are endemic, it is crucial to consider the animals' immunological status in relation to these diseases. The true prevalence of bovine anaplasmosis, the percentage of animals with protective antibodies against this TBD, and the diagnostic characteristics of three tests (multiplex polymerase chain reaction (mPCR), competitive-inhibition enzyme-linked immunosorbent assay (cELISA), and blood smear (BS)) were estimated using a Bayesian approach. A total of 620 samples were collected in two subtropical areas of Ecuador. A significant finding of this study is that approximately 70% of cattle in those endemic areas harboured protective antibodies against *Anaplasma marginale*. This elevated percentage may stem from persistent exposure with a high pathogen prevalence in ticks. The decline in cELISA specificity must be attributed to cross-reactivity with protective antibodies against *Anaplasma* spp. Therefore, it is crucial to interpret this test outcome alongside exposure history and clinical manifestations. The elevated apparent prevalence detected by cELISA and BS should be contextualised with mPCR results. The high seroprevalence and infrequent clinical outbreaks suggest that the pathogen has achieved endemic stability. This study provides valuable insights into the dynamics of anaplasmosis in endemic areas and may serve as a foundation for devising TBD control strategies in these areas.

Keywords: bayesian modeling; diagnostic tests; sensitivity; specificity; prevalence; anaplasmosis; Ecuador.

1. Introduction

Diseases transmitted by ticks in cattle, called tick-borne diseases (TBDs), occur throughout the world, especially in tropical and subtropical areas (Ogata et al. 2021). Bovine anaplasmosis is caused by obligate intra-erythrocytic bacteria called *Anaplasma*. The main species responsible of the disease in cattle are *Anaplasma marginale* and, to a lesser degree, *Anaplasma centrale* (Aubry and Geale 2011). This disease is usually transmitted by tick bites (*Rhipicephalus microplus*). Haematophagous insects, such as horse flies (*Tabanus* spp.), mosquitoes (*Psorophora* spp.), stable fly (*Stomoxys calcitrans*), *Haematobia irritans* and fomites (surgical instruments, or needles contaminated with blood) are also responsible for the transmission (Kocan et al. 2003, 2010; Orenge 2021). In areas of the USA, Africa, Central and South, where there are no tick vectors, mechanical transmission by *Tabanus* (horseflies) specimens is the main route of the spread of *A. marginale* (Hornok et al. 2008; Rodrigues et al. 2022). Vertical transmission has also been mentioned to occur under conditions of constant inoculation in endemic areas (Gonzalez Grau et al. 2013; Potgieter and van Rensburg 1987).

Epidemiologically, three types of zones are recognised in relation to the local disease status of tick-borne diseases: free, stable, and unstable zones. Free zones are those in which conditions are not favourable for vector development. Epidemiologically unstable zones are areas where the occurrence of the disease is determined by climatic conditions and/or livestock management. Stable zones are zones where animals have sufficient antibody levels to guarantee their protection and an asymptomatic disease is present (Amorim et al. 2014; Jonsson et al. 2012; Kessler et al. 1983; Madruga et al. 1984; Maya-Delgado et al. 2020). Although clinical signs and necropsy findings can guide the diagnosis of anaplasmosis, techniques that identify the presence of the pathogen are indispensable, particularly in unstable and epidemiologically free zones. Serious losses used occur when mature cattle with no previous exposure are moved into endemic areas or when animals are in endemically unstable areas (Tabor 2022).

All naïve animals are susceptible to the disease; however, clinical manifestations increase according to host factors: low immune status, cattle breed (*Bos taurus*), and host age (adult animals). Bovine anaplasmosis is characterized by fever, depression, weakness, icterus, anaemia, and often death in adult animals (Atif 2015; Kocan et al. 2010; Parodi et al. 2021; Sisson et al. 2023). Calves rarely develop clinical signs of acute disease in endemic areas. Following recovery from the clinical phase, animals may remain asymptomatic, persistently carrying the infection (challenge-immunized animals) (Rar, Tkachev, and Tikunova 2021; Ristic 2012; Tabor 2022; Young 2020).

Infections can be detected directly by PCR or blood smears or indirectly by serology (Kohn et al. 2011). The most common and routine technique for diagnosis is the microscopic examination of blood or organ smears (liver, kidney, heart, or lungs) with Giemsa stain. However, it only detects animals with acute infections as chronic-phase infections do not express a high level of parasitaemia (Corona et al.

2014; OIE 2015). On the other hand, although PCR allows us to determine the agent's presence and differentiate the species of *Anaplasma* present, it is a technique that cannot be performed on a large scale, achieved with serological tests (Eshetu 2015); high costs can make this test impractical, particularly when large number of samples are collected to estimate infection prevalence at animal level. A potential solution involves screening a large number of farms through pool testing, wherein sample pools are screened, making use of the high sensitivity and specificity of PCR; and when a pool is detected as positive, the positive individual sample(s) is (are) then sought within the original constituent samples (Dorfman 1943). Pooled testing provides a cost-effective alternative to testing individual animal samples. The accuracy of estimates obtained through pooled testing relies on factors such as the true prevalence, pool size, and number of pooled tests used (Christensen and Gardner 2000; Cowling, Gardner, and Johnson 1999; Speybroeck et al. 2012).

Serological assays for the anaplasmosis diagnosis include card agglutination test (CAT), indirect fluorescence antibody test (IFAT), and complement fixation tests (CFT), enzyme-linked immunosorbent assay (ELISA) including a competitive ELISA (cELISA) and indirect ELISA (iELISA) (Aubry and Geale 2011). Competitive ELISA and indirect ELISA use antigens with strong specificity and high sensitivity to detect IgG and IgM antibodies which may vary depending on several factors, such as exposure time, the pathogens or strains of *Anaplasma* spp. involved and the infection status of the animals (Zhang et al. 2022). The two serological tests currently preferred for diagnosing infected animals are cELISA and CAT (Aubry and Geale 2011; OIE 2015).

Imperfect sensitivity and specificity of existing diagnostic tests can lead to misclassification of some animals tested (Ekong et al. 2017; Rahman et al. 2013). Therefore, to estimate test characteristics under field conditions, sensitivity and specificity values should be obtained from prior information data or in case of absence, from expert opinion (*prior distribution*). Bayesian statistics allows us to determine the likelihood function using prior information about the parameters available in the observed data. Combining both the a priori distribution and the likelihood function using Bayes' theorem yields a *posterior distribution*, which reflects updated knowledge (Lesaffre, Speybroeck, and Berkvens 2007; Van de Schoot et al. 2021). In the context of epidemiology and diagnostic tests, Berkvens et al. (2006) described a Bayesian approach using probabilistic constraints to estimate true disease prevalence and diagnostic test characteristics when a set of diagnostic tests is applied to a set of individuals in a population. However, the issue of employing pooled testing in conjunction with individual assays has yet to be fully evaluated.

Concerning TBDs and the characteristics of the tests used for their detection in epidemiologically stable areas for anaplasmosis, what do the diagnostic test results mean for the conclusions about the health status of the animals? In theory, an animal is considered to have anaplasmosis when it exhibits clinical signs consistent with the disease caused by the pathogen for which it tested positive. In tests that detect antibodies, seropositivity alone does not necessarily indicate current illness; it signifies exposure

to the pathogen and the development of an immune response. However, if an animal is either seropositive or negative, and exhibits clinical signs that correspond to the disease, it suggests an active infection or anaplasmosis (Aubry and Geale 2011). For this reason, it is important to interpret the test results carefully and correlate them with the animal's clinical signs and history of exposure to make an accurate diagnosis and determine the appropriate course of treatment or management (Kocan et al. 2010; Springer et al. 2021; Walker 1998). In endemic areas, cattle usually become infected with *A. marginale* in early life, and losses due to anaplasmosis are minimal; similarly, these cattle remain as chronically infected carriers, allowing them to develop lifelong active immunity (challenge-immunized) without clinical disease (Kessler et al. 1983; Tabor 2022). Additionally, cows have high enough levels of antibodies to promote protection that can be passed to calves with the colostrum (Amorim et al. 2014). Thus, the objective of this study was to evaluate the diagnostic performance (sensitivity and specificity) of three diagnostic tests (cELISA, PCR, and BS) used for the detection of anaplasmosis in endemic dairy farms in Ecuador, applying a Bayesian modelling approach. To fulfil this objective, we built a conditional probabilistic model that allows the incorporation of a two-step approach for PCR. In this process, the initial PCR step is applied to pooled samples per farm, followed by a secondary step conducted only when a positive result emerges from the pooled sample, at which point individual animal testing ensues.

2. Materials and Methods

2.1. Ethics statement

This study was approved by the Research Committee of the Faculty of Veterinary Medicine and Zootechnics (COIF-FMVZ) of the *Universidad Central del Ecuador* -UCE (UCE-FMVZ-DEC-2023-0631-O). All animals were treated with care, and the usual farm management for blood sample collection was followed. Veterinarians conducted the sample collection, animals were not mistreated, and animal welfare was guaranteed. Adequate information was provided to farmers, who provided written consent before the commencement of sample collection from their animals. Each farm surveyed was assigned a unique code to ensure anonymity, incorporating numbers and letters to denote the specific farm and area visited.

2.2. Study area and sampling design

This study was part of the project “Socio-eco-epidemiology of ticks, tick-borne parasites, acaricide resistance and residual effects of acaricides in Ecuadorian tropical livestock: environmental, animal and public health impacts”. Between November 2020 and March 2021, a cross-sectional survey was conducted in two sub-tropical areas of Ecuador (**Figure 1**).

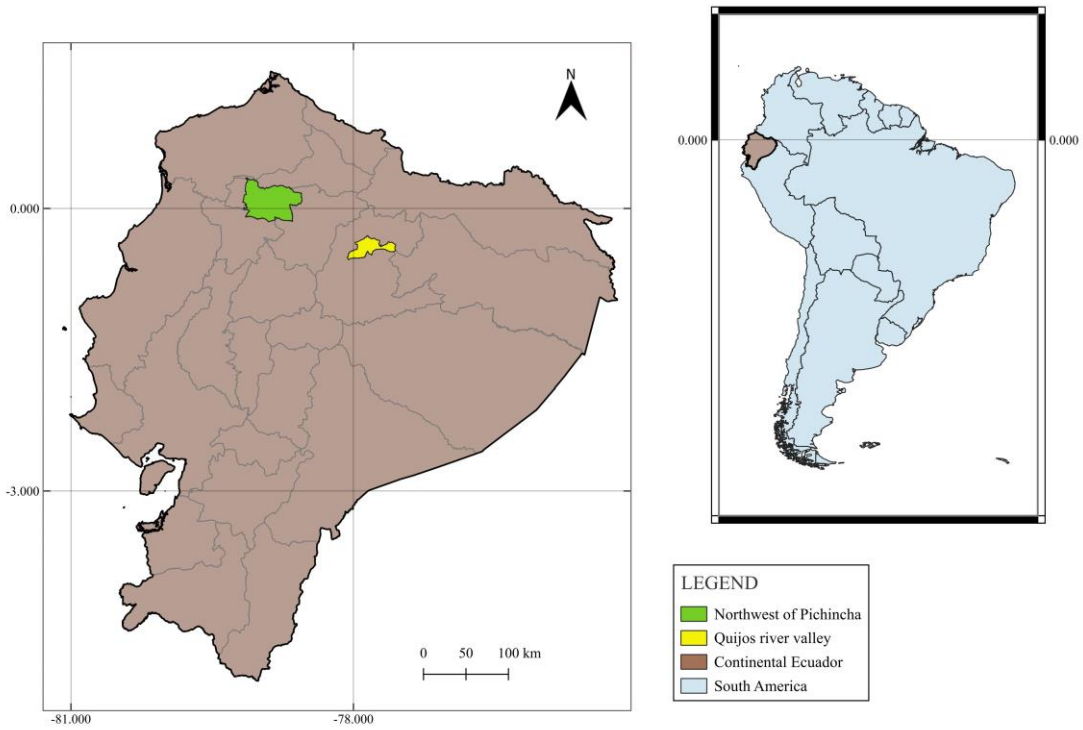


Figure 1. Study Areas

Area 1: Northwest of Pichincha Province, situated in the western foothills of the Andes (Coastal Region) between 0°18N, 79°10W and 0°09S, 78°33W. This zone experiences temperatures ranging from 19°C to 25°C at 600 metres above sea level (m.a.s.l.) to 1800 m.a.s.l (Echeverría 2017; HCPP 2000) and is crossed by Chocó Andino of Pichincha Biosphere Reserve (RBCAP 2019). Area 2: Quijos River Valley, located in the eastern foothills of the Andes (Amazon region) between 0°18S, 78°04W and 0°33S, 77°32W. Here, temperatures fluctuate between 16 and 24°C, with an altitude ranging from 1500 to 2000 m.a.s.l (Grijalva, Arévalo, and Wood 2004; PDOT El Chaco 2019; PDOT Quijos 2015). This area is in the middle of two protected areas, Cayambe Coca National Park and Sumaco Napo Galeras National Park (Cárdenas 2010). Five randomly selected animals were sampled at each farm without distinction of breed, age or sex. A total of 695 animals from 139 farms were sampled. Parameters such as temperature (°C), capillary refill time, heart rate, and respiratory rate were also recorded during clinical examination.

2.3. Processing of blood samples

Two tubes with about 4 ml of blood were collected from each animal by coccygeal venipuncture with disposable needles and plastic Vacutainer™ tubes (red top, no additives; and purple top, EDTA as an anticoagulant). Each blood sample was assigned a unique code, incorporating numbers and letters to denote the specific farm and area visited (animal code). Blood sample processing was done at the immunodiagnostic laboratory at the *Instituto de Investigación en Zoonosis* (CIZ) at the *Universidad Central del Ecuador*. Sera collected in red top Vacutainer™ tubes were separated by centrifugation (250

rpm). Each serum was stored in respectively labelled graduated cryovials. Haemolysed, lipaemic, icteric, and contaminated samples were removed from the study (SINAVE 2013). Animals with only one or two test results and/or farms where less than five animals were sampled are also excluded from the study. A cured and consolidated database was obtained with the results of 620 animals (89% sampled) belonging to 124 farms (89% sampled).

2.4. Microscopic examination

The microscopic examination consisted of the identification of the bacterium (*Anaplasma* spp.) in erythrocytes on blood smears (BS) stained with Giemsa. Blood samples collected in EDTA Vacutainer™ tubes were used to prepare thin BS. For each sample, two BS slides were made and identified with the animal code. The BS were fixed in methanol for 3 min and stained in Giemsa stain diluted at 10% with buffer solution for 25 min. Each BS prepared was examined under ×100 objective magnification and oil immersion on a Better Scientific microscope. Thorough searches were performed for small, rounded, deep purple intra-erythrocytic inclusions, approximately 0.3-1.0 µm in diameter. More than 30 microscopic fields per slide were observed before considering an animal as negative; a positive animal had at least one positive BS (parallel testing) (Aktas, Altay, and Dumanli 2006; Chauhan et al. 2015; Nasehi 2018; OIE 2015; Shabana, Alhadlag, and Zaraket 2018). A total of 1240 BS from 620 animals were examined for intra-erythrocytic inclusions using Giemsa stain. Figure 2 presents the result of a blood smear positive test.

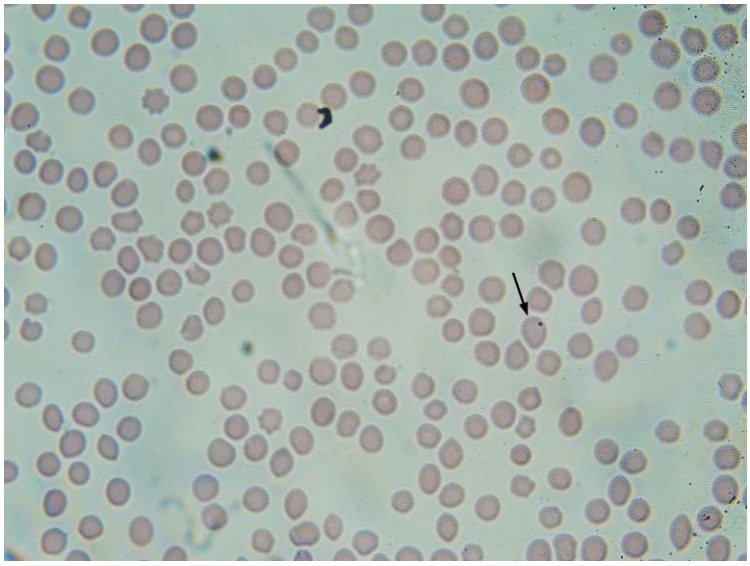


Figure 2. Giemsa-stained blood smear showing *Anaplasma* spp. in bovine erythrocytes of cattle

Photo credit, Kamilo Villarreal, Clinical Biochemistry Department, Faculty of Chemical Sciences, UCE

2.5. Serological tests

A competitive enzyme-linked immunosorbent assay (cELISA) based on antibody binding to major surface protein 5 (MSP5) of *A. marginale* was used. Sera were tested for specific antibodies against *A.*

marginale using a commercial ELISA kit, *Anaplasma* Antibody Test Kit cELISA v2 (Veterinary Medical Research and Development; VMRD, Inc.), following the manufacturer's instructions, catalog number 283–2 (**Appendix A**). Receiver operator characteristics (ROC) analysis was performed to determine the optimal cut-off value. Bovine blood samples from a disease-free zone were used as negative controls (N=70), and bovine blood samples positive for *A. marginale* by multiplex PCR (mPCR) were used as positive controls (N=43). These samples were obtained from the Animal Serum Bank of CIZ, Centro Experimental Uyumbicho of the Universidad Central del Ecuador, and the "*Hacienda El Prado" Selva Alegre"* of the *Universidad de las Fuerzas Armadas* (ESPE). The analysis was performed using R (R Core Team 2021), package *cutpointr* (Thiele and Hirschfeld 2021). The optimal cut-off was 35.76 with high sensitivity and specificity, 99 to 100%, respectively.

2.6. Molecular

Bovine blood samples were organised into pools of 5 individuals (k = 5) per farm. The testing procedure was a two-step approach: the first step consisted of applying a PCR to a pooled sample per farm. If the result of this test was negative, all the animals were declared negative. Conversely, if the result of the pooled test was positive, then the blood sample of each animal in the pool was individually tested by PCR.

2.6.1. DNA extraction. Genomic DNA was extracted from 400 µL of blood pools using the Column Pure Blood Genomic DNA kit (Applied Biological Materials Inc., Canada) in accordance with the manufacturer's instructions. The DNA was eluted in 60 µL of elution buffer and stored at -20°C until use.

2.6.2. Multiplex PCR for amplification of Anaplasma marginale and Anaplasma centrale. Based on previous studies, a set of specific primers for *Anaplasma* spp. were selected (**Table 1**). For the detection of *Anaplasma marginale* and *Anaplasma centrale*, the msp5 (714 bp) and msp4 (395 bp) genes were targeted, respectively (Reyna-Bello et al. 1998; Shkap et al. 2008).

The multiplex PCR reaction was performed as described by Guasumba (2022) (**Appendix B**).

Table 1. List of PCR primers used in the present study

Parasite	Gene target	Name	Primer sequence 5' – 3'	Product size (bp)	Reference
A. marginale	*msp5*	Ana 19A	GTGTTCCTGGGGTACTCCTA	714	Reyna-Bello et al. 1998
A. marginale	*msp5*	Ana 19B	TGATCTGGTCAGCCCCAGCT	714	
A. centrale	*msp4*	CentF	CATGGGGCATGAATCTGTG	395	Shkap et al. 2008
A. centrale	*msp4*	CentR	AATTGGTTGCAGTGAGCGC	395	

2.6.3. Individual PCR of Anaplasma marginale. Positive pools for *Anaplasma marginale* were subjected to individual PCR as described by Guasumba (2022) (**Appendix B**).

2.7. Conditional-probabilistic model description

The Bayesian analysis approach described by Berkvens et al. (2006) and Branscum et al. (2005) was used in WinBUGS 1.4.3 (Spiegelhalter et al. 1996) to estimate the sensitivities and specificities of the three tests and the true prevalence of anaplasmosis in cattle from subtropical areas of Ecuador. Additional calculations were performed in R version 4.2.0 (R Core Team 2021).

For our analysis, results of anaplasmosis diagnosis tests were sorted in the following order: mPCR (T1), cELISA (T2), and BS (T3). Tests were considered conditionally dependent. Under this assumption, a multinomial model including all possible interactions between the three tests requires 15 parameters (Θ_1-Θ_{15}) to be estimated. These included the prevalence (Θ_1), sensitivity (Θ_2), and specificity (Θ_3) of the T1; two conditional sensitivities and two conditional specificities of the T2; and four conditional sensitivities and four conditional specificities for the T3 (**Appendix C**).

Due to the two-step approach practiced for the pool testing (PCR), each test sample that is part of a pool can either be truly positive or truly negative (Speybroeck et al. 2012). Using the methodology described in detail by Speybroeck et al. 2012 and given a fixed pool sample size (represented by *k*), the calculation of the apparent prevalence (Θ_1') will depend on the probability of at least one positive result in the pool as showed in Equation 1:

$$\theta_1{}' = \frac{\theta_1}{1-(1-\theta_1)^k} \quad (1)$$

Sensitivity of PCR pool testing (Θ2') is now dependent of the number of positives in the group and from the PCR-test sensitivity (Θ_2) as shown in Equation 2:

$$\theta_2{}' = \frac{\sum_{i=1}^{k}\left[1-(1-\theta_2)^i\right]\frac{k!}{i!(k-i)!}\theta_1^{\,i}(1-\theta_1)^{k-i}}{1-(1-\theta_1)^k} \quad (2)$$

Thus, a combination of the two previous results represents the true positive rate under pool testing is:

$$\vartheta = \theta_1{}'\theta_2{}' \quad (3)$$

Considering the outcome of the three tests (Table 2), we have:

Table 2. The contingency table of the three tests

	cELISA$^+$		**cELISA$^-$**	
	BS$^+$	BS $^-$	BS$^+$	BS $^-$
mPCR$^+$	T1$^+$ T2$^+$ T3$^+$ 111	T1$^+$ T2$^+$ T3$^-$ 110	T1$^+$ T2$^-$ T3$^+$ 101	T1$^+$ T2$^-$ T3$^-$ 100
mPCR$^-$	T1$^-$ T2$^+$ T3$^+$ 011	T1$^-$ T2$^+$ T3$^-$ 010	T1$^-$ T2$^-$ T3$^+$ 001	T1$^-$ T2$^-$ T3$^-$ 000

Legend: Multiplex PCR, mPCR (T1); Competitive-inhibition enzyme-linked immunosorbent assay, cELISA (T2); Blood smear, BS (T3). Being negative results (0 or -), and positive results (1 or +), the expected cell probabilities under the conditional dependence.

Denoting by 0 negative results, positive test outcomes by 1, the expected cell probabilities (*P*) based on these three tests under the conditional dependence (the different conditional probabilities are listed in **Appendix C**) assumption are:

$$P(111) = \vartheta\,\theta_4\,\theta_8 + (1-\vartheta)\,(1-\theta_3)\,(1-\theta_7)\,(1-\theta_{15}) \quad (4)$$

$$P(110) = \vartheta\,\theta_2\,\theta_4\,(1-\theta_8) + (1-\vartheta)\,(1-\theta_3)\,(1-\theta_7)\,\theta_{15} \quad (5)$$

$$P(101) = \vartheta\,\theta_2\,(1-\theta_4)\,\theta_9 + (1-\vartheta)\,(1-\theta_3)\,\theta_7\,(1-\theta_{14}) \quad (6)$$

$$P(100) = \vartheta\,\theta_2\,(1-\theta_4)\,(1-\theta_9) + (1-\vartheta)\,(1-\theta_3)\,\theta_7\,\theta_{14} \quad (7)$$

$$P(011) = \vartheta\,(1-\theta_2)\,\theta_5\,\theta_{10} + (1-\vartheta)\,\theta_3\,(1-\theta_6)\,(1-\theta_{13}) \quad (8)$$

$$P(010) = \vartheta\,(1-\theta_2)\,\theta_5\,(1-\theta_{10}) + (1-\vartheta)\,\theta_3\,(1-\theta_6)\,\theta_{13} \quad (9)$$

$$P(001) = \vartheta\,(1-\theta_2)\,(1-\theta_5)\,\theta_{11} + (1-\vartheta)\,\theta_3\,\theta_6\,(1-\theta_{12}) \quad (10)$$

$$P(000) = \vartheta\,(1-\theta_2)\,(1-\theta_5)\,(1-\theta_{11}) + (1-\vartheta)\,\theta_3\,\theta_6\,\theta_{12} \quad (11)$$

In addition, animals that were tested positive for cELISA results but remained asymptomatic were termed challenged-immunized animals, with their rate calculated (**Appendix D**) as follows:

$$\frac{number\ of\ cELISA\ false\ positives}{number\ of\ cELISA\ false\ positives + number\ of\ cELISA\ true\ positives} \quad (12)$$

Different Bayesian models with different a priori information and assumptions for the estimation of the parameters of the multinomial model were used in conjunction with their evaluation criteria under Bayesian statistics (**Table 3**):

M1: specificity of T1=1

M2: specificity of T1=1, the sensitivity of T1 constrained uniformly (dunif[0.95,1.00]), beta prior on Θ_{12} in 0.98 (dbeta[235,5])

M3: specificity of T1=1, Θ_6=1

M4: specificity of T1=1, Θ_6=1, beta prior on Θ_{12} in 0.98 (dbeta[235,5])

M5: specificity of T1=1, sensitivity of T1 constrained uniformly (dunif[0.95,1.00]), Θ_{12}=1

M6: specificity of T1 constrained uniformly (dunif[0.99,1.00]), the sensitivity of T1 constrained uniformly (dunif[0.95,1.00]), beta prior on Θ_{12} in 0.98 (dbeta[235, 5])

M7: specificity of T1=1, Θ_6 = 1, uniform prior on Θ_5 = dunif[0.6, 1.00], beta prior on Θ_{12} in 0.98 (dbeta[235, 5])

M8: sensitivity of T1 constrained uniformly (dunif[0.95, 1.00]), uniform prior on Θ_4=dunif [0.8, 1.00], uniform prior on Θ_5= dunif[0.8,1.00], Θ_6 = 1, beta prior on Θ_{12} in 0.98 (dbeta[235, 5])

A summary of the models designed for this study with elicitation of the prior distributions is shown in Table 3.

Table 3. Models (M1 to M8) constructed and prior distributions used

	M1	M2	M3	M4	M5	M6	M7	M8
theta [1]	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1
theta [2]	0, 1	0.95, 1	0, 1	0, 1	0.95, 1	0.95, 1	0, 1	0.95, 1
theta [3]	1	1	1	1	1	0.99, 1	1	0, 1
theta [4]	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1	0.8, 1
theta [5]	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1	0.6, 1	0.8, 1
theta [6]	0, 1	0, 1	1	1	0, 1	0, 1	1	1
theta [7]	-	-	-	-	-	0, 1	-	0, 1
theta [8]	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1
theta [9]	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1
theta [10]	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1
theta [11]	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1	0, 1
theta [12]	0, 1	235, 5[a]	0, 1	235, 5[a]	1	235, 5[a]	235, 5[a]	235, 5[a]
theta [13]	0, 1	0, 1	-	-	0, 1	0, 1	-	-
theta [14]	-	-	-	-	-	0, 1	-	0, 1
theta [15]	-	-	-	-	-	0, 1	-	0, 1

Legend: Prior distributions are uniform (dunif), excluded for a than are beta distribution (dbeta).

2.7.1. Prior information for parameters. Estimations of the prevalence and test characteristics (sensitivities and specificities) of the three tests used are limited. Prior information was therefore introduced based on similar studies of bovine anaplasmosis in different countries found in the literature.

The sensitivity and specificity of PCR were 95.2% (95% CI: 85.2–99.1%) and 92.7% (95% CI:85.6–99.2%), respectively (Parodi et al. 2021). For some parameters, no available or objective prior information can be formulated, and it is necessary to leave prior information on these parameters as non-informative. In addition to these values, a priori information was also obtained from the consultation of expert opinion from the Department of Biomedical Sciences from the Institute of Tropical Medicine, Belgium; the Research unit of Epidemiology and Risk Analysis Applied to Veterinary Science from the University of Liege, Belgium; and from the Department of Biostatistics and Epidemiology at CIZ, Ecuador.

2.7.2. Sensitivity analysis. The influence of the prior information on the posterior estimates was assessed using sensitivity analysis (Branscum et al. 2005). A scenario was used for the chosen priors: perturbations of the prior interval (estimate - 0.0001, estimate + 0.0001). This model was run with the same number of chains, and similar diagnostic criteria were performed.

2.7.3. Model diagnostics. All models were run with a burn-in period of 10 000 iterations, and estimates were based on a further 40 000 iterations using three Markov chains. Model selection proceeded according to the methodology described by Berkvens et al. (2006) using the effective number of estimated parameters (*pD*), Bayesian P value (P value), and Deviance Information Criterion (DIC) as validation criteria. Moreover, the convergence of the model was also evaluated by trace plots, Kernel density plots, Brooks-Gelman-Rubin (BGR) convergence statistic plots, and autocorrelation plots (Berkvens et al. 2006; Gelman and Rubin 1992; Ntzoufras 2009). The WinBUGS codes used are presented in **Appendix E**.

2.7.4. Assessment of agreement between the tests. The level of agreement between the three diagnostic tests was calculated by Cohen's kappa coefficient for the agreement between each pair of tests. Using a 'two-by-two' contingency table. The kappa coefficient was calculated by using the psych package (Revelle 2020) under R environment. The following ranges were used for the interpretation of the kappa coefficient: poor agreement (<0.00), slight agreement (0.00–0.20), fair agreement (0.21–0.40), moderate agreement (0.41–0.60), substantial agreement (0.61–0.80), and almost perfect (0.81–1.00) (Landis and Koch 1977). In addition, the level of agreement was expressed in terms of indices of positive (P_{pos}) and negative (P_{neg}) agreement (Cicchetti and Feinstein 1990; Sanogo et al. 2013), and their Confidence intervals were calculated as described by Uebersax, 2018.

3. Results

3.1. Descriptive results and agreement between the tests

The mPCR results showed that 226 (AP = 36%) of 620 samples were positive for *A. marginale*, with no evidence of *A. centrale*. Of the 620 samples tested by cELISA and BS, 610 (AP=98%) and 500

(AP=81%) were positive for *Anaplasma* spp., respectively. The cross-classified test results obtained from the three individual tests during the epidemiological study are presented in **Table 4**.

During the clinical examination, the average temperature of the sampled animals was 38.3°C. Only 4% of these animals had a temperature above 39.5°C. The average heart rate of the animals was 75 beats per minute (bpm), and their respiratory rate was recorded at 28 breaths per minute. A capillary refill time of 1 to 2 seconds (s) was observed in 85% of the animals. However, 15% of the animals had a prolonged capillary refill time of over 2 seconds.

Table 4. Cross-classified test results for *Anaplasma* spp. in cattle of Ecuador based on mPCR, cELISA, and blood smear

mPCR	cELISA	BS	Number of samples
1	1	1	185
1	1	0	41
1	0	1	0
1	0	0	0
0	1	1	315
0	1	0	69
0	0	1	0
0	0	0	10
		Total	**620**

Legend: mPCR, multiplex polymerase chain reaction; cELISA, competitive-inhibition enzyme-linked immunosorbent assay; BS, blood smear.

The agreement and kappa value between cELISA–blood smear, cELISA–mPCR and mPCR – BS are shown in **Table 5**. The agreement level between cELISA with mPCR and BS tests were slight, in the other hand it was poor between the mPCR and BS. Concordance between cELISA - BS results was high (90%) in the positive results (P_{pos}), whereas the agreement on negative test results (P_{neg}) was low (15%). Concordances between cELISA - mPCR and mPCR- BS results were around 50% of the Ppos, whereas the agreement on Pneg was estimated to be 5% and 31%, respectively.

Table 5. Agreement between the tests

Test	Kappa coefficient (95% CI)	P_{pos} (95% CI)	P_{neg} (95% CI)
cELISA-BS	0.13 (0.06-0.20)[a]	0.90 (0.88-0.92)	0.15 (0.07-0.24)
cELISA-mPCR	0.02 (0.01-0.03) [a]	0.54 (0.50-0.58)	0.05 (0.02-0.08)
mPCR- BS	0.02 (-0.04-0.07) [a]	0.51 (0.47-0.55)	0.31 (0.26-0.36)

Legend: mPCR, multiplex polymerase chain reaction; cELISA, competitive-inhibition enzyme-linked immunosorbent assay; BS, blood smear; CI, confidence interval; Ppos, positive agreement; Pneg, negative agreement; [a], slight agreement.

3.2. Model selection and posterior estimates

The summary of the model selection criteria and the Bayesian estimates of model parameters from applying models M1 to M8 are presented in **Table 6**.

Table 6. Comparison of model diagnostic parameters used to estimate the true prevalence of anaplasmosis in cattle and sensitivity and specificity of three diagnostic tests

Model	*P* value	DIC	*pD*	Prevalence	mPRC		cELISA		BS	
					Se	Sp	Se	Sp	Se	Sp
M1	0.68	21.10	-10.03	0.47	0.81	1.00	0.98	0.03	0.79	0.21
M2	0.58	32.83	3.42	0.32	0.98	1.00	0.99	0.03	0.81	0.20
M3	1.00	72.23	3.07	1.00	0.45	1.00	0.99	1.00	0.82	0.91
M4	1.00	70.69	2.98	1.00	0.45	1.00	1.00	1.00	0.82	0.98
M5	0.56	32.58	3.40	0.32	0.98	1.00	0.99	0.02	0.80	0.20
M6	0.61	33.63	3.23	0.31	0.98	1.00	0.99	0.03	0.81	0.20
M7	1.00	70.68	2.97	1.00	0.45	1.00	1.00	1.00	0.82	0.98
M8	1.00	1556.02	3.00	0.98	0.95	0.90	1.00	0.94	0.81	0.93

Legend: mPCR, multiplex polymerase chain reaction; cELISA, competitive-inhibition enzyme-linked immunosorbent assay; BS, blood smear; M1 to M8, Bayesian models tested; Se, sensitivity; Sp, specificity; P value, Bayesian P value; DIC, Deviance Information Criterion; pD, the effective number of estimated parameters.

In the M1 model, the negative *pD* value suggests that the constraints are insufficient to estimate the model parameters. In models M3, M4, M7, and M8, the *pD* values calculated from the constraints imposed are positives and close to 3, which indicates that all parameters can be estimated. However, the Bayesian *P* values (1.00) suggest a lack-of-fit, which is also reflected in the badly estimated prevalence and sensitivities. In contrast, models M2, M5, and M6 have correct *pD* values, and the prevalences almost equal their true values. Model M5 has a Bayesian *P* value close to 0.5, the distribution kernel density (stemmed unimodal) and autocorrelation graphs showed good convergence (**Appendix F**), and it has the lowest DIC value of the three models, in addition, the DIC of this model was lower than 36.81, the maximum/optimum value obtainable from the data.

Table 7 summarises the estimated values for Se and Sp for the three tests. Model 5 estimated a true prevalence of *Anaplasma marginale* to be 32% (95% CrI: 27% - 38%) and the ratio of challenge-immunized animals (RCIA) to be 67% (95% CrI: 62%-73%). The results of the sensitivity analyses are shown in **Table 8** (Model 5'), which introduces perturbations of ± 0.0001 to the limits of all prior intervals, shows only minimal changes in estimated parameter values and their associated 95% credible intervals. In addition to these statistically insignificant changes, the Bayes P and *pD* values tended to zero, which underscores the robustness of the model to such perturbations.

Table 7. Posterior mean for the prevalence and the test characteristics (Model 5)

Test	Parameter	Posterior estimates (95% CrI)
	Prevalence	0.32 (0.27-0.38)
mPRC	Se	0.98 (0.95-1.00)
	Sp	1.00
cELISA	Se	0.99 (0.97-1.00)
	Sp	0.02 (0.01-0.04)
BS	Se	0.80 (0.75-0.85)
	Sp	0.20 (0.16-0.24)

Legend: mPCR, multiplex polymerase chain reaction; cELISA, competitive-inhibition enzyme-linked immunosorbent assay; BS, blood smear; CrI, credibility interval; Se, sensitivity; Sp, specificity.

Table 8. Posterior mean for the prevalence and the test characteristics based on a sensitivity analysis.

Model & tests	*P* value	DIC	*pD*	Prevalence (95% CrI)	Sensitivity (95% CrI)	Specificity (95% CrI)
Model 5'	0.00	24.64	0.00	0.32 (0.32,0.32)		
mPRC					0.98 (0.98-0.98)	1
cELISA					0.99 (0.99-0.99)	0.02 (0.02-0.02)
BS					0.81 (0.80-0.81)	0.20 (0.20-0.20)

Legend: Legend: mPCR, multiplex polymerase chain reaction; cELISA, competitive-inhibition enzyme-linked immunosorbent assay; BS, blood smear; CrI, credibility interval; Se, sensitivity; Sp, specificity; P value, Bayesian P value; DIC, Deviance Information Criterion; pD, the effective number of estimated parameters.

The proportion of animals that tested positive for cELISA but were free to the pathogen or the rate of challenged-immunized animals in these endemic areas was about 67% (95% CrI: 62%-73%), which can be interpreted as the rate of positive-false results coming from cELISA tests in these anaplasmosis endemic areas, meaning that there is a high rate of immunised animals.

4. Discussion

In Ecuador, more than 75% of cattle herds are situated in areas either infested or suitable to the development of cattle ticks (Gioia et al. 2018). Consequently, TBDs and anaplasmosis in particular, are commonly reported among cattle farms; yet diagnosing them accurately without laboratory confirmation remains challenging. Despite numerous studies on the prevalence of anaplasmosis in the country, published data on the sensitivity and specificity of serological tests and the true prevalence of the disease still need to be concluded. This highlights the significance of our research, which represents the first reported study employing a Bayesian analysis framework to estimate the true prevalence, sensitivity, and specificity of mPCR, cELISA, and BS tests in an endemic area.

Anaplasmosis presents several diagnostic challenges, including non-specific clinical signs, multiple disease presentation (mild, acute, peracute, and chronic), presence of co-infections, and limitations of serological tests (Kocan et al. 2012; Ristic 2012; Sanchez-Vicente and Tokarz 2023; Tabor 2022; Young 2020). Sub-inoculation of *Anaplasma*-infected red blood cells in splenectomised calves has been considered the "gold standard" for determining persistent *A. marginale* infections in cattle but it is not a routine test and is impractical on a large scale evaluation (Kocan et al. 2012; Sharma et al. 2015).

Bovine anaplasmosis is conventionally diagnosed by the identification of *Anaplasma* spp. in Giemsa-stained BS from clinically suspect animals during the acute phase of the disease. However, this method has several disadvantages, such as requiring experienced personnel and being tedious and time-consuming. In addition, it is not useful for detecting carriers (challenged-immunized animals) and presymptomatic animals; and does not distinguish between *A. marginale* and *A. centrale* (M'ghirbi et al. 2016; Noaman and Shayan 2010). In this study the BS method shows a reduced specificity of only 20%, attributable to a high rate of false positive animals. This limitation is attributed to the operator skills when performing the technique (good smear preparation, proper staining, and a well-trained microscopist), which may result in the unintended misidentification of inclusion bodies unrelated to *A. marginale* (Aquino et al. 2018; Noaman and Shayan 2010).

Molecular methods have been developed with high sensitivity and specificity; the polymerase chain reaction (PCR) assay is commonly used to identify and differentiate the *Anaplasma* species. PCR additions allow early detection during the prepatent period and identification of an "active" infection at a single time-point (Aubry and Geale 2011). The high cost of reagents and equipment, the need for skilled personnel, and the time involved in performing these tests are not practical for large-scale surveillance (Kocan et al. 2012; Noaman and Shayan 2010). A cost-effective alternative is the use of pools: if a pool is positive, one goes back to the original constituent samples to directly count the number of positives (Cowling, Gardner, and Johnson 2002; Dorfman 1943; Speybroeck et al. 2012). The model employed in this study incorporated this aspect, obtaining a high sensitivity (98%) and specificity (100%) for the mPCR technique. These results are in line which previous studies employing individual analysis (Ganguly et al. 2018; Parodi et al. 2021; Shabana et al. 2018), indicating the feasibility of such alternative.

Currently, the cELISA test is based on the detection of antibodies specifically directed towards major surface protein 5 (MSP-5);it is one of the most widely used diagnostic techniques used as a screening test to detect cattle infected with *A. marginale* due to its practicality and cost-effectiveness (Kocan et al. 2012). Despite its established high sensitivity (96%) and specificity (95%) in non-endemic environments (Knowles et al. 1996; Kocan et al. 2012; Torioni de Echaide et al. 2005), our study, that was conducted in endemic areas, uncovered a significant discrepancy. While sensitivity remained robust at 99% (95% CrI: 97-100%), specificity drastically declined to 2% (95% CrI: 1-4%). While a positive

ELISA result confirms the presence of the pathogen at some time, as it detects antibodies, it does not necessarily mean that the pathogen is present by the time the test is performed (Aubry and Geale 2011; Tana-Hernández et al. 2017). Molloy et al. (1999), using a competitive inhibition ELISA, reported that this test did not clearly differentiate between infected and non-infected herds in *A. marginale* endemic areas. In the present study, the Bayesian model also estimated the proportion of ELISA-positive animals, even if they were not currently infected. The proportion of carrier, persistently infected cattle, or challenge-immunized animals, as they were called in this study, was approximately 70% (67% [95% CrI: 62%-73%]). Thus, in this study, we attribute the lower specificity of the ELISA to the abundant presence of challenge-immunized animals, which possess lifelong immunity and remain asymptomatic following the challenge exposure.

In endemic areas, the transmission of *A. marginale* may occur via the transplacental route, and carrier cows may transfer antibodies through colostrum, providing the calf with passive protection for at least three months (Coetzee 2022; Gonzalez Grau et al. 2013; Potgieter and van Rensburg 1987). In these areas, calves are exposed to a continuous challenge of infected ticks throughout the year. They contract natural infections and often show mild symptoms or remain asymptomatic, developing immunity that could last a lifetime (Garry 2008; Kocan, Blouin, and Barbet 2000; Medley, Perry, and Young 1993). A study carried out in endemic areas of Ecuador determined that *A. marginale* prevalence in *R. microplus* was 27% (Maya-Delgado et al. 2020). Persistently infected cattle are robustly immune to reinfection and serve as major reservoirs of infection for mechanical, biological, or vertical transmission, contributing significantly to maintaining the state of endemicity (Knowles et al. 1996; Kocan et al. 2003, 2010, 2012; M'ghirbi et al. 2016; Torioni de Echaide et al. 1998, 2005). Therefore, confirming a positive cELISA result with PCR in the absence of clinical disease or a history of endemic anaplasmosis is advisable (Kocan et al. 2012), as a positive ELISA result may be the result of a previous infection and does not necessarily mean the animal is currently infected. Additionally, the decline in specificity can also be attributed to cross-reactivity observed between *Anaplasma phagocytophilum* and *A. marginale*, as documented by Dreher et al. in 2005. Although the presence of *A. phagocytophilum* in cattle has not been confirmed in Ecuador, a 2015-study by Pesquera et al. reported its presence in *R. microplus* ticks (the most abundant in Ecuador) collected from cattle near the study areas.

The level of agreement between diagnostic tests was measured through the kappa coefficient, P_{pos}, and P_{neg}. The kappa coefficient for different test pairs was in slight agreement. Our results suggest a good level of agreement when positive results for cELISA-BS were considered. The agreement was around 50% for sera, which tested positive for cELISA-mPCR and mPCR-BS. Positive animal status may vary depending on the disease presentation (acute or chronic) (Saegerman et al. 1999). The agreement between different tests pairs was low for sera, which tested negative. Consequently, the level of agreement of negative results correlated with the relatively low specificity observed for the mELISA and blood smear, possibly due to the high prevalence of persistently infected cattle or challenged-

immunized animals (Torioni de Echaide et al. 1998). Animals that tested positive by cELISA but negative by mPCR might be attributed to carrier animals with circulating levels of *A. marginale* below levels detectable by PCR (Gioia et al. 2018). An *A. marginale* cattle carrier can have bacteraemia levels ranging from 0.0025% to 0.000025% of infected erythrocytes. Given that mPCR assays are capable of detecting approximately 0.0001% of infected erythrocytes, mPCR only detects a proportion of carrier animals (Figueroa et al. 1993; French et al. 1998). These results are consistent with a study by Hairgrove et al. (2015) and Gioia et al. (2018), who linked positive cELISA and negative RT-qPCR results to antibodies that remain in the blood after the decrease or clearance of the parasite. In our investigation, no animal tested positive for PCR and negative for cELISA, suggesting the absence of early infections. Previous research has shown that PCR is capable of detecting infections in the early stages (up to 15 days) that cELISA may miss due to low antibody loads (Gioia et al. 2018; Hairgrove et al. 2015).

Therefore, the selection of the diagnostic method(s) will depend on what we want to determine. On the one hand, a blood smear can reveal the presence of *Anaplasma* spp. in animals showing acute phase symptoms (clinical cases) to detect bovine anaplasmosis. Similarly, in these cases, the infection can also be diagnosed by serological demonstration of antibodies with confirmation by molecular detection methods (Aubry and Geale 2011). On the other hand, serological tests are particularly useful for epidemiological applications, such as prevalence studies and control programs. Antibody prevalence can indicate the existence of endemic stability, but it is important to interpret individual animal results with caution (Kivaria et al. 2012; Molloy et al. 1999; De Vos 1991). To detect infection in cattle and identify carrier animals for movement to disease-free areas, highly sensitive diagnostic techniques are required (Corona et al. 2014). Even end-point PCR may not detect the presence of *Anaplasma* in certain cases, so ELISA using msp5 recombinant protein is a recommended option (OIE 2015; Torioni de Echaide et al. 1998). Our study revealed that mPCR is very sensitive. However, its use alone is not advised, given the possible lack of performance in animals with low intracellular bacteria load. This limitation is not attributed to using pools, as a prior study utilizing the same cELISA commercial kit and real-time PCR revealed that 29% (40/140) of cELISA-positive animals were PCR-positive (Parvizi et al. 2020). Hence, the simultaneous use of ELISA and mPCR is advisable.

The true prevalence of *A. marginale* infection was estimated to be 32% in tropical dairy farms. The estimated true prevalence is lower than the seroprevalence detected by cELISA (98%) and BS (81%) methods but close to mPCR (36%). Preliminary studies on the Ecuadorian coast found similar results (86%) using cELISA (Fernandez 2018) and 66% in the Amazonian region using iELISA (Medina et al. 2017). Studies conducted in the Ecuadorian Amazon using a nested PCR (nPCR) determined a 44% prevalence for *Anaplasma marginale* (Caroa 2020) and 64% (Muñoz et al. 2020). Although this technique is capable of identifying a greater number of carrier animals, it poses specificity problems for routine use (OIE 2015). Other studies using PCR have found a prevalence for *A. marginale* of around 85% on the Ecuadorian coast (Escobar et al. 2015; Muñoz et al. 2020; Tana-Hernández et al. 2017).

Nevertheless, these studies were confined to a limited number of farms. Moreover, a study conducted by Chávez-Larrea et al. (2023) in two slaughterhouses reported a 60% prevalence for *A. marginale* using PCR. Similarly, the range of apparent prevalence was wide when using blood smears, i.e. from 19 to 39% in the coastal region (Luzurraga 2015; Macías and Villavicencio 2022; Minga 2019; Oñate 2015) and from 2 to 50% in the Amazon region (Caroa 2020; Celi 2010; Guamán-Quinche, Sarango-Guamán, and Guerrero-Pincay 2020; Muñoz et al. 2017; Vargas and De la Cueva 2014). However, the most important finding of this study was the detection of protective antibodies against *Anaplasma marginale* in about 70% of dairy cattle in endemic areas.

The clinical examination determined that most animals presented clinical parameters compatible with those of normal adult bovines. Only a small percentage of them (4%) presented body temperatures above 39.5°C. Such elevated body temperature could be attributed to various factors, including potential infectious, inflammatory processes or stress induced by handling during management (Mota-Rojas et al. 2021). The rare presence of clinical disease outbreaks, added to the antibody prevalence (98%), allows us to presume that the pathogen has reached a state of endemic stability in the study areas (Raboloko et al. 2020).

Although the animals in this zone are protected against the disease (challenged-immunized animals), it is common for farmers to implement certain practices to increase productivity on their farms, such as introducing animals from areas where the disease is not present. Kocan et al. (2012) emphasized the importance of maintaining challenge-immunized animals to keep endemic stability and avoid introducing susceptible cattle, which could increase the risk of acute disease. Additionally, since there is no reliable method to eliminate persistent *A. marginale* infections in cattle, animal movements should be restricted as a precautionary measure to protect animals in areas where the disease is not endemic (Coetzeea et al. 2005; Curtis et al. 2021; Kocan et al. 2010). Maintaining minimal tick infestations is critical not only for optimizing animal production but also for preserving the endemicity of anaplasmosis, thus playing a paramount role in disease management within these regions (Jonsson, Bock, and Jorgensen 2008). The importance of exposing calves to persistent tick infestation is emphasized (Lagranha et al. 2024; Mahoney et al. 1981; Mahoney and Ross 1972). This requires housing the calves in paddocks where they can come in contact with ticks, as zero grazing may cause a decline in endemic stability in the area (Jonsson et al. 2012; Peter et al. 2005; Rubaire-Akiiki et al. 2006).

5. Conclusions

In endemic zones for TBD, it is imperative to consider the immunological status of the animals under assessment with respect to these TBDs. Diagnostic tests must be validated for application in endemic populations, and it is crucial to interpret test results in conjunction with the exposure history and clinical signs. A significant finding of this study was the estimation that approximately 70% of dairy

cattle harboured protective antibodies against *Anaplasma marginale* in those endemic areas. Such high proportion may stem from persistent exposure with a high pathogen prevalence, as observed in previous studies, or from early primary challenges. The high seroprevalence and infrequent clinical outbreaks suggest that the pathogen has achieved endemic stability. Thus, any control program in these areas must strike the right balance such as implementing measures for tick control that enhance cattle productivity without compromising the delicate equilibrium required for maintaining endemic stability. In addition, avoiding practices like introducing animals from disease-free areas and/or inadequate calf immunization can disrupt endemic regions, leading to sporadic cases of clinical anaplasmosis. Moreover, it is imperative to highlight that the areas studied are endemic to *A. marginale*, which underlines the potential risk associated with the introduction of other species, such as *A. centrale*, or additional tick-borne pathogens, such as *Babesia* spp. that could cause an outbreak of TBDs. Furthermore, under scenarios of global warming, inadequate knowledge about anaplasmosis management can lead to risky management practices, such as the movements of carrier animals or improper handling of contaminated instruments, which can exacerbate the spread of TBDs to areas where they were previously absent (Marques et al. 2020).

Appendices

Appendix A. cELISA protocol

Appendix B. mPCR protocol

Appendix C. Conditional probabilities and test result probabilities, without considering that the mPCR was obtained by pooling

Appendix D. Ratio of challenge-immunized Animals

Appendix E. The WinBUGS code (MODEL 5) used to estimate true prevalence of anaplasmosis and test characteristics for mPCR, cELISA, and blood smear

Appendix F. Kernel density and Gelman Rubin statistic of the parameters estimated using the model 5

6. References

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7. Supplementary File

Appendix A. cELISA Protocol

1. Thaw the serums to room temperature (23 ± 2°C).
2. Prepare the conjugate: dilute 1 part of the antibody-peroxidase conjugate with 99 parts of the conjugate dilution buffer.
3. Prepare the wash solution: dilute 1 part of the wash solution with 9 parts of distilled water.
4. Load the positive control to the first well and the next 3 wells load with the negative controls.
5. Transfer 50 µl of the controls and serum samples from the transfer microplate to the antigen-coated plate as quickly as possible using a pipette. Tap the microplate so that the samples touch the bottom of the wells.
6. Incubate the microplate for 1 hour at room temperature (23 ± 2°C).
7. Wash: after 1- hour of incubation, remove the contents of the wells and tap the microplate 4 times on absorbent paper. Then wash 2 times with wash solution and tap the microplate on absorbent paper after each wash.
8. Conjugate: add 50µ of the prepared conjugate to each well. Tap the microplate so that the conjugate touches the bottom of the wells.
9. Incubate the microplate for 20 minutes at room temperature (23 ± 2°C).
10. Wash: wash the microplate 4 times.
11. Add 50µl of the substrate solution to each well. Tap the microplate so that the substrate touches the bottom of the wells.
12. Incubate the microplate for 20 minutes at room temperature (23 ± 2°C) and avoid exposure to light.
13. Add 50µl of the wash solution to each well. Tap the microplate so that the substrate mixes with the wash solution at the bottom of the wells.
14. Read the result: Read the microplate on the spectrophotometer by setting the device to 620 nm.

Appendix B. PCR Protocol

Composition of master mix for PCR amplification

Reagent	Multiplex PCR			Individual PCR		
	Initial concentration	Final concentration	Volume per reaction (µL)	Initial concentration	Final concentration	Volume per reaction (µL)
PCR buffer	5X	1X	4	5X	1X	3
MgCl2	25 mM	1.2 mM	0.96	25 mM	1.2 mM	0.72
dNTPs	25 mM	0.3 mM	0.24	25 mM	0.2 mM	0.12
Ana 19A	10 µM	0.2 µM	0.4	10 µM	0.23 µM	0.35
Ana 19B	10 µM	0.2 µM	0.4	10 µM	0.23 µM	0.35
CentF	10 µM	0.3 µM	0.6	-	-	-
CentR	10 µM	0.3 µM	0.6	-	-	-
Taq polymerase	5 U/µL	0.14 U/µL	0.56	5 U/µL	0.033 U/µL	0.1
DNA	-	-	5	-	-	3
H20	-	-	7.24	-	-	7.36
Total volume			20 µL			15 µL

millimolar (mM), micromolar (µM), microliter (µL), units per microliter (U/µL)

PCR cycling conditions for multiplex PCR and individual PCR of *Anaplasma marginale*

		Multiplex PCR			Individual PCR		
	Step	Temperature	Time	Number of cycles	Temperature	Time	Number of cycles
Step 1	Initial denaturation	94	3 min	1	94	4 min	1
	Denaturation	94	30 s		94	45 s	
Step 2	Annealing	54	45 s	35	64	30 s	35
	Extension	72	1 min		72	1 min	
Step 3	Final extension	72	10 min	1	72	10 min	1

minute, min; second, s

Appendix C

In the following codes, D+ (D-) indicates that a subject is diseased (disease-free); the actual prevalence of anaplasmosis is represented by TP; the sensitivity and specificity of mPCR by Se1 and Sp1, respectively. Being mPCR (T1), cELISA (T2), blood smear (T3), negative results (0 or -), and positive results (1 or +), the expected cell probabilities (P) under the conditional dependence assumption are:

Conditional probabilities

$\theta_1 = P(D^+) = TP$
$\theta_2 = P(T1^+|D^+) = Se1$
$\theta_3 = P(T1^-|D^-) = Sp1$
$\theta_4 = P(T2^+|D^+, T1^+)$
$\theta_5 = P(T2^+|D^+, T1^-)$
$\theta_6 = P(T2^-|D^-, T1^-)$
$\theta_7 = P(T2^-|D^-, T1^+)$
$\theta_8 = P(T3^+|D^+, T1^+, T2^+)$
$\theta_9 = P(T3^+|D^+, T1^+, T2^-)$
$\theta_{10} = P(T3^+|D^+, T1^-, T2^+)$
$\theta_{11} = P(T3^+|D^+, T1^-, T2^-)$
$\theta_{12} = P(T3^-|D^-, T1^-, T2^-)$
$\theta_{13} = P(T3^-|D^-, T1^-, T2^+)$
$\theta_{14} = P(T3^-|D^-, T1^+, T2^-)$
$\theta_{15} = P(T3^-|D^-, T1^+, T2^+)$

Test result probabilities, without considering that the mPCR was obtained by pooling.

$pr[1] = P(111) = \theta_1\,\theta_2\,\theta_4\,\theta_8 + (1-\theta_1)\,(1-\theta_3)\,(1-\theta_7)\,(1-\theta_{15})$
$pr[2] = P(110) = \theta_1\,\theta_2\,\theta_4\,(1-\theta_8) + (1-\theta_1)\,(1-\theta_3)\,(1-\theta_7)\,\theta_{15}$
$pr[3] = P(101) = \theta_1\,\theta_2\,(1-\theta_4)\,\theta_9 + (1-\theta_1)\,(1-\theta_3)\,\theta_7\,(1-\theta_{14})$
$pr[4] = P(100) = \theta_1\,\theta_2\,(1-\theta_4)\,(1-\theta_9) + (1-\theta_1)\,(1-\theta_3)\,\theta_7\,\theta_{14}$
$pr[5] = P(011) = \theta_1\,(1-\theta_2)\,\theta_5\,\theta_{10} + (1-\theta_1)\,\theta_3\,(1-\theta_6)\,(1-\theta_{13})$
$pr[6] = P(010) = \theta_1\,(1-\theta_2)\,\theta_5\,(1-\theta_{10}) + (1-\theta_1)\,\theta_3\,(1-\theta_6)\,\theta_{13}$
$pr[7] = P(001) = \theta_1\,(1-\theta_2)\,(1-\theta_5)\,\theta_{11} + (1-\theta_1)\,\theta_3\,\theta_6\,(1-\theta_{12})$
$pr[8] = P(000) = \theta_1\,(1-\theta_2)\,(1-\theta_5)\,(1-\theta_{11}) + (1-\theta_1)\,\theta_3\,\theta_6\,\theta_{12}$

Appendix D

	D^+				D^-			
	$cELISA^+$		$cELISA^-$		$cELISA^+$		$cELISA^-$	
	Blood smear$^+$	**Blood smear $^-$**	**Blood smear$^+$**	**Blood smear $^-$**	**Blood smear$^+$**	**Blood smear $^-$**	**Blood smear$^+$**	**Blood smear $^-$**
mPCR$^+$	*a* $P(D^+ \cap T1^+ \cap T2^+ \cap T3^+)$	*b* $P(D^+ \cap T1^+ \cap T2^+ \cap T3^-)$	*c* $P(D^+ \cap T1^+ \cap T2^- \cap T3^+)$	*d* $P(D^+ \cap T1^+ \cap T2^- \cap T3^-)$	*e* $P(D^- \cap T1^+ \cap T2^+ \cap T3^+)$	*f* $P(D^- \cap T1^+ \cap T2^+ \cap T3^-)$	*g* $P(D^- \cap T1^+ \cap T2^- \cap T3^+)$	*h* $P(D^- \cap T1^+ \cap T2^- \cap T3^-)$
mPCR$^-$	*i* $P(D^+ \cap T1^- \cap T2^+ \cap T3^+)$	*j* $P(D^+ \cap T1^- \cap T2^+ \cap T3^-)$	*k* $P(D^+ \cap T1^- \cap T2^- \cap T3^+)$	*l* $P(D^+ \cap T1^- \cap T2^- \cap T3^-)$	*m* $P(D^- \cap T1^- \cap T2^+ \cap T3^+)$	*n* $P(D^- \cap T1^- \cap T2^+ \cap T3^-)$	*o* $P(D^- \cap T1^- \cap T2^- \cap T3^+)$	*p* $P(D^- \cap T1^- \cap T2^- \cap T3^-)$

Legend: D+ (D-), Diseased (Disease-free); Multiplex PCR, mPCR (T1); Competitive-inhibition enzyme-linked immunosorbent assay, cELISA (T2); Blood smear (T3) ; -, Negative results; +, Positive results.

$a = \theta_1\,\theta_2\,\theta_4\,\theta_8$ $\quad e = (1-\theta_1)\,(1-\theta_3)\,(1-\theta_7)\,(1-\theta_{15})$ $\quad i = \theta_1\,(1-\theta_2)\,\theta_5\,\theta_{10}$ $\quad m = (1-\theta_1)\,\theta_3\,(1-\theta_6)\,(1-\theta_{13})$

$b = \theta_1\,\theta_2\,\theta_4\,(1-\theta_8)$ $\quad f = (1-\theta_1)\,(1-\theta_3)\,(1-\theta_7)\,\theta_{15}$ $\quad j = \theta_1\,(1-\theta_2)\,\theta_5\,(1-\theta_{10})$ $\quad n = (1-\theta_1)\,\theta_3\,(1-\theta_6)\,\theta_{13}$

$c = \theta_1\,\theta_2\,(1-\theta_4)\,\theta_9$ $\quad g = (1-\theta_1)\,(1-\theta_3)\,\theta_7\,(1-\theta_{14})$ $\quad k = \theta_1\,(1-\theta_2)\,(1-\theta_5)\,\theta_{11}$ $\quad o = (1-\theta_1)\,\theta_3\;\;\theta_6\,(1-\theta_{12})$

$d = \theta_1\,\theta_2\,(1-\theta_4\,)\,(1-\theta_9)$ $\quad h = (1-\theta_1)\,(1-\theta_3)\,\theta_7\,\theta_{14}$ $\quad l = \theta_1\,(1-\theta_2)\,(1-\theta_5)\,(1-\theta_{11})$ $\quad p = (1-\theta_1)\,\theta_3\,\theta_6\,\theta_{12}$

$$Ratio\ of\ challenge-immunized\ animals\ (RCIA) = \frac{number\ of\ false\ positives}{number\ of\ ELISA\ positives}$$

$$RCIA = \frac{e+f+m+n}{a+b+i+j+e+f+m+n}$$

$$RCIA = \frac{1-\theta_7+\theta_3\theta_7-\theta_1+\theta_1\theta_7-\theta_1\theta_3\theta_7-\theta_3\theta_6+\theta_1\theta_3\theta_6}{\theta_1\theta_2\theta_4+\theta_1\theta_5-\theta_1\theta_2\theta_5+1-\theta_7+\theta_3\theta_7-\theta_1+\theta_1\theta_7-\theta_1\theta_3\theta_7-\theta_3\theta_6+\theta_1\theta_3\theta_6}$$

Appendix E

Appendix E1 presents the WinBUGS code (MODEL 5) used to estimate true prevalence of anaplasmosis and test characteristics for mPCR, cELISA, and blood smear.

```
anaplasma_animal <- model {
r[1:8] ~ dmulti(pr[1:8], n)

th1prime <- th[1]/(1-pow(1-th[1], k))
for (i in 1:k)
{
  se_p[i] <- (1 - pow(1 - th[2], i)) * exp(logfact(k))/(exp(logfact(i))*exp(logfact(k-i))) * pow(th[1], i) * pow(1-th[1], k-i)
}
ap <- th1prime * sum(se_p[]) / (1 - pow(1-th[1], k))

pr[1] <- ap * th[2]    *    th[4]    *    th[8]
pr[2] <- ap * th[2]    *    th[4]    * (1-th[8])
pr[3] <- ap * th[2]    * (1-th[4])  *    th[9]
pr[4] <- ap * th[2]    * (1-th[4])  * (1-th[9])
pr[5] <- ap * (1-th[2])    *   th[5]    *    th[10]    + (1-ap) * (1-th[6]) * (1-th[13])
pr[6] <- ap * (1-th[2]) *  th[5]         * (1-th[10]) + (1-ap) * (1-th[6]) * th[13]
pr[7] <- ap * (1-th[2])    * (1-th[5]) *    th[11]
pr[8] <- ap * (1-th[2])    * (1-th[5]) * (1-th[11]) + (1-ap) * th[6]

r2[1:8] ~ dmulti(pr[1:8],n)
for (i in 1:8)
{
  d[i] <- r[i]*log(max(r[i],1)/(pr[i]*n))
  d2[i] <- r2[i]*log(max(r2[i],1)/(pr[i]*n))
}
bayesp <- step(sum(d[]) - sum(d2[]))

tp <- th[1]
se[1] <- th[2]
sp[1] <- th[3]
se[2] <- th[2] * th[4] + (1-th[2]) * th[5]
sp[2] <- th[6]
se[3] <- th[2] * (th[4] * th[8] + (1-th[4]) * th[9]) + (1-th[2]) * (th[5] * th[10] + (1-th[5]) *  th[11])
sp[3] <- th[6] + (1-th[6]) * th[13]
```

```
RCIA<- (1 - th[7] + (th[3]*th[7]) - th[1] + (th[1]*th[7]) + (th[1]*th[3]) - (th[1]*th[3]*th[7]) - (th[3]*th[6]) - (th[1]*th[3]) + (th[1]*th[3]*th[6]) ) / ( (th[1]*th[2]*th[4]) + (th[1]*th[5]) - (th[1]*th[2]*th[5]) + 1 - th[7] + (th[3]*th[7]) - th[1] + (th[1]*th[7]) + (th[1]*th[3]) - (th[1]*th[3]*th[7]) - (th[3]*th[6]) - (th[1]*th[3]) + (th[1]*th[3]*th[6]) )

th[1] ~ dunif(0.00, 1.00)
th[2] ~ dunif(0.95, 1.00)
th[3]<-1
th[4] ~ dunif(0.00, 1.00)
th[5] ~ dunif(0.00, 1.00)
th[6] ~ dunif(0.00, 1.00)
th[7] ~ dunif(0.00, 1.00)
th[8] ~ dunif(0.00, 1.00)
th[9] ~ dunif(0.00, 1.00)
th[10] ~ dunif(0.00, 1.00)
th[11] ~ dunif(0.00, 1.00)
th[12]<-1
th[13] ~ dunif(0.00, 1.00)
th[14] ~ dunif(0.00, 1.00)
th[15] ~ dunif(0.00, 1.00)
}
list(r=c(192,44,0,0,325,70,0,10),n=641, k=5)
```

Appendix E2 presents the unrestricted WinBUGS code used to estimate true prevalence of anaplasmosis and test characteristics for mPCR, cELISA, and blood smear.

```
anaplasma <- model {
r[1:8] ~ dmulti(pr[1:8], n)
th1prime <- th[1]/(1-pow(1-th[1], k))

for (i in 1:k)
{
  se_p[i] <- (1 - pow(1 - th[2], i)) * exp(logfact(k))/(exp(logfact(i))*exp(logfact(k-i))) * pow(th[1], i) * pow(1-th[1], k-i)
}
se_p_tot <- sum(se_p[]) / (1 - pow(1-th[1], k))
ap <- th[1] * se_p_tot / (1-pow(1 - th[1] * se_p_tot, k))

pr[1] <- ap * th[2] * th[4] * th[8] + (1-ap) * (1-th[3]) * (1-th[7]) * (1-th[15])
pr[2] <- ap * th[2] * th[4] * (1-th[8]) + (1-ap) * (1-th[3]) * (1-th[7]) * th[15]
pr[3] <- ap * th[2] * (1-th[4]) * th[9] + (1-ap) * (1-th[3]) * th[7] * (1-th[14])
pr[4] <- ap * th[2] * (1-th[4]) * (1-th[9]) + (1-ap) * (1-th[3]) * th[7] * th[14]
```

```
pr[5] <- ap * (1-th[2]) * th[5] * th[10] + (1-ap) * th[3] * (1-th[6]) * (1-th[13])
pr[6] <- ap * (1-th[2]) * th[5] * (1-th[10]) + (1-ap) * th[3] * (1-th[6]) * th[13]
pr[7] <- ap * (1-th[2]) * (1-th[5]) * th[11] + (1-ap) * th[3] * th[6] * (1-th[12])
pr[8] <- ap * (1-th[2]) * (1-th[5]) * (1-th[11]) + (1-ap) * th[3] * th[6] * th[12]

r2[1:8] ~ dmulti(pr[1:8],n)
for (i in 1:8)
{
  d[i] <- r[i]*log(max(r[i],1)/(pr[i]*n))
  d2[i] <- r2[i]*log(max(r2[i],1)/(pr[i]*n))
}
bayesp <- step(sum(d[]) - sum(d2[]))

tp <- th[1]
se[1] <- th[2]
sp[1] <- th[3]
se[2] <- th[2] * th[4] + (1-th[2]) * th[5]
sp[2] <- th[3] * th[6] + (1-th[3]) * th[7]
se[3] <- th[2] * (th[4] * th[8] + (1-th[4]) * th[9]) + (1-th[2]) * (th[5] * th[10] + (1-th[5]) *  th[11])
sp[3] <- th[3] * (th[6] * th[12] + (1-th[6]) * th[13]) + (1-th[3]) * (th[7] * th[14] + (1-th[7]) * th[15])

RCIA<-  (1 - th[7] + (th[3]*th[7]) - th[1] + (th[1]*th[7]) + (th[1]*th[3]) - (th[1]*th[3]*th[7]) - (th[3]*th[6]) - (th[1]*th[3]) + (th[1]*th[3]*th[6]) )  /  ( (th[1]*th[2]*th[4]) + (th[1]*th[5]) - (th[1]*th[2]*th[5])  + 1 - th[7] + (th[3]*th[7]) - th[1] + (th[1]*th[7]) + (th[1]*th[3]) - (th[1]*th[3]*th[7]) - (th[3]*th[6]) - (th[1]*th[3]) + (th[1]*th[3]*th[6]) )

th[1] ~ dunif(0,1)
th[2] ~ dunif(0,1)
th[3] ~ dunif(0,1)
th[4] ~ dunif(0,1)
th[5] ~ dunif(0,1)
th[6] ~ dunif(0,1)
th[7] ~ dunif(0,1)
th[8] ~ dunif(0,1)
th[9] ~ dunif(0,1)
th[10] ~ dunif(0,1)
th[11] ~ dunif(0,1)
th[12] ~ dunif(0,1)
th[13] ~ dunif(0,1)
th[14] ~ dunif(0,1)
th[15] ~ dunif(0,1)
}
list(r=c(185,41,0,0,315,69,0,10),n=620, k=5)
```

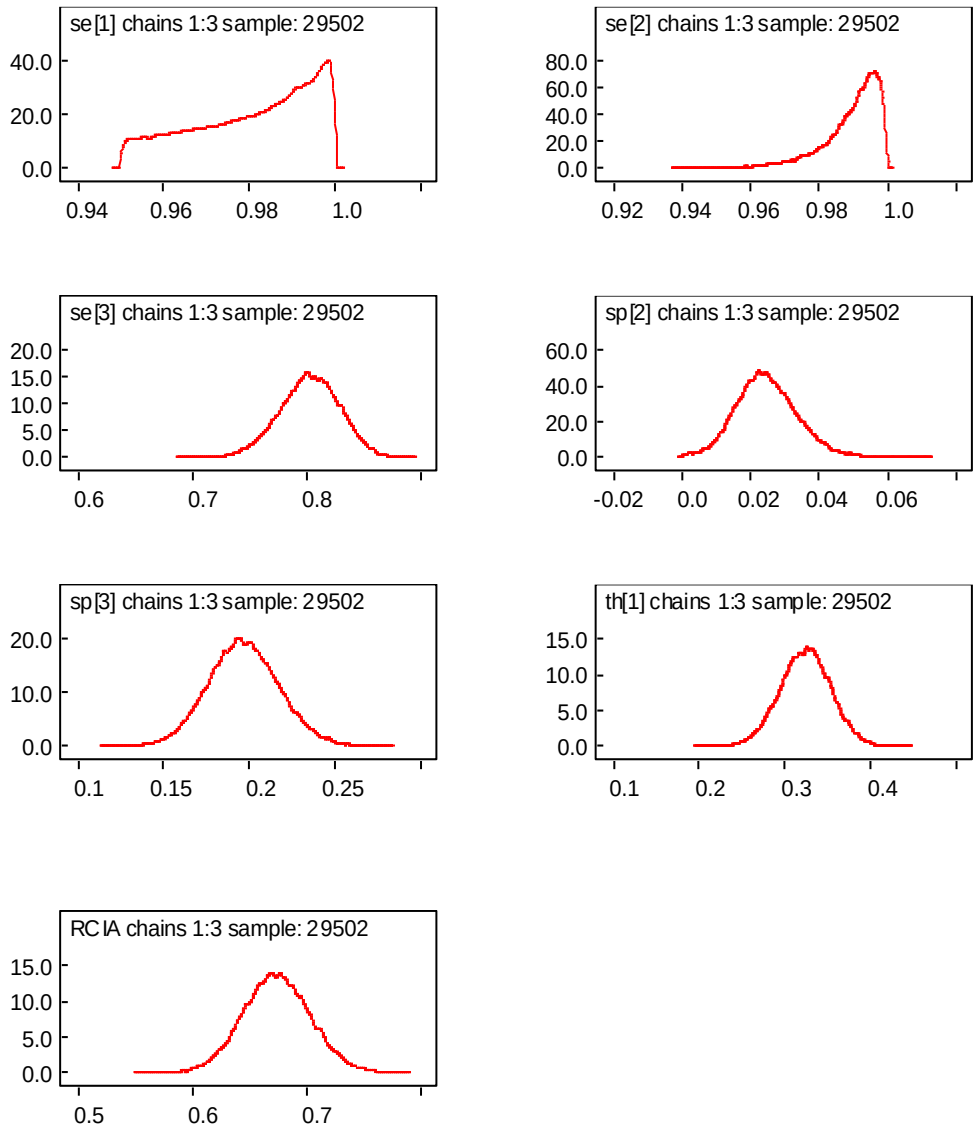
Appendix F

Kernel density and Gelman Rubin statistic of the parameters estimated with Model 5

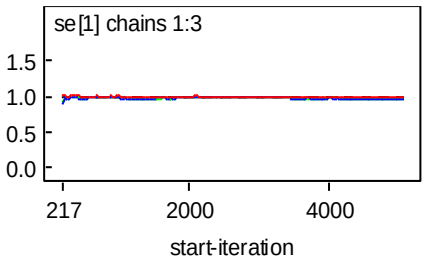
In the following graphs generated in WinBUGS, the sensitivity and specificity of mPCR are represented by y se[1] and sp[1], respectively, of cELISA by se[2] and sp[2] respectively, and of blood smear by se[3] and sp[3] respectively. The true prevalence of anaplasmosis is represented by th[1], and the rate of challenge-immunized animals by RCIA.

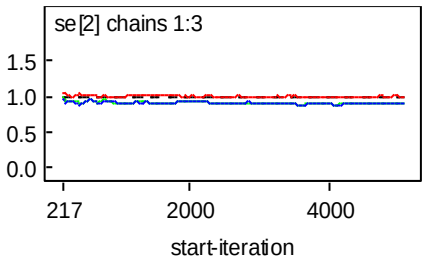
MODEL 5

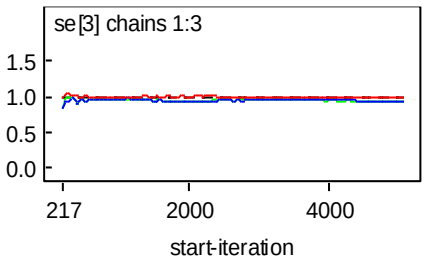
Kernel density

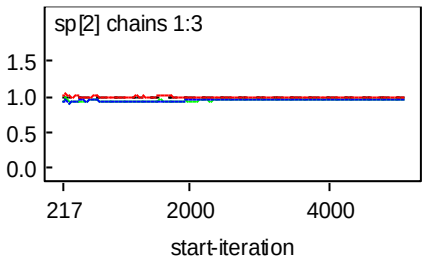


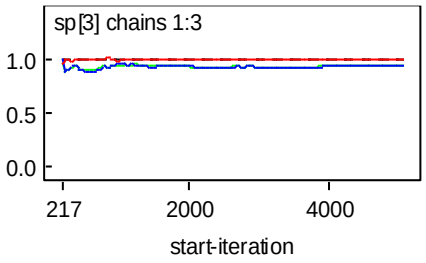
Gelman Rubin statistic

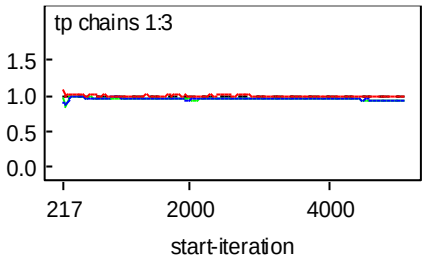


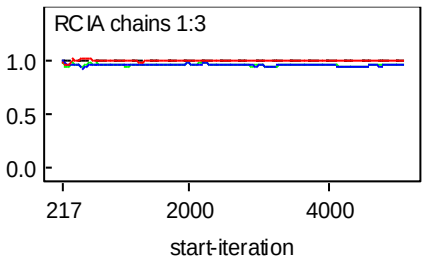












Experimental section

Study 5

Evaluating the Human Risks of Consumption of Bovine Products with Ivermectin Residues in Ecuador

Submitted in Foods

Valeria Paucar-Quishpe, Darío Cepeda-Bastidas, Richar Rodríguez-Hidalgo, Ximena Pérez-Otáñez, Cecilia Perez, Sandra Enríquez, Erika Guzman, Fernanda Ulcuango, Jorge Grijalva, Sophie O. Vanwambeke, Lenin Ron-Garrido, Claude Saegerman,

Preamble

Bovine products like meat and milk are important parts of diets worldwide, providing essential nutrients and contributing significantly to the global food supply. However, the widespread use of veterinary pharmaceuticals in cattle, such as ivermectin for controlling parasites, raises significant public health concerns regarding the safety of these drugs. Ivermectin is widely used to treat and control a wide variety of livestock parasites. However, its misuse and failure to adhere to withdrawal times may lead to residues in bovine products. This study aims to investigate the presence of ivermectin residues in cattle milk, meat, liver, urine, and faeces. Additionally, it will evaluate the risk of consuming ivermectin-contaminated cattle meat, liver, or milk.

Human Risks of Consuming Bovine Products with Ivermectin Residues in Ecuador

Valeria Paucar-Quishpe[1,2], Darío Cepeda-Bastidas[3], Richar Rodríguez-Hidalgo[2,4], Ximena Pérez-Otáñez[2,5], Cecilia Perez[4], Sandra Enríquez[2], Erika Guzman[3], Fernanda Ulcuango[3], Jorge Grijalva[4], Sophie O. Vanwambeke[5], Lenin Ron-Garrido[2,4¶], Claude Saegerman[1¶]

[1] Research Unit of Epidemiology and Risk Analysis applied to Veterinary Science (UREAR-ULiège), Fundamental and Applied Research for Animals & Health (FARAH) Center, Faculty of Veterinary Medicine, University of Liege, 4000 Liège, Belgium. claude.saegerman@uliege.be

[2] Instituto de Investigación en Zoonosis (CIZ), Universidad Central del Ecuador, 170521 Quito, Ecuador. avpaucar@uce.edu.ec; xfperez@uce.edu.ec; ienriquez@uce.edu.ec

[3] Facultad de Ciencias Agrícolas, Universidad Central del Ecuador, 170521 Quito, Ecuador. dacepedab@uce.edu.ec; ezguzman@uce.edu.ec, fvulcuango@uce.edu.ec

[4] Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, 170521 Quito, Ecuador. rrodriguez@uce.edu.ec; bcperez@uce.edu.ec; jgrijalva@uce.edu.ec; ljron@uce.edu.ec

[5] Georges Lemaitre Centre for Earth and Climate Research, Earth & Life Institute, 1348 UCLouvain, Louvain-la-Neuve, Belgium. sophie.vanwambeke@uclouvain.be

* Correspondence author: e-mail: claude.saegerman@uliege.be; ljron@uce.edu.ec

¶ These authors contributed equally to this work.

Abstract: Ivermectin is an antiparasitic commonly used in livestock. However, its use can result in the presence of residues in products and excretions of treated animals. The objective of the present study was to determine the presence of ivermectin residues in cattle meat, liver, milk, faeces, and urine. In addition, the risk of consuming residues of ivermectin was evaluated quantitatively using a deterministic approach based on the acceptable daily intake, the daily consumption of meat, liver, and milk, and the residues in bovine food items. The results evidenced the presence of ivermectin residues (decreasing order) in 68% of faeces samples (27/40) and around 3% in the liver (1/30), milk (2/70), and urine (1/39) samples. No residue was present in meat samples. The results obtained from the estimated daily intake of ivermectin show that the consumption of residues was low, and the risk was assessed as negligible. In addition, the results obtained from the chronic dietary exposure show that the consumption of residues was low, and the risk was assessed as rare to very rare. However, in a one health perspective, the presence of residues in faeces opens the door to further research since the risk is present for the environment and non-target species, and ivermectin residues in bovine products (different approach), despite being banned in dairy cattle (dry and milking period), is being used.

Keywords: Ivermectin residues, milk, meat, liver, urine, faeces, risk assessment, consumption

1. Introduction

The livestock sector plays a critical role in global food supply and security, providing essential nutrition through products like meat, milk, and eggs. These products account for 18% of global calorie intake and 34% of protein consumption, significantly enhancing human diets (FAO, CIRAD, & ILRI, 2020). Meat and meat-derived products are an energy-dense source of high-quality protein enriched in micronutrients such as vitamin B12, iron, zinc, selenium and phosphorus (Pereira & Vicente, 2013). Milk and dairy products contribute significantly to calcium, phosphorus, iodine, riboflavin, and vitamins A and B_{12} (Kliem & Givens, 2011). In 2023, global milk production reached 965.5 million tons and meat production 76.6 million tons (FAO, 2024b, 2024a).

In Ecuador, the livestock sector is a vital component of the agricultural economy. The country has a livestock population of 3.7 million heads (INEC, 2024), with 81% of this population raised by small producers who manage 20 or fewer cattle heads (Agrocalidad, 2018). Cattle husbandry in Ecuador primarily relies on grazing, with about 80% located in tropical or subtropical areas (Pourrut *et al.*, 1983; Torres *et al.*, 2014). These areas create proper conditions conducive to diseases caused by endo- and ectoparasites, which are the leading causes of illness and production losses (Bianchin *et al.*, 2007).

Various drugs are used to manage cattle internal and external parasites; one of the most widely used is avermectins. Avermectins are a class of macrocyclic lactones produced by the soil actinomycete *Streptomyces avermitilis* (Campbell *et al.*, 1983; D'Auria *et al.*, 2023). This drug was discovered in 1973 and introduced to massive commercial success in the animal health market since 1981 (Campbell *et al.*, 1983). The most widely utilized derivative of avermectin is ivermectin. Five years after its introduction, it was sold in 46 countries and administered to 320 million cattle heads (Pecenka & Lundgren, 2019). Its success in the livestock market is due to its strong activity on a wide variety of nematode and arthropod parasites (Campbell *et al.*, 1983). Ivermectin is used to treat billions of cattle heads, helping to boost the production of food and leather products, as well as keeping cattle healthy around the world (Crump & Omura, 2011).

Although the health benefits of ivermectin are particularly important for livestock, its high level of faecal excretion represents a potential environmental risk (González Canga *et al.*, 2009; Vokřál *et al.*, 2019). Additionally, the indiscriminate use of these drugs can result in trace amounts of residues and their metabolites persisting in edible tissues and animal products, such as meat, liver, and milk. (**Figure 1**), which may pose potential health risks to people who consume these products (Sanders, 2007). To ensure that levels of acaricides and/or their metabolites in food of animal origin remain below thresholds hazardous to consumers, the livestock industry must strictly comply with mandated withdrawal periods to reduce residue levels. Ivermectin, in particular, is banned in milk production due to its highly lipophilic nature, which causes its residues to persist in milk and dairy products (Dedavid e Silva *et al.*, 2024; Escribano *et al.*, 2012). Consequently, most countries have implemented strict regulations that

establish maximum residue limits (MRLs) in food products to ensure their safety for consumption. However, to respect the withdrawal periods, farmers must dispose of the milk and not slaughter the animal during the resting period, which represents an additional cost to tick control (De Meneghi, Stachurski, & Adakal, 2016). Dairy farmers continue to rely on the use of pesticides to control pests and increase productivity, looking only at the immediate advantages of pest control without considering the potential short and long-term risks of residue accumulation.

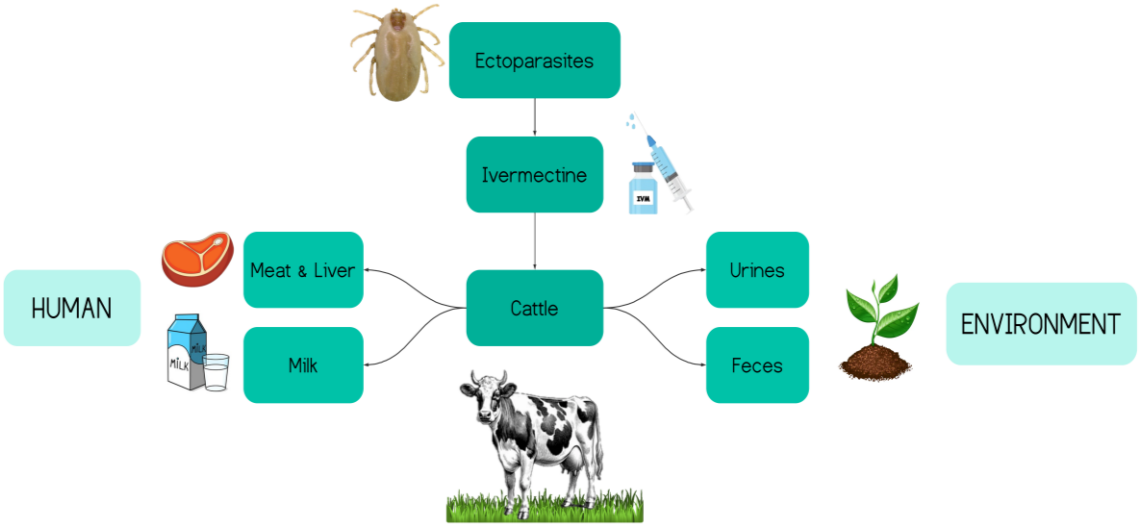


Figure 1. Interactions between ivermectin treatment, cattle products, environmental and human

This challenge is particularly acute for small dairy farmers, who must compete with larger producers that enjoy better market access and lower production costs. Compared to large industries, small farmers often grapple with daily issues such as inadequate infrastructure, limited resources, and insufficient access to veterinary care, making it difficult for them to produce high-quality milk at competitive costs (Paucar *et al.*, 2023). In Ecuador, inconsistent supervision leads many small producers to operate in informal markets where quality controls are minimal (CIL, 2023). Economic pressures and competition further exacerbate the situation, often compromising milk quality and posing potential health risks to consumers. In this context the objectives of this study were 1) to determine the prevalence of ivermectin residues in small-scale dairy farms located in two subtropical areas of Ecuador in foods of bovine origin such as milk, meat, liver, and excretions like urine, and faeces; and 2) to assess the risk of consuming those foods of bovine origin through the measurement of ivermectin concentrations in these food items.

2. Materials and Methods

2.1. Ethics statement

This study was conducted as part of the project “Socio-eco-epidemiology of ticks, tick-borne parasites, acaricide resistance and residual effects of acaricides in Ecuadorian tropical livestock:

environmental, animal and public health impacts". The study protocol was reviewed and approved by the Human Research Ethics Committee (COIF-FMVZ) of the *Universidad Central del Ecuador* (Code: 017-DOC-FMVZ-2023), ensuring that all ethical guidelines were followed.

2.2. Study area

This research was conducted in two livestock areas of Ecuador. Area 1, known as the "Northwest of Pichincha," is situated in the province of Pichincha and includes the localities of *Nanegal, Nanegalito, Pacto, Gualea, San Miguel de los Bancos* and *Pedro Vicente Maldonado*. This area is part of the Chocó Andino Biosphere Reserve, characterised by forests, rivers, waterfalls, and a diverse range of flora and fauna (RBCAP, 2019). Area 2, known as the "Quijos River Valley," is located in the province of Napo and comprises the localities of San Francisco de Borja, Sumaco, Linares, Sardinas, El Chaco, and Baeza. This area, situated in the foothills of the Andes Mountains and the high jungle of the Amazon region, is part of the protected areas of the Antisana Ecological Reserve, Cayambe Coca National Park, and Sumaco Napo Galeras National Park (Cárdenas, 2010; Guamán *et al.*, 2019). The residents in both areas are mainly involved in ecotourism, agriculture (tropical fruits, sugar cane, cacao, coffee, palm heart), fish farming (tilapia, trout), and cattle breeding (Benavides, 2022; Cabezas et al., 2019; Grijalva, Arévalo, & Wood, 2004; RBCAP, 2019).

Livestock in these areas primarily consists of small and medium-sized cattle herds dedicated to dairy or dual-purpose production. The farmers in these areas frequently use dairy breeds such as Brown Swiss, Holstein, Jersey or their crosses (Benavides Ortiz *et al.*, 2016; Guamán *et al.*, 2019; Paucar *et al.*, 2021). Feeding is mainly through grazing, but there is also the use of supplemental feeding based on concentrates or agro-industrial byproducts (Paucar *et al.*, 2023; Torres, 2012). The cattle population in the study areas is around 100,000 heads distributed across 4,087 herds (Agrocalidad, 2023).

2.3. Sampling and chemical analysis

From 2021 to 2023, samples of milk, beef, and liver were collected from the two study areas. Raw cow milk samples (N=70) were obtained from small milk tanks designated for collection. Each milk collector tank represented one farm; and holds approximately 40 litres from 5 to 7 cows. The selection of milk-tank samples was mainly based on sample accessibility and followed the primary route taken by the milk collection trucks. Meat (N=46) and liver (N=30) samples were collected at local slaughterhouses in each area. Additionally, samples of urine (N=39) and faeces (N=40) were collected. Each of these samples consisted of a pool of 6 cows from the farm. As one local slaughterhouse in Area 2 was closed, samples were acquired from a nearby slaughterhouse where animals from the study areas were relocated (**Figure 2**). The sampling process was carried out randomly, and official animal movement guides were reviewed to ensure that the animals were from the study areas.

Raw milk samples (100 ml) were collected in polyethene plastic vials, and meat and liver samples (100 gr) were stored individually in zip-lock plastic bags. All samples were kept in a cooler with ice blocks until they were transported to the laboratory. Analyses were conducted in the EcuaChemLab Chemical and Microbiological Laboratory of Ecuador, which is accredited according to NTE INEN ISO/IEC 17025. The concentration of the B1a component of ivermectin was analysed using a High-Performance Liquid Chromatographic method with Electron Affinity Fluorescence Quenching (HPLC-EAFQ), with a limit of detection (LOD) of <10 µg/kg.

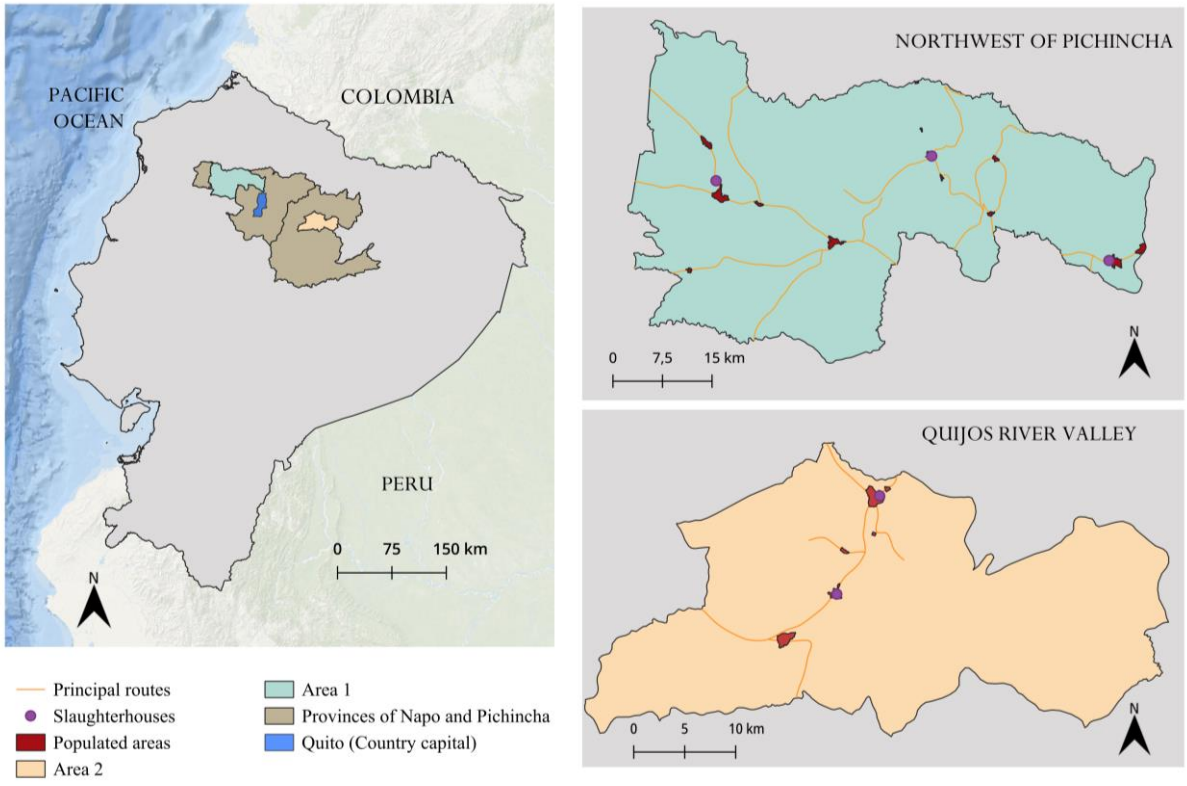


Figure 2. Location of study areas and sampling sites

2.4. Food consumption survey

A questionnaire was used to estimate milk, meat, and liver consumption. The questionnaire was validated by national and international experts in the field. It was pilot tested with a small group of volunteers, who commented on the clarity of the questions. The participants interviewed were men or women inhabitants of the study areas, heads of household, and over 18 years of age in 2024. The data were collected in paper-and-pencil format and contained questions on socio-demographic information (gender, age, number of persons living in the household), and consumption habits of foods of bovine origin at the household level (**Figure 2**). The sample size was estimated using household data from the INEC (2010) census; with a total of 17,194 households as the population size reference, a confidence level of 95%, and a margin of error of 5%. Consequently, the study included a sample size of 631

households. Information on beef, liver, and milk consumption was expressed in grams, with the conversion factor of 1 ml of milk corresponding to 1.03 g (Guerrero-Beltrán et al., 2010).

2.5. Risk to consumer's health

For human health, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established for ivermectin an acceptable daily intake (ADI) for consumers of 0-10µg/kg of body weight (FAO/WHO, 2023).

Concentrations of ivermectin residues measured in milk, meat, and liver were compared to the Maximum Residue Level (MRL) for human consumption. MRL are 10µg/kg in milk, 30µg/kg in muscle, and 800µg/kg in liver (FAO/WHO, 2023). There are several approaches for chronic exposure assessment and risk assessment. The World Health Organization (WHO) and the European Food Safety Authority (EFSA) recommend three scenarios for dealing with contamination data below the quantification limits. These approaches are named lower bound that induced underestimation (LB), middle bound that induced overestimation (MB), and upper bound that induced most overestimation (UB) (EFSA, Carrasco Cabrera, & Medina Pastor, 2022; FAO/WHO, 2023). In this study, we use the MB, where results below the limit of detection are replaced by LOD/2.

There is no international consensus on the age groups of consumers (Cohen Hubal *et al.*, 2014). In this study, the risk was assessed for two groups: (1) individuals younger than 10 years and (2) individuals older than 10 years in such a way that this group included adolescents and adults, following the recommendations of the American Academy of Pediatrics (Hagan, Shaw, & Duncan, 2017).

Two scenarios were analysed: (A) for the overall study population and (B) specifically for people who consume foods of bovine origin.

The estimated daily intake (EDI) of ivermectin residues by the consumer is:

$$\textit{Estimated Daily Intake (EDI)} = \frac{\textit{contamination (mg/kg)} \quad x \quad \textit{consumption (g)}}{\textit{bw (kg)}} \qquad \textbf{(Eq. 1)}$$

Where *contamination* is the average concentration of ivermectin in the meat, liver, and milk. *Consumption* stands for the daily average consumption of these products in the study region; and *bw* represents the body weight. The average *bw* in the study area for a person (man or woman) under 10 years of age was 13.49kg (standard error (SE): 7.80kg) and 68.39kg (SE: 15.70) for a person (man or woman) over 10 years of age. These data were obtained from a database provided by a local health clinic (N= 15223).

JECFA uses the global estimate of chronic dietary exposure (GECDE) for chronic dietary exposure assessment to veterinary drug residue (Arcella *et al.*, 2019; FAO/WHO, 2012). According to JECFA, the ivermectin' GECDE recommendation level for adults and the elderly is lower than 0.72

µg/kg *bw* per day, which represents 7.2% of the upper bound of the ADI of 10 µg/kg *bw*. The GECDE recommendation level for children is lower than 0.93 µg//kg *bw* per day, which represents 9.3% of the upper bound of the ADI of 10 µg/kg *bw* (FAO/WHO, 2023).

The Global Estimate Chronic Dose Exposure (GECDE) to ivermectin residues for the population in the studies areas is the highest exposure calculated using the 97.5th percentile consumption figure for a single food selected from all the foods plus the mean dietary exposure from all the other relevant foods (FAO/WHO, 2012, 2014, 2022):

$$\textit{High exposure from each animal product} = \textit{97.5}^{th}\ \textit{percentile consumption} \times \textit{Median residue} \quad \textbf{(Eq. 2)}$$

$$\textit{Global Estimated Chronic Dietary Exposure (GECDE)} = \textit{Highest exposure from one animal product} + \textit{Total mean exposure from all other products} \quad \textbf{(Eq. 3)}$$

3. Results and Discussion

3.1. Ivermectin residues

Of the total samples analysed (N=225), the presence of ivermectin residues was determined in 68% of faeces samples (27/40; values between 0.03 to 0.42mg/kg) and around 3% in liver (1/30; value of 0.34 mg/kg), milk (2/70; values of 0.09 and 0.44 mg/kg), and urine (1/39; value of 0.06 mg/kg) samples. No residue over the LOD was detected in the 46 meat samples (**Figure 3**). The average value of ivermectin in faeces reached 0.118mg/kg, 0.006mg/kg in urines, 0.016mg/kg in milk, 0.018mg/kg in liver, and 0.005mg/kg in meat.

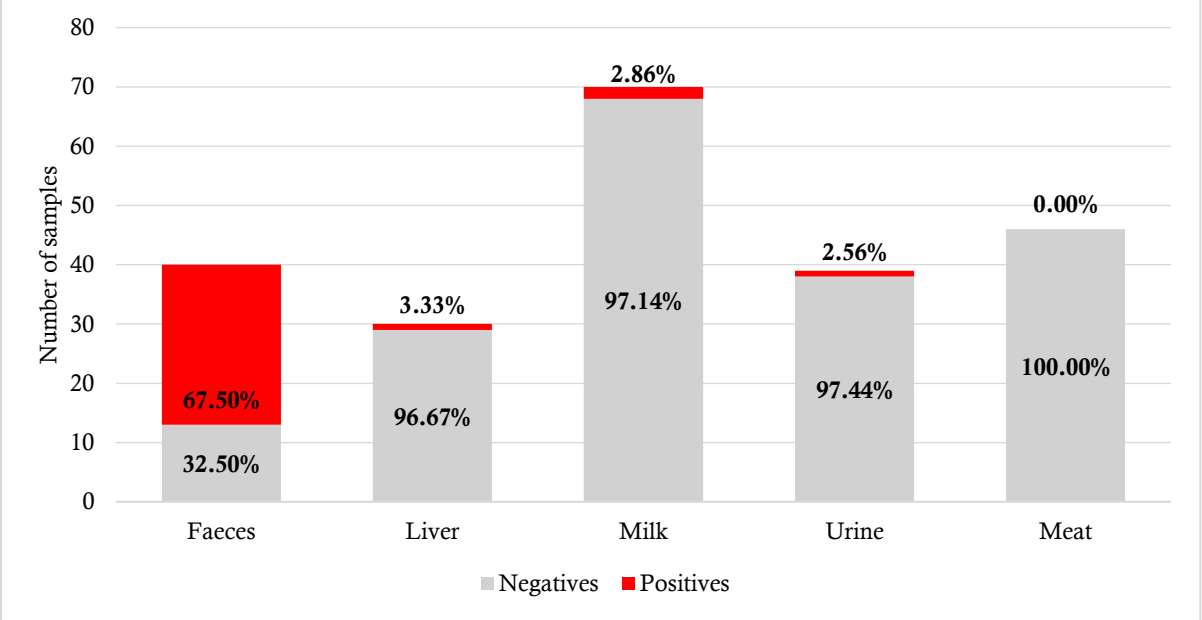


Figure 3. Prevalence of ivermectin residues in the analysed samples (decreasing order)

This study is the first to detect ivermectin in foods of bovine origin such as milk, meat, and liver and excretions like urine and faeces from small-scale dairy farms. While HPLC did not detect residues above the Maximum Residue Limit (MRL) in meat and liver samples, the positive milk samples did exceed the MRL established by FAO (FAO/WHO, 2023). Regionally, a study conducted in the Brazilian retail dairy market informed that ivermectin is substantially used in dairy cows; the authors reported that 46% of milk samples had some level of residues detected/quantified. Although these residues did not exceed the maximum residue limit (MRL), their presence in nearly half of the samples is concerning (Novaes *et al.*, 2017).

Although the Codex Alimentarius sets an MRL of 10 µg/kg for ivermectin, other regulatory bodies, such as the European Medicines Agency (EMA, 2013) and Health Canada (2024), do not approve the use of ivermectin in dairy cattle, resulting in no established legal MRL for milk in these regions. Moreover, another study conducted in the areas of the present study indicates that ivermectin was used in dairy cattle at a rate of 50% (Paucar *et al.*, 2022). The discovery that 68% of faeces samples contain ivermectin residues strongly confirms the widespread use of this drug among local farmers. Despite the fact that in this study, the number of samples over the MRL was small, and there are no additional studies conducted in the field where this is studied, there are several studies conducted on raw milk and meat, identifying the presence of antibiotics and heavy metals (Brito, 2017; De la Cueva *et al.*, 2021; Puga-Torres *et al.*, 2022, 2024). Additionally, some undergraduate research projects have investigated the occurrence and elimination of antiparasitics, such as eprinomectin, ivermectin, and fipronil, in meat and milk. Considering that two-thirds of the milk in Ecuador is marketed informally, where quality controls are minimal and represent the most accessible market for small producers (CIL, 2023), there is a pressing need for larger-scale studies to assess risk and ensure food safety and quality accurately. These comprehensive investigations will yield a better understanding of the prevalence of ivermectin residues in milk, thereby informing regulatory decisions aimed at safeguarding public health. Additionally, the detection of ivermectin in faeces underscores the importance of further research into its environmental impact (e.g. non-target species). Low doses of ivermectin residues have been shown to cause significant short- and long-term ecological effects, including alterations in dung beetle populations, disruption of manure degradation processes, and changes in soil properties and functions (Verdú *et al.*, 2018). It is important to note that the absence of ivermectin residues in meat does not automatically ensure food safety. The sampling was conducted in farms with some level of sanitary control. However, in Ecuador, approximately 36% of foods of bovine origin come from informal slaughtering (clandestine or homemade) (Mendoza, 2017). Furthermore, only larger farms have better access to official slaughterhouses, while smaller producers depend on intermediate dealers, who collect the animals from the different farms and transport them to livestock markets or directly to slaughterhouses (Castillo & Carpio, 2019). Sampling local butcher shops could help determine the safety of the food reaching consumers.

3.2. Consumption of cattle products

It was determined that most of the households surveyed consumed meat and milk (91% and 97%, respectively), but only 30% consumed liver. The daily consumption (DC) average of meat, liver, and milk, considering two scenarios, is shown in **Table 1**.

Table 1. Daily consumption of meat, liver, and milk in g/person/day.

Product	Households (%)	Less or equal to 10 years old			Higher than 10 years old		
		Consumers (%)	DC [A]	DC [B]	Consumers (%)	DC [A]	DC [B]
Milk	97	98	94.76	98.24	97	95.85	102.15
Meat	91	91	5.88	6.45	92	30.95	35.62
Liver	30	32	0.14	0.35	30	0.75	2.27

Legend. (DC) Daily consumption; (A) Scenario with the average consumption of the inhabitants of the study area; (B) Scenario with the average consumption only of the inhabitants that consume each food of bovine origin

Based on the survey data, the annual consumption in the study area is 10 kg of meat and 0.26 kg of liver for the whole population. There has yet to be any previous data available regarding the amount of liver consumption at the local or national level. However, meat consumption is close to the national average of 13 kg *per capita* (Ritchie & Roser, 2019). The national *per capita* consumption is similar to that of neighbouring countries like Colombia (14 kg) but lower than the reported consumption in South American countries such as Argentina (48 kg) or Brazil (35 kg), both of which are substantial consumers of meat globally (Ritchie & Roser, 2019).

Furthermore, our findings reveal an annual milk consumption per person of 32 litres, which is significantly lower than the national *per capita* average of 110 litres (Baquerizo & Córdova, 2022) and well below the recommended 220 litres by the FAO (FAO, 2011). Previous studies in the country indicate that the highest *per capita* milk consumption is in the Highlands region of Ecuador. In contrast, in the Amazonian and Coastal regions, where the study areas are situated, consumption is much lower, reaching a quarter of the consumption in the Highlands areas (Bermeo, 2015).

3.3. Risk assessment of contaminated bovine products for the consumer

The risk assessment in this study was estimated based on the amount of ivermectin present in milk, liver, and meat, the consumption of these foods in the study areas, and data evaluated by JECFA (FAO/WHO, 2023). For milk, 2 samples (0.09 mg/kg and 0.44mg/kg) had an ivermectin concentration above the MRL (0.01mg/kg), and 68 samples below the LOD (0.01 mg/kg). For the liver, one sample (0.34mg/kg) had an ivermectin concentration between the LOD (0.01 mg/kg) and the MRL (0.8mg/kg) and 45 samples below the LOD (0.01 mg/kg). For meat, all samples had ivermectin concentrations under the MRL (0.03mg/kg) and the LOD (0.01 mg/kg).

Considering (1) that the body weight (*bw*) of a person (man or woman) under 10 years of age (Avg. 13.49) and for a person (man or woman) over 10 years of age (Avg. 68.39); (2) the individual consumption data; (3) and the results of the estimated amount of ivermectin, the estimate daily intake (EDI) of ivermectin residues through milk, meat and liver were close to zero (between 0.02 and 0.0935 ug/kg *bw*/day), i.e. the lower limit of the ADI (0 µg/kg *bw*) and also largely lower to the upper limit of the ADI (10 µg/kg *bw*) (FAO/WHO, 2023). Furthermore, the GECDE for ivermectin residues was between 0.0029% (Scenario A) and 0.2959% (Scenario B) of ADI for person older than 10 years, i.e. lower than the 7.2% recommended. In addition, the GECDE for ivermectin residues was between 0.9131% (Scenario A) and 1.0246% (Scenario B) of ADI for person younger than or equal to 10 years, i.e. also largely lower than the recommended level of 9.3% (FAO/WHO, 2023). (**Table 2**). A complementary stochastic modelling (data not shown) confirmed the same picture with only one EDI simulation among the 10,000 simulations with value higher of ADI in one person younger than or equal to 10 years. Indeed, the expression of the risk should be qualified as rare to very rare (Büchter *et al.*, 2014).

Although no long-term toxicity studies have been conducted with repeated doses in humans or other laboratory animals, studies with abamectin in mice (94 weeks) have found carcinogenic effects (JECFA, 2016). In addition, short-term studies (4 weeks) in young rats have shown increased sensitivity to ivermectin due to an underdeveloped blood-brain barrier (Lankas, Minsker, & Robertson, 1989). Given its recent use during the COVID-19 pandemic, clinical effects, including neurotoxicity, gastrointestinal symptoms and musculoskeletal complaints, have been reported. Patients taking high doses of veterinary ivermectin reported neurotoxicity with altered mental status. On the other hand, patients taking lower doses of ivermectin over a prolonged period reported milder toxicity, with no cases of severe altered mental status (Hoang *et al.*, 2022).

It is important to note that milk consumption in the study areas is about a quarter of the national average. This suggests that risk assessments could yield different results in regions with higher milk consumption. While no immediate risk was identified, the potential danger remains, and measures should be implemented to ensure food safety. Given that milk is a basic product in the basic food basket and provides essential micro- and macronutrients, particularly crucial during infancy and childhood when bone mass growth is critical, ensuring its safety is paramount (Vragović, Bažulić, & Njari, 2011).

There may be other, potentially more dangerous scenarios, such as the consumption of milk from cows recently treated with ivermectin, particularly if consumed during the short-term withdrawal period. While this study focused on the average consumption of foods of bovine origin in the study areas, the scenario mentioned is certainly plausible among small farmers, where the accumulation of milk consumption over several days could lead to hazardous or toxic situations.

4. Conclusions

Although this study identified ivermectin residues as a negligible risk for the study population, it highlights the potential danger and underscores the need for further research. There may be other, potentially more dangerous scenarios, such as the consumption of milk from cows recently treated with ivermectin, particularly if consumed during the short-term withdrawal period. Future studies should focus on the risk analysis of consuming milk containing residues of antiparasitics, antibiotics, or heavy metals. Additionally, given the high presence of ivermectin in faecal samples, it is crucial to investigate its effects on tropical livestock, where this drug is extensively used for controlling ectoparasites. This study emphasizes the importance of collaborative and intersectoral efforts. Veterinary professionals, public health experts, biologists, and ecologists must work together to address this issue, ensuring good animal health, food safety, and human health through sustainable and environmentally friendly livestock practices.

Table 2. Daily intake of ivermectin and chronic dietary exposure

		Food of bovine origin	Median residue concentration (ug/kg)	Mean residue concentration (ug/kg)	Consumption Percentile 97.5th (kg/day)	Consumption means (kg/day)	*bw* (kg)	EDI (ug/bw/day)	EDI (ug/kg bw/day)	Exposure (ug/kg bw/day)		GECDE	
										97.5th	Mean	ug/kg bw/ day	%ADI
A	Less or equal to 10 years old	Milk	5.0000	12.4571	0.2404	0.0948	13.4935	1.1804	0.0875	0.0891	0.0351	0.0891	0.0891
		Meat	5.0000	5.0000	0.0118	0.0059	13.4935	0.0294	0.0022	0.0044	0.0022	0.0022	0.0044
		Liver	5.0000	16.1667	0.0008	0.0001	13.4935	0.0022	0.0002	0.0003	0.0001	0.0001	0.0003
		TOTAL						1.2120	0.0899	0.0937	0.0373	0.0913	0.0937
A	Higher than 10 years old	Milk	5.0000	12.4571	0.3668	0.0958	68.3891	1.1940	0.0175	0.0268	0.0070	0.0268	0.0268
		Meat	5.0000	5.0000	0.0724	0.0309	68.3891	0.1547	0.0023	0.0053	0.0023	0.0023	0.0053
		Liver	5.0000	16.1667	0.0056	0.0008	68.3891	0.0122	0.0002	0.0004	0.0001	0.0001	0.0004
		TOTAL						1.2609	0.0200	0.0325	0.0093	0.0291	0.0325
B	Less or equal to 10 years old	Milk	5.0000	12.4571	0.2697	0.0982	13.4935	1.2238	0.0907	0.0999	0.0364	0.0999	0.0999
		Meat	5.0000	5.0000	0.0133	0.0065	13.4935	0.0323	0.0024	0.0049	0.0024	0.0024	0.0049
		Liver	5.0000	16.1667	0.0011	0.0004	13.4935	0.0057	0.0004	0.0004	0.0001	0.0001	0.0004
		TOTAL						1.2618	0.0935	0.1053	0.0389	0.1025	0.1053
B	Higher than 10 years old	Milk	5.0000	12.4571	0.3668	0.1022	68.3891	1.2725	0.0186	0.0268	0.0075	0.0268	0.0268
		Meat	5.0000	5.0000	0.0733	0.0356	68.3891	0.1781	0.0026	0.0054	0.0026	0.0026	0.0054
		Liver	5.0000	16.1667	0.0072	0.0023	68.3891	0.0367	0.0005	0.0005	0.0002	0.0002	0.0005
		TOTAL						1.4873	0.0217	0.0327	0.0102	0.0296	0.0327

Legend. (A) Scenario with the average consumption of the inhabitants of the study area; (B) Scenario with the average consumption only of the inhabitants that consume foods of bovine origin; Estimate daily intake (EDI); Acceptable daily intake (ADI); Global estimated chronic dietary exposure (GCDE); body weight (*bw*).

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Chap. 4 - General discussion and perspectives

Challenges in Ecuadorian Tropical Livestock

In Ecuador, the production systems vary depending on the agroecozones. In the Highlands region (Andes Mountains), livestock production focuses on dairy production, while the coastal and Amazon regions concentrate on dual-purpose and meat production (Vera, 2006). The tropical zones of this study are located in the foothills of the eastern (Amazon region) and western (Coastal region) Andes of Ecuador, and according to a survey (**Study 1**), are characterized as systems where milk production predominates (72%), followed by dual purpose (27%) and beef (1%). These production systems tend to use dairy cattle phenotypic characteristics, which are more associated with *Bos taurus* (93%), with breeds such as Holstein, Brown Swiss, or Jersey cattle, crossbreds, and purebreds. The type of cattle present in these areas is more similar to the Andes breeding systems and, to a lesser extent, to the breeding systems managed in the Coast and Amazonia, where cattle of Brangus, Brahman, Charolais, and Nelore breeds or crossbreeds predominate (Roche *et al.*, 2022). In general, zebu cattle (*Bos indicus*) are better suited for tropical regions than European cattle (*Bos taurus*). This is attributed to their ability to tolerate hot environments, which is facilitated by their low metabolic rate and high transpiration capacity. They can also feed on low-quality forages, which is typical of tropical regions. Due to their skin thickness, coat type, hair density, coat colour, and skin secretions, zebu cattle are more resistant to parasite attacks like ticks (Cooke *et al.*, 2020; Hansen, 2004; Mattioli *et al.*, 2000; Hossain, Khan, & Hashem, 2017; Shyma *et al.*, 2015). However, in Ecuador, there is no specialized animal breeding program aimed at enhancing the adaptation of either taurine or zebu breeds to the country tropical conditions. As a result, the sperm utilized in artificial insemination primarily originates from imported reproductive material from developed countries. These countries breeding programs do not place a high priority on adapting to tropical challenges. Furthermore, the prevalent use of natural mating complicates the selection of desirable traits that enhance tropical adaptability.

In these production systems, as in the rest of the country, pastures are the main source of feed for ruminants (Torres *et al.*, 2023). Cattle graze on the available pastures, which are typically supplemented with concentrate or cut-and-carry forage. The predominant pasture is Dallis grass (*Paspalum dilatatum*) and several species of the genus *Brachiaria*. These short-cycle pastures (28-42 day rest) have been introduced by farmers in order to increase farm productivity and, little by little, have been displacing other long-cycle species such as imperial grass (*Axonopus scoparius*) (Grijalva, Arevalo, *et al.*, 2004). It is important to note that these cattle farming practices take place in close proximity to several ecological reserves (Chocó Andino of Pichincha Biosphere Reserve and the Cayambe Coca and Sumaco Napo Galeras National Parks), where one of the main problems is the increase in deforestation and the advance of the agricultural frontier (Torres *et al.*, 2022, 2023). FAO has determined that in Latin America, land that was cleared and burned was converted to growing crops and grazing livestock. As the forest area is reduced, the livestock population and grazing areas have

increased rapidly. Although grazing areas have increased as the population has grown, they still do not meet the nutritional needs of livestock, causing overgrazing that accelerates nutrient loss and soil erosion (FAO, 2005). To improve this situation, local farmers continue to invade the forests or look for other ways of supplying feed, such as moving the animals outside the farm boundaries to grazing lots (external paddocks) that are generally leased to other farmers in the area. Likewise, the farming systems in the tropical study areas are generally non-technified systems in 61% of the cases. Typical in these systems, milking is done manually (74%), and although there is the presence of corrals, installations (60%), and artificial insemination (47%) and veterinary service is mainly due to the support of local governments, which subsidize the implementation of these systems. Although cattle raising is the primary source of income, it is complemented by the cultivation of palm hearts, bananas, coffee, cocoa, tree tomatoes, and lulu for commercial or self-consumption.

Researchers have suggested that the use of typologies of farmers can improve the effectiveness of rural development programs for agriculture (Emtage, Herbohn, & Harrison, 2006). Typologies are a way of classifying and grouping farms based on their characteristics, resulting in more homogenous groups. This helps us understand the diversity within the agricultural system (Alvarez *et al.*, 2018; Larouche, 2011). According to our results, there are three main groups: small, medium, and large farms. According to **Study 2**, small farms (Type 1) are generally non-technified farms that rely on family labour and require the rental of external paddocks to feed their cattle. Type 2 farms are similar to Type 1 but farmers also grow and sell crops; Type 3 farms are medium-sized farms, non-technified, who use permanent labour; and Type 4 farms are medium, semi-technified farms that rely on family labour. In contrast, Type 5 farms are large-sized and technified and use permanent labour. Identifying the farming system through these typologies allows for targeted and effective interventions, such as the development of extension services, credit facilities, and innovation and technology adoption strategies tailored to the requirements of each farmer group. This knowledge is vital, as it is not feasible to have policies and programs customized for each farm (Alvarez *et al.*, 2018; Awoke Eshetae *et al.*, 2024; Emtage *et al.*, 2006). Studies carried out by Torres (2008) and Paucar *et al.*, (2021) have shown that about 80% of the livestock in Ecuador are composed of small and medium farmers, making it crucial to develop health programs that focus on this target.

In the economic analysis (**Study 2**) carried out in the study area, it was determined that in all the groups, the main expenses in livestock farming are the labour force and supplementary livestock feed. Even though the systems are based on paddocks with introduced and improved pasture species, farmers still allocate 26% of total costs to the purchase of concentrate feed and mineral salts, compared to only 0.36% allocated to pasture management. Studies have reported that sustainable and profitable pasture-based dairy farming depends on the type of grass, grassland management, and stocking rate, along with other factors such as soil type, climatic conditions, and dairy cow genetics (Hanrahan *et al.*,

2018; Shalloo, Creighton, & O'Donovan, 2011). While the use of supplementary feeding in grazing dairy systems can lead to increased production costs, it is essential in tropical livestock farming, especially in areas with forest soils that have high erodibility and limited fertility (FAO, 2005; Pimentel & Kounang, 1998; Silva *et al.*, 2017; Southgate & Whitaker, 1992). So, in this context, integrating supplemental feeding and proper paddock management is the key to meeting the nutritional needs of cows and generating good efficiency and profitability in the dairy unit (Hanrahan *et al.*, 2018).

It is crucial to understand that animal nutrition is not just about providing energy and nutrients for animal production. It also plays a significant role in maintaining the integrity of the skin as a physical barrier. In the process of tolerating intruders (ticks and pathogens of TBDs), the skin plays a crucial role in immunity and its microbiome (Boulanger & Wikel, 2021). Proteins, minerals, and vitamins contribute to nutritional support, help immunity, and maintain skin tissues (Nelson, 1984). Deficiencies in these can result in a high level of parasitic infestation as natural protective mechanisms do not develop in the animal. Deficiencies of vitamin A copper and zinc are associated with hair loss, rough hair coat, scaling of hyperkeratinized epidermis, and dermatitis, among others (Miller & Miller, 1962; Nelson, 1984). Additionally, deficiencies in vitamins A and D result in decreased skin immune response (Gingrich & Barrett, 1975; Nelson, 1984). In tropical grazing systems, supplementation with minerals or protein banks (Alemán *et al.*, 2020; Grijalva O. & Oñate, 2000) can improve nutritional status and production while decreasing tick density in animals (Githaka *et al.*, 2022). Underinvestment in pasture management was reflected in the study; small and medium farmers spend more money on paying rent for external paddocks than on maintaining their pastures. Strategies to improve productivity include fertilizer application (chemical and organic), modification of grazing duration and frequency, grass and legume mixtures, stocking rate manipulation, and grass allocation (Boval & Dixon, 2012; Cibils & Coughenour, 2001). However, livestock producers need to be made aware of existing strategies and the importance of grassland maintenance to optimize forage quality and animal health. The high costs involved in implementing some of these strategies and the search for environmentally friendly alternatives also hinder their implementation.

Access to veterinary services described in **study 1** is consistent with the money invested in this study (**study 2**), representing approximately 1.25% of the total costs of livestock production. It was observed that while veterinary control is present in 67% of the farms, the majority are concentrated in Zone 1 of the study area, where such services are often subsidized by state or local governments. However, a broader study conducted across Ecuador (see **Appendix 3**), revealed that only 28% of farms have access to veterinary control nationwide (Paucar *et al.*, 2021). Although the relationship between veterinary control as a protective factor for the presence of disease is still unclear, in Ecuador, farms that reported having veterinary control did not present a lower presence of diseases (Paucar *et al.*, 2021) or tick infestation (Paucar *et al.*, 2022). For such control to be effective, it must be carried out by a

professional with solid experience and complemented with laboratory tests. In these areas, public veterinary control is not always carried out by professionals and often focuses more on technical aspects of production and reproduction than on sanitary control, potentially contributing to the limited impact on disease presence. In contrast, the expenditure on drug purchases (**study 2**) accounts for 8% of costs. This is due to the easy accessibility to antibiotics, antiparasitics, vaccines, and hormones in agricultural warehouses across Ecuador, where veterinary prescriptions usually are not required, and drugstore staff are not mandated to possess veterinary medical education. The findings underscore the complex landscape of veterinary services and disease management in Ecuadorian livestock farming, highlighting the need for enhanced, consistent veterinary oversight, improved regulation of drug distribution, and greater emphasis on preventative measures to safeguard livestock health and productivity.

Although agriculture is crucial to Ecuador economy, the country livestock sector requires increased support from the authorities. Despite an official price of 0.42 USD per litre for raw milk (MAGAP, 2013), farmers receive an average of 0.39 USD per litre, according to our study. This data is consistent with the national average reported in 2021 (Corporación Financiera Nacional B.P., 2023). Only large, technified farms manage to surpass the base price by receiving bonuses for quality and the adoption of Best Management Practices (BMPs), generally recognised by regional industries accessible to these farms. Conversely, small and medium farms encounter difficulties in market access, often channelling their production to local industries or cheese factories, informal markets where payments significantly fall below established rates with reduced sanitary and quality control (Fierro, Carrera, & Ordóñez, 2020). In Ecuador, only 53% of litres produced are destined for the formal industry (CIL, 2018). The low remuneration recorded in this study may also be associated with the fact that data were collected during the COVID-19 pandemic, which resulted in a 69.5% decrease in farmers' income from milk sales. As a result, milk collectors decreased the payment per litre of milk, limiting the volume of milk to be commercialized or with delays in the payment. Farmers often face challenges in adapting to a rapidly changing market coupled with environmental pressures. All these consequences are even more evident in small and medium farms. This is due to the unclear guidelines and incentives that promote investment, innovation, and sustainable practices in the sector. Additionally, farmers also struggle to access financing and credit to invest in their operations and improve their production capacity. The lack of policy coherence also contributes to market inefficiencies and price volatility, making it difficult for farmers to plan and invest in long-term (Nataly *et al.*, 2023; Piñeiro *et al.*, 2020; Thorsøe *et al.*, 2020).

Tick infestation and acaricide resistance

Around 80% of the world cattle population is threatened by ticks (FAO, 2004b), particularly the cattle tick *Rhipicephalus microplus*, which is a significant risk to livestock in tropical and subtropical regions worldwide. **Study 1** provided information on the tick species that are prevalent in the study areas, with *R. microplus* being the predominant species found in 98% of the surveyed farms.

In addition, *Ixodes boliviensis* was found on three farms, *I. montoyanus* on two farms, and *Amblyomma mixtum* on one farm. These findings are consistent with previous studies conducted in Ecuador, which have consistently identified *R. microplus* as the primary tick species affecting Ecuadorian livestock. Although this species was found in this study at an altitude ranging from 600 to 2,000 meters above sea level (m.a.s.l), its presence has been reported in Ecuador up to an altitude of 2,469 m.a.s.l (Chávez Larrea *et al.*, 2021).

Study 1 determined that 96% (95% CI: 90–98) of the visited farms had ticks. A semi-quantitative visual assessment was employed to classify the level of infestation into high and low categories. At the farm level, 41% of farms exhibited a high infestation level, while at the animal level, 38% of the sampled animals were highly infested. Knowing the level of tick infestation allows us to identify risk factors associated with high infestation levels. The risk factors associated with tick infestation are critical to develop effective management strategies. The study found that factors such as breed (*Bos taurus*), advanced age, animals in "*thin"* condition, and milking cows increase the susceptibility to tick infestation. The study also revealed that livestock management practices, such as the absence of advanced technology and the competence of the personnel responsible for preparing and applying acaricides, contribute to tick exposure and infestation levels. A high level of infestation (**Study** 3) was also related to the use of organophosphate acaricides, frequent (every one or two weeks) or irregular (every five or more weeks) acaricide treatments, low perception of the efficacy of injectable acaricide treatments, reporting of TBDs cases on the farm, and extensive grazing practices. These factors have been consistently reported as risk factors for tick infestation in previous studies (Cruz-González *et al.*, 2023; Jonsson, Mayer, & Green, 2000; Miyama *et al.*, 2020; Piper *et al.*, 2010; Rehman *et al.*, 2017; Taye, Assefa, & Hika, 2015). The semi-quantitative visual method used in **Study 1** to determine the level of tick infestation was found to be effective and correlated well with previously identified risk factors. This method was also utilized in **Study 3** with participating farmers and was well received. As a result, farmers can use this method to monitor tick infestations, allowing them to implement targeted control measures, improve animal health, reduce economic losses, and ensure better compliance with health regulations.

Tick Control

In order to control the presence of ticks, livestock farmers in Ecuador and around the world are heavily dependent on chemical acaricides (FAO, 2024). Both **studies 1 and 3** described tick control practices. It was determined that the main method for tick control is chemical control via spraying utilizing a backpack sprayer, which small farmers consider a convenient and economical form of control. Other methods of acaricide application are pour-on, swabbing, and injections. Data obtained in these studies, as in other research (Abbas *et al.*, 2014; Githaka *et al.*, 2022), underscore significant challenges in acaricide management. These challenges include the unawareness of the chemical

differences between different acaricide brands (persistent use), incorrect dosage, improper application, the mixing of different chemical classes available in the market, and the lack of knowledge of life cycles. These findings emphasize the critical need for improved education and training among farmers to enhance the efficacy and sustainability of tick control strategies.

Despite the availability of six active ingredients on the market (amides, alpha-cypermethrin, macrocyclic lactones, organophosphates, fipronil, and fluazuron), this study identified that the farms surveyed utilised 67 different trade names. Combined with limited knowledge on proper acaricide rotation, it resulted in only 17% of farmers correctly rotating acaricides. The potential risks of such practice are significant, as it can lead to the development of resistance in ticks, rendering the acaricides ineffective. Furthermore, 83.33% of farmers used co-formulated acaricides, a strategy justifiable under ideal conditions but potentially ineffective when facing resistance to an active ingredient. In addition, 25% of farmers manufacture their co-formulated acaricides by mixing different commercial acaricides to try to maximize and prolong the acaricidal effect (Mugabi, Mugisha, & Ocaido, 2010; Vudriko *et al.*, 2016). The complexity of acaricide dosage emerged as a significant issue. Although 75% of farmers reported using syringes for dosing, only 10% administered the correct dosage. Despite awareness of incorrect dosing, the majority persisted in their practices. Moreover, only 5% of farmers employed reliable methods to determine animal weight and subsequently applied treatment based on this.

With respect to organophosphate acaricides, although banned in 32 countries due to their harmful effects on health and the environment, active ingredients such as dichlorvos and trichlorfon are available in Ecuador without any veterinary prescription (Agrocalidad, 2021; Bejarano, 2017). Organophosphates were predominantly used in a technique called swabbing, which involves dissolving an overdose of the acaricide (up to five times the recommended dose) in water, cooking oil, or engine oil and applying it with a wipe cloth to the most affected areas (Byaruhanga *et al.*, 2015; Moyo & Masika, 2009).

In **Study 3**, when assessing knowledge about ticks, it was evident that although there was very basic knowledge, it was mostly gained from hands-on experience or passed down through generations. However, more technical aspects of tick knowledge, were limited. For instance, it was evident that farmers lacked knowledge about the correct management of acaricides. Although the farmers had practical knowledge acquired in their daily lives on the farm, it was not always the most adequate, underscoring the importance of continuing education. This practical knowledge, acquired through their experiences, should serve as the cornerstone for education programs. By incorporating traditional knowledge and introducing contemporary techniques, these programs can enhance livestock management practices (Wanzala et al., 2005). The design of such programs should be interactive and communicative, utilising participatory approaches that significantly increase the impact of information

assimilation and the effective implementation of acaricidal control strategies (Hu, 2020). Farmer field schools are a form of adult education where farmers learn optimally from observation and experimentation in the field (Berg, 2004). In addition, focus groups in which people can share experiences in acaricide control can be useful. Vudriko *et al.*, (2018), mention that sharing practical evidence of correct tick control practices allowed farmers to reflect, realize, and commit to making positive changes.

Although the farmer-owner has the final decision on acaricide management, this decision may be influenced by suggestions or advice from other stakeholders such as veterinarians, pharmaceutical industry stakeholders, other farmers, and authorities. Therefore, education programs must involve all decision-makers and stakeholders in acaricide control. These education programs should be directed according to the target audience. Training field veterinarians and the pharmaceutical industry should be more technical and in-depth so that they can transmit this information to farmers. Special attention should be paid to the control in veterinary warehouses, which are the first place of consultation for small and medium farmers. According to the regulations in Ecuador, the sale of acaricides must be made prior to the presentation of a professional prescription (Agrocalidad, 2016). In Ecuador, people who sell them are not necessarily trained, in most cases, and the sale of these products is done freely. Therefore, it is essential for the regulatory agency to perform constant control. Sellers should follow constant training on resistance. This training should focus on understanding resistance and the crucial role it plays in combating acaricide and drug resistance.

Acaricide resistance

The overdependence and improper use of chemical acaricides by farmers trying to control tick populations have led to the emergence of resistance. **Study 2** investigated resistance to three primary acaricides used in Ecuador: amitraz, alpha-cypermethrin, and ivermectin. In this study, the larval package test revealed resistance rates of 53%, 50%, and 37% in *R. microplus* to alpha-cypermethrin amitraz, and ivermectin, respectively. Globally, *R. microplus* populations have developed resistance to multiple acaricide compounds, predominantly synthetic pyrethroid, organophosphates, amides, fipronil, and ivermectin (Dzemo, Thekisoe, & Vudriko, 2022). The main acaricide to which most of the farms studied exhibited resistance was alpha-cypermethrin. This acaricide is available in the market as spraying and pour-on formulations; there are also commercial co-formulations containing one (organophosphates) or two (organophosphates and phenylpyrazolones) additional active ingredients. Elevated levels of resistance to synthetic pyrethroids have been documented in several countries, and it has been noted as the most aggressively marketed acaricide over the past fifteen years (Dzemo *et al.*, 2022; Kumar, Sharma, & Ghosh, 2020).

This study represents a significant advancement in understanding acaricide resistance in two provinces of Ecuador. However, it underscores the need for further research, as data on ticks and acaricide resistance in a substantial portion of the country is lacking. Mapping tick resistance to acaricides is a starting point for identifying acaricide resistance hotspots and resistant chemical classes (Vudriko *et al.*, 2018). Additionally, studies are crucial to evaluate resistance to commonly used acaricides, such as organophosphates, phenylpyrazoles (fipronil), and benzoylphenyl ureas (fluazuron). **Study 3** related these acaricides to poor use practices associated with dosing. Organophosphates and other acaricides used in spraying were often administered in overdose due to their price affordability, while pour-on acaricides were often under-dosed due to their high cost. Even though the application of acaricides in pour-on is a relatively recent control method in Ecuador, their improper use could lead to long term resistance. This has been observed in other Latin American countries since 2007 for fipronil (Cuore *et al.*, 2007) and 2014 for fluazuron (Reck *et al.*, 2014), ongoing surveillance and investigation are essential for Ecuador.

Knowledge of the risk factors associated with acaricide resistance faced by tropical livestock described in this study highlights the need for action at several points, from the training of livestock farmers to the promotion of research through government institutions, universities, or non-governmental organizations (NGOs). Since there are no laboratories in the country that allow farmers to carry out resistance tests, the creation of a Research Centre for tick and TBDs control is a priority. In addition, the dissemination of information through farmer field schools (Sones, Duveskog, & Minjauw, 2003) on tropical livestock management (tropical livestock breeds and pastures), proper acaricide application, acaricide rotation, understanding the instructions on acaricide bottles, acaricide safety tips, and alternative control methods can help to greatly reduce acaricide use and preserve the functionality of the remaining acaricides. In addition, the dissemination of informational material through easy-to-understand manuals targeted to farmers can be very useful in reinforcing and disseminating recommended practices for acaricide control (George *et al.*, 2021; Vudriko *et al.*, 2018). This study, as part of the "Ticks and TBDs" project, contributed to the development of a manual (**Appendix 2**) aimed at farmers that was disseminated in the study areas, and it is planned to work on a technical manual for veterinarians and technicians.

Alternative acaricide control practices

While chemical control remains the main method for tick control, **Study 3** revealed that a small group of farmers with a relatively good knowledge of tick biology sought alternatives to chemical control and experimented alternative methods such as grazing management, manual tick removal, biological control (entomopathogenic fungi), and herbal extracts. However, farmers who relied exclusively on alternative control methods without a proper chemical treatment management experienced similar infestation levels to those who did not use them. This underscores the importance

of adequate chemical acaricide management for successful tick control. Along with the methods mentioned above, the Gavac® vaccine became available in 2022, but its usage has yet to expand due to its cost and uncertain effectiveness within the country (Tinoco, 2022). When correctly implemented, these alternative control methods, along with several others outlined in the literature, have proven highly effective (Abbas *et al.*, 2014; Giglioti *et al.*, 2011; Hernández, 2005; Obaid *et al.*, 2022; Valle, Caicedo, & Masapanta, 2020; WingChing Jones, 2015). Although they do not replace the need for chemical control, they do significantly reduce its use (Domínguez, Torres, & Rosario-Cruz, 2016). By integrating these techniques into an Integrated Tick Management (ITM) framework, a comprehensive combination of tools and strategies can be implemented to manage tick infestations while also maintaining optimal levels of animal production (FAO, 2004b; Humblet, Losson, & Saegerman, 2020; Rodriguez-Vivas *et al.*, 2018).

Well-rounded training initiatives are essential in supporting the success of an ITM approach. This approach involves a comprehensive strategy that includes both chemical and non-chemical control methods. Such programs must go beyond simply instructing farmers on the proper use of chemical acaricides and explore the various control options that exist. It is common for farmers to overlook alternative measures due to their lack of availability (De la Fuente *et al.*, 2023).

Integrated tick management, in addition to combining multiple control strategies, involves the incorporation of technical expertise. The combination of the practical experience of farmers with the technical knowledge of professionals ensures that control measures are effective and sustainable. Farmer field schools play a crucial role in this process, serving as platforms where farmers can learn on ITM techniques, share their experiences, and develop skills through hands-on training. Farmer field schools enable individual and group learning through reflection, analysis, and problem-solving by farmers in their particular conditions. By equipping farmers with knowledge and involving them in decision-making, ITM has proven to improve the effectiveness of tick control practices (Minh, Larsen, & Neef, 2010).

The economic impact of the ticks in cattle

The health and productivity of livestock are essential for the success of the industry. However, parasites, especially ticks, can be a significant obstacle to achieve these goals. They not only cause health problems in animals but also lead to reduced production and expensive control measures (Manjunathachar *et al.*, 2014; WAAA, 2018). In Ecuador, approximately 3 million cattle are at risk of tick infestations and TBDs, which can have a significant impact on their health and the economy (Roche *et al.*, 2022; Rodríguez-Hidalgo *et al.*, 2017). Studies 2 and 3 examined acaricide control practices and their economic impact on livestock.

Study 2 calculated the costs associated with acaricide treatment, encompassing the expenses of acaricides and labour force. The findings revealed that the average annual acaricide treatment accounts for a significant 4.23% of the total costs in livestock production, translating to an average of $19.41 per animal. These costs are not uniform and vary depending on the level of farm technification, herd size, and the severity of tick infestation. Technified farms, for instance, had lower expenses on acaricide treatment (1.30%) compared to semi-technified farms (3.43%) and non-technified farms (6.24%). Similarly, farms with high infestation incur higher costs (4.28%) than those with low rates of infestation (2.74%). Among farms with similar characteristics, the cost fluctuates based on the control practices employed. For instance, farms using pour-on acaricides, co-formulated acaricides, mixing different acaricides, or manual tick removal face higher treatment costs. In farms practising manual tick removal, the annual treatment cost of an adult animal escalates to USD 23.85, and at the farm level, 5.75% of the production costs are allocated. However, this apparent cost has to be considered in the long term since this practice will reduce high tick loads in the future. The data obtained in this study confirmed that ticks have a significant impact on small and medium-sized farms, which invest the most money in attempting to control them. Small and medium-sized farms are generally more affected by this cost compared to large farms, which benefit from economies of scale and receive advice from veterinarians representing pharmaceutical companies. Small and medium-sized farms often have to pay higher unit costs as they purchase their inputs from veterinary warehouses where the staff is generally not well trained in parasite control. Based on these results, it is crucial to develop control programs where authorities provide financial assistance, and regulations that support sustainable and economically viable tick control measures. Additionally, farmers can greatly benefit from creating or joining livestock organizations. These organizations can help small and medium-sized farmers to increase their bargaining power when negotiating prices and conditions with suppliers, leading to better offers and discounts. By improving their collective bargaining power, livestock organizations can also reduce barriers to enter the markets and enhance their negotiating position with buyers (Markelova *et al.*, 2009). Well-consolidated livestock organizations can effectively defend the interests of their members, influence policy decisions, and secure subsidies and grants, thus further reducing costs. Given that small and medium-sized producers often rely on advice from untrained personnel in agricultural warehouses, pooling resources within the collective allows for the hiring of qualified veterinarians. These professionals can perform sanitary controls, provide expert guidance, and train farmers on best practices in Good Animal Husbandry Practices (GAHP).

Furthermore, in **study 3**, the behaviour of farmers when using an acaricide treatment was examined. It was found that acaricides used in spraying cost less than USD 0.55 per milligram (ml) and are usually used in high doses, while pour-on acaricides can cost up to USD 3.62 per ml and are typically used in low doses. Although price differences may influence a farmer's decision on which product to buy and use (Obaid *et al.*, 2022), relying solely on this factor does not provide a comprehensive

assessment of a product profitability over time. Therefore, training programs that incorporate cost considerations should be implemented, and farmers should be educated on the most economical, effective, and sustainable acaricide practices in the long term. In addition, knowing which practices increase the cost of treatment can raise awareness so that they are no longer practised or invested in, which can be beneficial for reducing future costs.

Although these studies did not quantify the direct economic losses related to tick infestation, **Study 3** delved into farmers' perceptions regarding the economic impact and losses attributed to ticks through both surveys and participatory meetings. It was revealed that 96% of surveyed farmers perceived ticks as contributors to economic losses. Among the most commonly reported effects during participatory meetings were: decreased milk production (95%), weight loss (88%), presence of TBDs (83%), and skin/coat/lesions (55%). Furthermore, when evaluating the economic losses, the heaviest weight in the proportional piling was attributed to the reduction of milk production. This observation can be attributed to the fact that the study areas are predominantly dairy regions, where the primary source of income for farmers is the sale of milk. The perceptions that farmers have about the damage caused by ticks are supported by experimental research. Studies have shown that 105 ticks per animal can lead to a 23% reduction in daily milk production, while an infestation of 40 ticks per animal per day can result in a loss of 20 kg of weight per year (Frisch *et al.*, 2000; Manjunathachar *et al.*, 2014).

Understanding how farmers perceive the impact of ticks is crucial in their decision-making process (Alarcon *et al.*, 2014). This perception of the negative effects caused by ticks can foster openness and willingness among farmers to embrace new control methods, such as integrated tick control programs. Additionally, their perceptions are closely tied to their knowledge about ticks (Meijer *et al.*, 2015), making it essential to focus on the topics that should be taught and delivered in educational programs.

Health impact of the ticks on cattle

Ticks pose a significant threat due to their capacity to transmit diseases, some of which potentially fatal to their hosts. Ticks are vectors of a diverse array of pathogens, including bacteria, viruses, and parasites, culminating in a spectrum of TBDs. The distribution of TBDs is dictated by the presence of specific tick vectors for each disease. For instance, *Rhipicephalus* spp. are responsible for transmitting anaplasmosis and babesiosis worldwide, while *Amblyomma* spp. carry heartwater (cowdriosis) in Africa. Theileriosis is common in tick-infested areas throughout the world. East Coast fever (*Theileria parva*), transmitted by *Rhipicephalus appendiculatus*, is a serious problem restricted to Central and East Africa. In contrast, tropical theileriosis (*T. annulate*), transmitted by *Hyalomma* spp., occurs in North Africa, southern Europe, the Middle East, India, and Asia (Blowey & Weaver, 2011; Johnson, 2023; McLeod R, 1999). While strides have been made in diagnosing TBDs, accessibility to

cutting-edge diagnostic tools remains a challenge, particularly in field settings. Understanding the co-occurrence of tick species and diseases in a given area can significantly enhance diagnostic accuracy (Minjauw & Mcleod, 2003).

In Ecuador, *R. microplus* is known to be present in 24 of the 24 provinces of Ecuador (Bustamante–Ordóñez, Bustamante–Guzmán, & Rivera, 2024; Gioia *et al.*, 2018; Maya-Delgado *et al.*, 2020; Pérez-Otáñez *et al.*, 2024). Additionally, multiple studies have documented the presence of pathogens such as *Anaplasma marginale*, *Babesia bovis*, and *Babesia bigemina* (Chávez Larrea *et al.*, 2021; Escobar *et al.*, 2015; Gioia *et al.*, 2018; Maya-Delgado *et al.*, 2020; Moreira, 2018; Soto, 2010; Tana-Hernández *et al.*, 2017; Vasco, 2014). In study 4, the presence of *Anaplasma* spp. was investigated using three different methodologies: multiplex polymerase chain reaction (mPCR), blood smear analysis, and competitive enzyme-linked immunosorbent assay (cELISA). The results obtained from mPCR confirmed the presence of *A. marginale*, while *A. centrale* was not isolated. Seroprevalence rates of anaplasmosis were 98% and 81% through blood smear and cELISA, respectively. Bayesian analysis revealed a 32%-real animal prevalence of *Anaplasma*, with near to 70% of animals considered challenged-immunized animals (chronically infected or carriers). These findings indicate that there is an endemic stability scenario for anaplasmosis, which is in line with the definition provided by Dreyer et al., 1998, who define this state when the number of seropositive animals exceeds 81%. On the other hand, an unpublished study (Paucar-Quishpe *et al.*, 2024) utilizing mPCR to evaluate the prevalence of babesiosis revealed the existence of *Babesia bigemina* in 0.97% and *B. bovis* in 2.42% of the animals. Furthermore, seroprevalence rates of babesiosis were documented at 2.42% and 4.35% through blood smear and sandwich ELISA, correspondingly. Conversely, the lower seroprevalence of babesiosis suggests a more unstable epidemiological condition for this disease.

Endemic stability in TBDs is defined as an epidemiological state in which clinical disease is rare despite high levels of infection. In the case of anaplasmosis, the following factors have been fundamental for the existence of endemic stability: a) the presence of chronically infected cattle; b) passive immunity gained from colostrum, first and innate immunity after; c) the possibility for *A. marginale* to be transmitted mechanically by arthropods other than ticks and surgical fomites and needles (Aubry & Geale, 2011; Bock *et al.*, 2004; Eisler *et al.*, 2003; Jonsson *et al.*, 2012; Lorusso, 2014). For Lorusso, (2014), this latter factor can considerably contribute to the spread of the infection within a confined area. Conversely, babesiosis is only transmitted by ticks, and animals that recover from acute babesiosis become carriers for a few months (*B. bigemina*) or a number of years (*B. bovis*) (Bock *et al.*, 2004). These simple differences are important not only for an infected individual but also for the host population to reach a condition of endemic stability. The relative likelihood of the emergence of endemic stability varies greatly between diseases, being widespread and common for

Anaplasma spp. and less common for *Babesia* spp. (Eisler *et al.*, 2003; Jonsson *et al.*, 2012; Norval *et al.*, 1984).

Tick control and its link to TBDs

While eradicating ticks might seem like an attractive option, it would be extremely challenging in Ecuador. The distribution and abundance of ticks, along with pathogen transmission dynamics, are intricately linked to various factors such as host presence, suitable habitats, and both direct and indirect anthropogenic influences (Babayani & Makati, 2021). Instead, adopting ITM strategies is a practical and sustainable approach. Such an approach ensures that tick populations are managed effectively while mitigating the risk of TBDs outbreaks in a sustainable, environmentally compatible, and cost-effective manner (Willadsen, 2006). In integrated tick control, combining several complementary control measures will allow one to get the most out of each one without relying too much on a single component (Humblet *et al.*, 2020). Thus, even if one measure loses effectiveness, the overall control plan remains impactful (Graf *et al.*, 2004; Mondal, Sarma, & Saravanan, 2013).

There are several control measures for TBDs, which vary according to geographic location and specific diseases. One potential measure is the use of vaccines; however, in Ecuador, no vaccines are currently available for TBDs. Another strategy is maintaining TBDs-free herds, which involves identifying and eliminating positive animals. For anaplasmosis, since antimicrobial therapy mainly controls active infections and its efficacy in carrier animals remains uncertain, this approach would require eliminating carrier animals, a costly and challenging option for the region (Aubry & Geale, 2011; Coetzee, 2022).

Therefore, the main recommendation in the study areas would be to maintain low levels of tick infestation to allow optimal animal production and preserve a stable endemicity of anaplasmosis as described in **Study 4**. Endemic stability is obtained when calves are continuously exposed to infected ticks, allowing them to acquire robust immunity with a low probability of disease (age-reversed immunity). This reduces the probability of disease development in adult animals (Lagranha *et al.*, 2024; Mahoney *et al.*, 1981; Mahoney & Ross, 1972). The rare occurrence of clinical disease in calves is attributed to the protective role of colostrum and vertical transmission from dams under constant inoculation in endemic areas (Gonzalez Grau *et al.*, 2013; Potgieter & van Rensburg, 1987). Since immunity can be lost if older animals are not continuously exposed to infection, it is essential to ensure that both young and adult cattle are regularly exposed to ticks (Jonsson *et al.*, 2012; Peter *et al.*, 2005; Rubaire-Akiiki *et al.*, 2006). In Ecuador, where cattle are grazed in paddocks, farmers should be trained to expose cattle at an early age. Zero grazing can lead to a general loss of endemic stability in the area (Jonsson *et al.*, 2012; Peter *et al.*, 2005; Rubaire-Akiiki *et al.*, 2006).

Given that livestock farming in tropical zones is characterized by 1) grazing as the main food source for cattle and 2) relatively uniform environmental conditions, we can approach the management of anaplasmosis and babesiosis differently. Anaplasmosis can be managed by maintaining endemic stability. In contrast, for babesiosis, which has a low prevalence in the study areas, the absence of a vaccine in the country and the availability of treatment that can eliminate acute infections and carrier states, maintaining disease-free herds could be a viable option (Bock *et al.*, 2004; OIE, 2021,). In this context, for both anaplasmosis and babesiosis, it is recommended to manage closed areas. This means restricting the exchange (entry or departure) of animals from free zones to these endemic areas (Lorusso, 2014). If animal movements are necessary, two diagnostic tests are essential: PCR to detect animals in the prepatent period and active infections and cELISA to identify carrier animals. In cases where animals exhibit signs indicative of tick-borne diseases (TBDs), in addition to the techniques mentioned above, the identification of *Anaplasma* spp. on Giemsa-stained blood smears is an option. This comprehensive diagnostic approach ensures the accurate detection of both active infections and carrier states, thereby minimizing the risk of disease transmission during animal movements.

Although Ecuador generally has a tropical climate, there are tick-free zones near the Andes, where dairy production is relevant. Under global warming scenarios, risky management practices, such as the movement of animals to these areas, could facilitate the spread of ticks and TBDs (Chávez Larrea *et al.*, 2021), leading to significant economic losses for the industry. Therefore, it is essential to develop tick and TBD control programs tailored to the specific needs of different regions. Additionally, evaluating the need to introduce TBD vaccines in the country is a crucial step in controlling these diseases (Marques *et al.*, 2020).

One health

One Health is not a new concept, but it has received renewed attention due to the frequency and severity of threats linking the health of humans, animals, plants, and the environment. One Health is described as an integrated approach that aims to sustainably balance and optimize the health of humans, animals, plants, and ecosystems, noting in particular that human health depends on a healthy and functioning ecosystem (Agrebi, 2023; FAO *et al.*, 2022). Ticks represent a global challenge, underscoring the interconnected nature of animal, human, and environmental health. These ectoparasites not only impact the health and productivity of livestock but also pose significant risks to human health through zoonotic transmission. Moreover, the uncontrolled use of acaricides is a significant concern due to its potential impact on environmental pollution and the potential for drug residues to contaminate milk and meat products. (Graf *et al.*, 2004; Mondal *et al.*, 2013).

In **study 3**, the problems that farmers are exposed to when applying acaricide treatments are described. Among these challenges, it is notable that none of the surveyed farmers utilized all

recommended Personal Protective Equipment (PPE), only 48% of them adhered to basic hygiene practices like showering and changing clothes, 13% washed their hands and changed clothes, 17% only washed their hands after spraying treatments, and 22% continued their work in the field without any posterior clean. In addition, 16% of farmers reported having at least one of the following signs: dizziness, vomiting, reddening of the skin, tearing, red eyes, and difficulty breathing after spraying animals. Moreover, in desperate attempts to control severe infestations, some farmers resort to perilous practices such as using mixtures of acaricides with burnt oil or administering overdoses of acaricides, particularly organophosphates. Increased use of highly toxic chemicals, low-risk awareness, improper use of PPE, and careless handling and spraying are problems that have already been described in other studies (Human Rights Council, 2017; Hutter *et al.*, 2018; Moyo & Masika, 2009; Muñoz-Quezada *et al.*, 2017). However, it is crucial to recognize that while pesticide exposure is a global concern, farmers in low and middle-income tropical regions are disproportionately affected due to inadequate safety regulations, monitoring, and training programs (Buralli *et al.*, 2020; Lewis *et al.*, 2016).

The control of cattle ticks often involves the use of antiparasitic drugs like ivermectin. However, its improper and indiscriminate use increases the incidence of environmental contamination and food residues (Henrioud, 2011). **Study 5** conducted an analysis to detect the presence of ivermectin residues in samples taken from farms and slaughterhouses in the study areas. Samples of faeces, urine, milk, meat, and liver were tested using HPLC. The results revealed that 68% of the faeces samples contained ivermectin residues. In contrast, only 3% of the milk, urine, and liver samples showed detectable residues, and no residue was found in the meat samples. The high presence of residue-positive faecal samples is attributed to the fact that more than 90% of ivermectin is eliminated through this route, with smaller amounts being eliminated through urine (less than 2%) and milk (just over 1%) (González Canga *et al.*, 2009). Although the sampling was not conducted on the same animals, the presence of residues in faeces, combined with the findings of Study 1, which reported ivermectin use on 50% of dairy farms, suggests the use of this drug in dairy cattle. Given that the use of ivermectin is prohibited in dairy cattle, it is crucial to carry out larger or longitudinal studies to determine the scope of this issue and to assess if ivermectin residue levels in these products pose any risk to consumers. Additionally, considering the presence of residues in faeces and, urine, and the potential implications of ivermectin residues on beetle populations and ecosystem functioning, as well as water source contamination (Verdú *et al.*, 2018), further research is necessary to understand the extent of ivermectin contamination in the environment fully.

Given that the acceptable daily intake (ADI) for ivermectin, as established by the FAO (FAO/WHO, 2023), is 680 μg/person/day for adults (bw=68 kg) and 130 μg/person/day for children (bw=13 kg), the assessed risk is negligible, being less than 1% of the ADI. Furthermore, the GECDE for ivermectin residues in the study areas is also lower than 7.2% for a person older than 10 years and

9.3% for a person younger than or equal to 10 years (FAO/WHO, 2023). However, it should be emphasized that this corresponds to rural areas where it was determined that milk consumption is a quarter of national consumption, so it should not be underestimated since the hazard is present. This information not only highlights the need for future research but also suggests that Good Animal Husbandry Practices (GAHP) programs may need modification. Although economic recognition is given for farms with good livestock practices, less than 1% (167 out of 280,000) of cattle farms have received this certification nationwide. Efforts should be made to increase participation to these programs. Economic incentives, which are currently only applied to dairy farms, could be extended to beef farms to encourage more farms to enter the program of GAHP (Paucar *et al.*, 2021). Moreover, the existing program should be revised to include the analysis of antibiotic, antiparasitic, and heavy metal residues in bovine products as criteria for certification (Agrocalidad, 2012). This would ensure a more comprehensive approach to food safety and public health.

Although this study did not detect the presence of ivermectin residues in meat, it is recommended that future studies employ a more comprehensive sampling strategy, including samples from local butcher shops. This is crucial as some meat consumed in homes and restaurants originates from informal slaughterhouses. Consequently, this study does not guarantee food safety for consumers in the study area. Currently, only 56% of the 823 operational formal slaughtering plants meet the Slaughterhouse Under Official Inspection (MABIO) certification standards, which ensure that the protein is safe and processed at authorized facilities (Agrocalidad, 2020). Additionally, four provinces in Ecuador need more official cattle slaughterhouses, compelling small and medium cattle farmers to incur higher costs to transport their animals to neighbouring provinces, sell their livestock at lower prices to intermediaries, or resort to home or clandestine slaughtering. Therefore, the creation and improvement of active slaughterhouses should be a priority, along with updating inspection programs to include testing for antiparasitic, antibiotic, and heavy metal residues, is vital to guarantee safe and high-quality food for Ecuadorian consumers.

In general, it is essential to implement holistic measures that include: 1) training programs for farmers and veterinarians on the correct management of acaricides, with an emphasis on adhering to recommended withdrawal periods; 2) regular residue monitoring and analysis in slaughterhouses and milk collection centres; and 3) launching consumer awareness campaigns that highlight the dangers of drug residues and the importance of purchasing products from reliable stores; 4) promoting interdisciplinary collaboration among veterinarians, public health experts, healthcare professionals, biologists, and ecologists. This collaboration is vital for ensuring holistic approaches to animal health, food safety, and human health through the adoption of sustainable and environmentally friendly livestock practices.

Perspectives and conclusions

According to these and many other studies conducted in tropical regions around the world, livestock animals face numerous challenges that significantly impact their welfare and productivity. Understanding the factors and challenges that contribute to tick proliferation in tropical livestock systems is crucial to develop effective management strategies that safeguard animal health, protect human health, and promote environmental sustainability.

This work aimed to investigate the levels of tick infestation and the various tick control practices employed by farmers. Sustainable methods such as biological control, manual removal, and the use of medicinal plants were found to be used, as well as more risky approaches such as inappropriate application, overdose, and the mixture of acaricides. In the areas studied, where ticks are prevalent, frequent and intensive chemical control has led to the emergence of resistance to acaricides, which imposes additional financial burdens, especially on small and medium-sized farms. The study identified the presence of anaplasmosis and babesiosis, highlighting the endemic nature of anaplasmosis and the potential risk of disease outbreaks due to uncontrolled animal movements (biosecurity) or climate change (surveillance). In addition, the study also analysed the presence of ivermectin residues in milk, meat, liver, urine, and faeces. The high concentration of residues in faeces indicates the need to assess their impact on soil biodiversity, a concern that has been highlighted in previous research. It is also important to investigate the presence of acaricides in natural water sources in order to fully understand the potential environmental effects of tick control practices. Conversely, despite the isolation of low residues in milk and liver and no residue in meat, these results provide a starting point for future research. Such research should use different methodologies and examine other commonly used acaricides to assess their prevalence and impact comprehensively.

This work, along with numerous other investigations conducted in tropical regions worldwide, highlights the multifaceted challenges that livestock face, profoundly impacting their welfare and productivity. Understanding the intricate factors contributing to tick proliferation, acaricide resistance, and TBDs in tropical livestock systems is essential for devising effective management strategies that not only safeguard animal health but also protect human health and promote environmental sustainability.

Recommendations

Effective tick control management requires collaboration and coordination among various stakeholders involved in livestock production (**Figure 14**). This process involves the participation of various stakeholders, including farmers, veterinarians, zootechnicians, pharmaceutical companies, researchers, and regulatory agencies. Here are recommendations proposed for engaging stakeholders in tick control and TBD management:

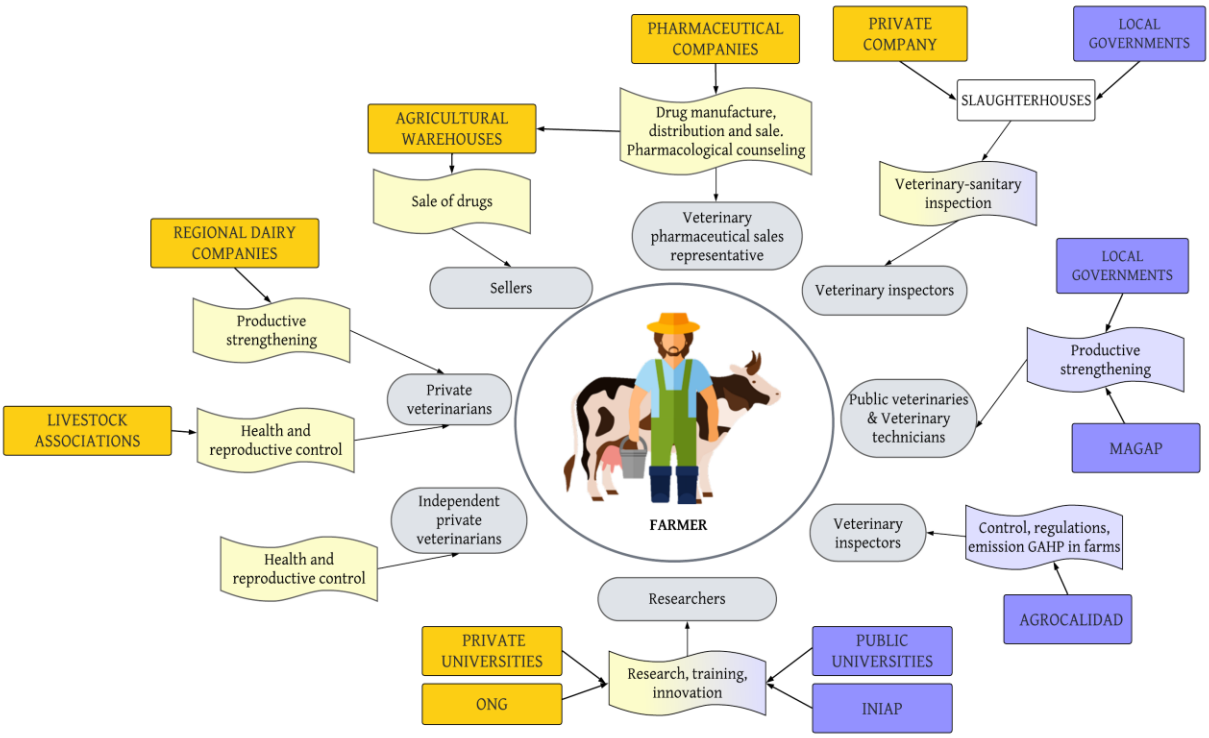

Figure 14: Stakeholder mapping

Legend: Public stakeholders are in blue, and private stakeholders are in yellow. Agencia de Regulación y Control Fito y Zoosanitario (AGROCALIDAD); Ministerio de Agricultura, Ganadería, Acuacultura y Pesca (MAGAP); Instituto Nacional de Investigaciones Agropecuarias (INIAP); Non-governmental organisation (ONG).

SHORT-TERM INTERVENTIONS (1 YEAR)

Farmers

- Implementation of sanitary records, which include periodic assessment of the level of infestation in livestock and acaricide management.
- Participate in workshops and training on tick control offered by agricultural extension services or universities.
- Join livestock organisations to share knowledge and resources related to tick control.

Veterinarians and zootechnicians

- Participate in continuous training programmes on integrated tick management and parasite control.

Authorities

- Provide training and dissemination of information on tick control to farmers and veterinarians.

- Allocate funds to universities and public institutions to support scientific investigations on tick biology, ecology, disease transmission dynamics, and control methods.
- Implement regular inspections of agricultural warehouses to ensure compliance with regulations and standards related to acaricide sales and distribution.
- Launch campaigns to educate consumers about the risks associated with consuming drug residues and the importance of purchasing products from accredited stores.

Researchers

- Participate in some networks on ticks and TBDs in order to share experiences, ideas, and practices, among others.
- Investigate alternative strategies for tick control, such as entomopathogenic fungi, vaccines, and manual removal, among others.
- Compile all existing information on ticks and TBDs in the country to determine the spatial distribution maps of ticks and TBDs.

MEDIUM-TERM INTERVENTIONS (2-3 YEARS)

Farmers

- Establish livestock organisations: well-established livestock organisations enable their members to pool financial resources to contract veterinary services and purchase inputs at reduced prices through bulk purchasing.

Veterinarians and zootechnicians

- Acquire skills in sustainable animal management, tick collection methods, acaricide resistance testing and communication techniques.

Authorities

- Restrict the use of highly toxic acaricides (organophosphates).
- Implement surveillance and monitoring systems to assess the prevalence and distribution of tick populations throughout Ecuador.
- Renew genetic improvement programs for tropical cattle by abandoning the use of *Bos taurus* dairy breeds, which are not suitable for tropical climates. Instead, the introduction (artificial insemination) of breeds adapted to a tropical environment should be prioritised (*Bos indicus*).

- Promote multi-sectoral coordination: Encourage collaboration between government agencies and stakeholders to develop tick control policies.

Researchers

- Conduct cost-benefit analyses to evaluate the economic feasibility and sustainability of integrated tick control strategies that minimize the use of acaricides.
- Investigate the presence of resistance to other acaricides on the market.
- Perform research studies to determine the presence of acaricide (organophosphates, amides, alpha-cypermethrin, fipronil, fluazuron) residues in milk or meat from animals treated.
- Assess the potential impacts of climate change on tick distribution, tick infestation, and TBDs.

LONG-TERM INTERVENTIONS (> 4 YEARS)

Farmers

- The adoption of integrated tick management (ITM) adapted to the reality of the farm and according to the production system.

Veterinarians and zootechnicians

- Collaborate in setting up ITM programmes and transferring sustainable knowledge to farmers.

Researchers

- To assess the impact of acaricide residues on human health and the ecosystem, as well as to investigate acaricide resistance and the impact of climate change on tick distribution.

Authorities

- Establish a "Tick and TBD Control Research" centre with equipment and trained personnel for resistance testing, residue detection and diagnosis of tick-borne diseases.
- Implement a systematic monitoring programme (Slaughterhouse Veterinary Inspectors and AGROCALIDAD) in slaughterhouses to sample and analyse meat for residues of acaricides and other veterinary drugs.
- Develop policies that promote sustainable tick control practices and offer incentives for the adoption of integrated tick management approaches within Good Animal Husbandry Practices (GAHP).

Limitations

The COVID-19 pandemic presented additional constraints and challenges during the study. Movement restrictions and social distancing measures may have impacted data collection methods and participant recruitment. Since the method used in this sampling was snowball sampling, the pandemic may have limited the number of participants recruited into the study. Laboratory capacity limits also caused delays in sample processing, potentially affecting the timely analysis of collected data. While efforts were made to ensure high-quality data, there is a possibility of bias, as individuals who were more accessible or participated more actively during the pandemic may have been overrepresented in the study population. Despite these challenges, the study was conducted with care, to minimize bias and to achieve accurate results.

Chap. 5 - References

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Chap. 6 - Appendixes

Appendix 1

Table S1. Activities and studies carried out in the thesis

PART OF THE STUDY	TITLE OF THE SCIENTIFIC PAPER	FIELDWORK ACTIVITIES
1. Assessment of farmers' knowledge, perceptions of ticks and TBDs and the different practices they use to control these parasites.	Study 1: The Associated Decision and Management Factors on Cattle Tick Level of Infestation in Two Tropical Areas of Ecuador. Study 3: Farmers' adoption, knowledge, and perceptions of tick control measures on dairy farms in subtropical areas of continental.	– Cross-sectional survey: Socio-eco epidemiology of ticks and TBDs. – Animal sampling.
2. Quantitative assessment of the economic impact of ticks in Ecuador.	Study 2: An economic evaluation of cattle tick acaricide-resistances and the financial losses in subtropical dairy farms of Ecuador: A farm system approach	– Cross-sectional survey: Socio-eco epidemiology of ticks and TBDs. – Animal sampling. – Interview of agricultural warehouses. – Participatory Meeting.

Table S1. *Cont.*

PART OF THE STUDY	TITLE OF THE SCIENTIFIC PAPER	FIELDWORK ACTIVITIES
3. Determine the presence of several blood pathogens in cattle.	Study 4: What is the value of testing for tick-borne diseases in cattle in endemic areas? A case study of bovine anaplasmosis	− Cross-sectional survey: Socio-eco epidemiology of ticks and TBDs. − Animal sampling.
4. Assessment of human exposure to acaricide residues in food of bovine origin.	Study 5: Human Risks of Consuming Bovine Products with Ivermectin Residues in Ecuador	− Animal sampling. − Bovine product sampling. − Cross-sectional survey: Consumption of bovine products.

Appendix 2

Integrated tick control manual

MIEMBROS DEL PROYECTO TICKS & TBD

Coordinador Sur - Universidad Central del Ecuador

Richar Rodríguez-Hidalgo.

Coordinadora Norte - UCLouvain

Sophie Vanwambeke.

Colaborador Norte - LIÈGE université

Claude Saegerman.

Colaboradores Sur - Universidad Central del Ecuador

Lenin Ron, Cecilia Pérez, Dario Cepeda, Sandra Enríquez, Susana Arciniegas, Jorge Grijalva.

Estudiantes de doctorado

Valeria Paucar-Quishpe.
Ximena Pérez-Otáñez.

Estudiantes de maestría

Daniela Balseca.

Estudiantes de pre grado

Bryan Benavidez, Carlos Rivera, Linette Jácome, Tania Tinoco, Luis Sandoval, Pamela Llasha, Yadira Guasumba, Kamilo Villareal, Richard Orozco, Daniela Serrano, José Buitrón, Jeanina Abadeano, Priscila Alomoto, Ma. Elisa López, Ximena Condo, Erika Guzman, Fernanda Ulcuango & Joselin Egas.

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Appendix 3

Bayesian Estimation of the Prevalence and Test Characteristics (Sensitivity and Specificity) of Two Serological Tests (RB and SAT-EDTA) for the Diagnosis of Bovine Brucellosis in Small and Medium Cattle Holders in Ecuador

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Bayesian Estimation of the Prevalence and Test Characteristics (Sensitivity and Specificity) of Two Serological Tests (RB and SAT-EDTA) for the Diagnosis of Bovine Brucellosis in Small and Medium Cattle Holders in Ecuador

Valeria Paucar [1,2], Jorge Ron-Román[3], Washington Benítez-Ortiz[1], Maritza Celi[1], Dirk Berkvens[4], Claude Saegerman [2,*] and Lenin Ron-Garrido [1,5,*]

1 Centro Internacional del Zoonosis (CIZ), Universidad Central del Ecuador, Quito 170521, Ecuador;adriana_vale@live.com (V.P.); wbenitez@uce.edu.ec (W.B.-O.); mceli@uce.edu.ec (M.C.)
2 Research Unit of Epidemiology and Risk Analysis Applied to Veterinary Science (UREAR-ULg), Fundamental and Applied Research for Animals & Health (FARAH) Center, Faculty of Veterinary Medicine, University of Liege, 4000 Liège, Belgium
3 Grupo de Investigación en Sanidad Animal y Humana (GISAH), Carrera Ingeniería Agropecuaria, Departamento de Ciencias de la Vida y la Agricultura, Universidad de las Fuerzas Armadas ESPE, Sangolquí 171103, Ecuador; jwron@espe.edu.ec
4 Department of Biomedical Sciences, Institute of Tropical Medicine, 2180 Antwerp, Belgium;
5 ddbdb@telenet.be
Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, Quito 170521, Ecuador
* Correspondence: claude.saegerman@uliege.be (C.S.); ljron@uce.edu.ec (L.R.-G.)

Abstract: In Ecuador, a national program for bovine brucellosis control has been in implementation since 2008. Given the costs, small- and medium-sized livestock holders are not completely committed to it. The objective of this study was to determine true prevalence (TP) of bovine brucellosis in small- and medium-sized herd populations, as well as the diagnostic sensitivity and specificity of the Rose Bengal (RB) test and the sero-agglutination test (SAT)-EDTA using a Bayesian approach. Between 2011 and 2016, 2733 cattle herds were visited, and 22,592 animal blood samples were taken in nineteen provinces on mainland Ecuador. Bayes-p and deviance information criterion (DIC) statistics were used to select models. Additionally, risk-factor analysis was used for herds according to their brucellosis test status. True prevalence (TP) in herds was estimated by pool testing. National seroprevalence of farms was 7.9% (95% CI: 6.79–9.03), and TP was 12.2% (95% CI: 7.8–17.9).
Apparent prevalence (AP) in animals was 2.2% (95% CI: 1.82–2.67), and TP was 1.6% (95% CrI: 1.0–2.4). Similarly, the sensitivity of the RB was estimated at 64.6% (95% CrI: 42.6–85.3) and specificity at 98.9% (95% CrI: 98.6–99.0); for the SAT-EDTA test, sensitivity was 62.3% (95% CrI: 40.0–84.8) and 98.9% (95% CrI: 98.6–99.1) for specificity. Results of the two tests were highly correlated in infected and uninfected animals. Likewise, high spatial variation was observed, with the Coastal Region being the zone with the highest TP at 2.5%. (95% CrI: 1.3–3.8%) in individual animals and 28.2% (95% CI: 15.7–39.8) in herds. Risk factors include herd size, type of production (milk, beef, and mixed), abortions recorded, and vaccination. The results of this study serve to guide authorities to make decisions based on parallel testing at the beginning of a bovine brucellosis program for small livestock holders to increase sensitivity level of the screening tests in Ecuador.

Keywords: bovine; modelling; brucellosis; diagnosis; sensitivity; specificity; true prevalence; Bayes

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