

Tick-borne diseases in subtropical dairy farming: The role of calf rearing strategies in antibody and antigen prevalence

Enfermedades Transmitidas por Garrapatas en la Ganadería Lechera subtropical: El Papel de las Estrategias de Crianza de Terneros en la Prevalencia de Anticuerpos y Antígenos

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Abstract

Tick-borne diseases (TBDs), such as anaplasmosis and babesiosis, pose significant challenges to dairy farming in subtropical regions, where balancing tick control with immune development is essential to maintaining herd health. This study evaluated the immune responses of calves raised under confined and traditional systems in Ecuador. Serum samples from 124 cows and their calves were analysed using ELISA assays to detect *Anaplasma marginale* antibodies and *Babesia bovis* antigens. The results showed a significantly higher seroprevalence of *A. marginale* antibodies in calves reared under traditional systems (98 %) compared to those raised under confinement (32 %) ($p < 0.01$),

Daniela Balseca, Valeria Paucar-Quishpe and Claude Saegerman contributed equally to this study.

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Funding information

Académie de recherche et d'enseignement
supérieur

Associate Editor: Maureen Laroche

suggesting that early exposure to infected ticks enhances immune response development. In contrast, antigen levels for *B. bovis* were low overall (1 % Ag-prevalence), suggesting limited pathogen circulation, although the Ag-prevalence was slightly higher in traditional systems (2 %). In confined systems, maternal antibody transfer appeared to play a more prominent role, whereas calves in traditional systems developed a more active, independent immunity. These findings highlight the importance of calf-rearing strategies that incorporate controlled tick exposure alongside targeted management practices to enhance herd resilience and mitigate economic losses. A balanced approach considering endemic stability could improve long-term protection against TBDs in sub-tropical dairy farming systems.

KEYWORDS

Anaplasma, antibodies, antigens, babesia, calves, Ecuador, rearing systems

INTRODUCTION

Tick-borne diseases (TBDs) in cattle such as anaplasmosis and babesiosis, pose a significant challenge to cattle farming worldwide but particularly in tropical and subtropical regions (Jonsson et al., 2008). The causative agents are the bacteria *Anaplasma* spp. and the protozoan *Babesia* spp. Currently, eight species of *Anaplasma* have been described: *A. marginale*, *A. phagocytophilum*, *A. centrale*, *A. ovis*, *A. bovis*, *A. platys*, *A. odocoilei* and *A. capra*. Regarding *Babesia*, the most prevalent species in subtropical and tropical regions of the world are *Babesia bovis* and *B. bigemina*, but more than 100 different species have been described (Rar et al., 2021; Tate et al., 2013). Both genera of pathogens inhabit the host's erythrocytes and are primarily transmitted through the bite of the tick *Rhipicephalus microplus* or *Rhipicephalus annulatus* (Aubry & Geale, 2011; Boulanger et al., 2019; CFSPH, 2013). *Anaplasma* can also be transmitted by hematophagous insects, such as horse flies, mosquitoes and stable flies, as well as through iatrogenic routes (Kocan et al., 2003, 2010; Khaluhi et al., 2021). Anaplasmoses and Babesioses share common clinical signs in cattle, including fever, weakness, depression and anaemia, with severity depending on the parasite species and host factors such as immune status, breed and age (Parodi et al., 2021; Sisson et al., 2023; Vieira et al., 2019).

The most severe economic and health impacts of these TBD are observed in adult cattle, which exhibit higher mortality rates, while calves generally experience milder clinical signs. (Kessler et al., 1983; Tabor, 2022). Thus, understanding the epidemiological dynamics of TBDs in tropical regions is important for implementing effective control strategies that balance disease prevention with long-term herd immunity.

In endemic stability areas for TBD, the continuous exposure to ticks and other blood-feeding parasites during early life allows calves to develop immunity without experiencing severe disease. Most young animals become naturally infected, resulting in long-lasting protection. As a result, clinical disease is minimal, and economic losses are reduced (Kessler et al., 1983; Paucar-Quishpe et al., 2025; Tabor, 2022). However, when strict tick control measures are

implemented without considering immunity dynamics, the number of susceptible adult cattle may increase, making herds more vulnerable to severe outbreaks. This unintended consequence highlights the need for a strategic approach that integrates tick control with controlled exposure to maintain herd resistance to TBDs (Koual et al., 2023; Oliveira et al., 2011; Perez et al., 1994; Ribera Cuéllar et al., 2000). In unstable endemic areas, not all cattle acquire early immunity, resulting in frequent outbreaks in both calves and adult animals. These areas often have high mortality rates in adult animals when new animals are introduced without previous exposure, resulting in increased costs of treatment and management of the disease (Barros et al., 2005; Betancur & Giraldo-Ríos, 2019; Kessler et al., 1983).

In this context, a sustainable approach to TBD management should encourage controlled exposure of calves to ticks to stimulate early immune development and enhance long-term resistance (Tabor, 2022). While vaccines against anaplasmosis and babesiosis are available, their availability and widespread implementation remain limited in many regions, necessitating the use of alternative management and control practices. (ParaBoss, 2022). Such an approach promotes the development of protective immunity early in life, thereby lowering the likelihood of severe disease later in adulthood. This approach, known as challenge immunisation, has been widely recognized as a cost-effective and practical method in endemic areas (Betancur & Giraldo-Ríos, 2019; Paucar-Quishpe et al., 2025). However, its successful implementation requires farmer awareness, education and policy support to optimize disease control while preserving natural immunity within cattle populations.

In tropical and subtropical areas of Ecuador, anaplasmosis and babesiosis are prevalent diseases that significantly impact cattle farming. Specialized dairy farming is relatively new in subtropical regions of the country, where farmers have adopted or attempted to adopt traditional rearing methods from temperate zones, where tick pressure is lower and tick-borne diseases (TBDs) are less of a concern. In tropical dairy production zones, calves are typically reared using one of two main approaches: confined systems or traditional systems. In confined systems, calves are separated from their dams at birth and

raised in pens or stalls, minimising their exposure to ticks and, consequently, to tick-borne diseases. In contrast, in traditional systems, calves remain with their dams in open pastures, where they are more exposed to tick infestations and face a higher risk of contracting TBDs.

In this context, the aim of this study was to evaluate the presence and levels of antibodies against *Anaplasma marginale* and antigens of *Babesia bovis* in cows and their calves. Additionally, the study aimed to compare immune responses between calves raised under traditional and confined rearing systems. The findings aim to provide evidence-based insights to help farmers design calf-rearing programs that enhance calf protection against tick-borne diseases, improve herd resilience and reduce health-related economic losses.

MATERIALS AND METHODS

Study design

A cross-sectional observational and convenience sampling was conducted within the framework of the broader research project titled ‘Socioecopidemiology of ticks, tick-borne diseases, acaricide resistance, and residual effects of acaricides in tropical Ecuadorian livestock farming: environmental impacts and impacts on animal and public health’. Fifty-three out of 95 dairy farms participating in the project were sampled across 12 locations. Those farms met the inclusion criteria and were enrolled in the study. The eligibility requirements were as follows: (1) the presence of both dam and calf on the farm, (2) the calf being 3 months of age or younger, and (3) the farmer providing signed informed consent to participate. The study included 124 cows and their calves (124 pairs). Given the exploratory nature of the study and the field-based constraints, a formal a priori sample size calculation was not performed. However, the final sample size was considered sufficient to estimate and compare proportions between rearing systems using differences in seroprevalence.

A schematic representation of the farm classification according to calf-rearing system is presented in Figure 1. In traditional systems, calves remain with their dams in paddocks either full-time or part-time, with the option of overnight housing in pens. In contrast, the confined system entails the immediate separation of calves at birth and individual housing in pens to ensure regulated care, including controlled administration of milk and feeding. In both systems, calves are allowed to suckle directly from the dam during milking, a common practice in low-technology systems to stimulate milk let-down and facilitate milking; however, in higher-technological farms, milk for calves usually is provided from the bulk tank.

Antigen-detection assays for *Anaplasma marginale* were not available in Ecuador during the study period. In a similar way, for *Babesia bovis* the only commercially available diagnostic option was an antigen-detection ELISA, where positive results are indicative of active infection. Antibody-based assays for *Babesia* have only recently become commercially available and were not accessible at the time of sampling.

Study area

Between June 2023 and September 2023, 53 out of 95 dairy farms participating in the project were sampled across 12 locations in the subtropical dairy areas of northern Ecuador: Pedro Vicente Maldonado (539 m.a.s.l.), San Miguel de los Bancos (1074 m.a.s.l.), Pacto (1268 m.a.s.l.), Gualea (1348 m.a.s.l.), Nanegalito (1598 m.a.s.l.), Sumaco (1871 m.a.s.l.), Sardinas (2172 m.a.s.l.), San Francisco de Borja (2287 m.a.s.l.), Linares (2339 m.a.s.l.), Tumbaco Valley (2400 m.a.s.l.), San Fernando (2719 m.a.s.l.) and Uyumbicho (2735 m.a.s.l.) (Topographic Map, 2024). These locations are distributed across three main regions of continental Ecuador: The Quijos River valley in Napo province, which serves as an entrance to Ecuadorian Amazon; part of the dairy basin of Pichincha province located in the inter-Andean valley of Guayllabamba, and northwestern of Pichincha Province, corresponding to the foothills of the country's subtropical western region. The two subtropical regions are humid zones; however, they differ in rainfall patterns. In the Quijos River valley precipitation is constant throughout the year, resulting in a perception that tick infestation is uniform over time. In contrast, northwestern Pichincha presents a more defined dry season, typically from June to November, which has been associated with increased tick infestation during these months. These areas exhibit a high prevalence of ticks and are endemic for tick-borne diseases (TBD). Within the inter-Andean Guayllabamba Valley, ticks occur throughout the region; however, higher-altitude areas are characterized by cooler temperatures, lower relative humidity and reduced annual rainfall; this region is generally less favourable for tick persistence, resulting in unstable endemicity of TBD. These ecological differences may influence tick population dynamics and host exposure patterns. (Paucar et al., 2024).

Data collection

Calf rearing system was categorized into traditional and confined systems. Following this classification of calf rearing systems, blood samples were collected from all cows with calves up to 3 months old at each farm. All animals received proper care, and the typical farm practices for blood sample collection were followed. Veterinarians conducted the sample collection without mistreating the animals, ensuring animal welfare. Approximately 4 mL of blood was drawn from each animal using disposable needles and red-top Vacutainer™ tubes (no anticoagulant) via coccygeal venipuncture for cows and jugular venipuncture for calves. For each sample, a unique code was assigned and transported under refrigeration to the Immunodiagnostic Laboratory at the Instituto de Investigación en Zoonosis (CIZ) at the Universidad Central del Ecuador for analysis. The serum was separated by centrifugation at 2500 rpm, then transferred into individually labelled cryovials and frozen at $-20^{\circ}\text{C} \pm 3^{\circ}\text{C}$ until serological analysis. A curated and consolidated database was compiled, containing the results from 124 cows and their respective calves. Additionally, for each farm, a health record form was completed for each animal, and a questionnaire on animal management practices was administered to

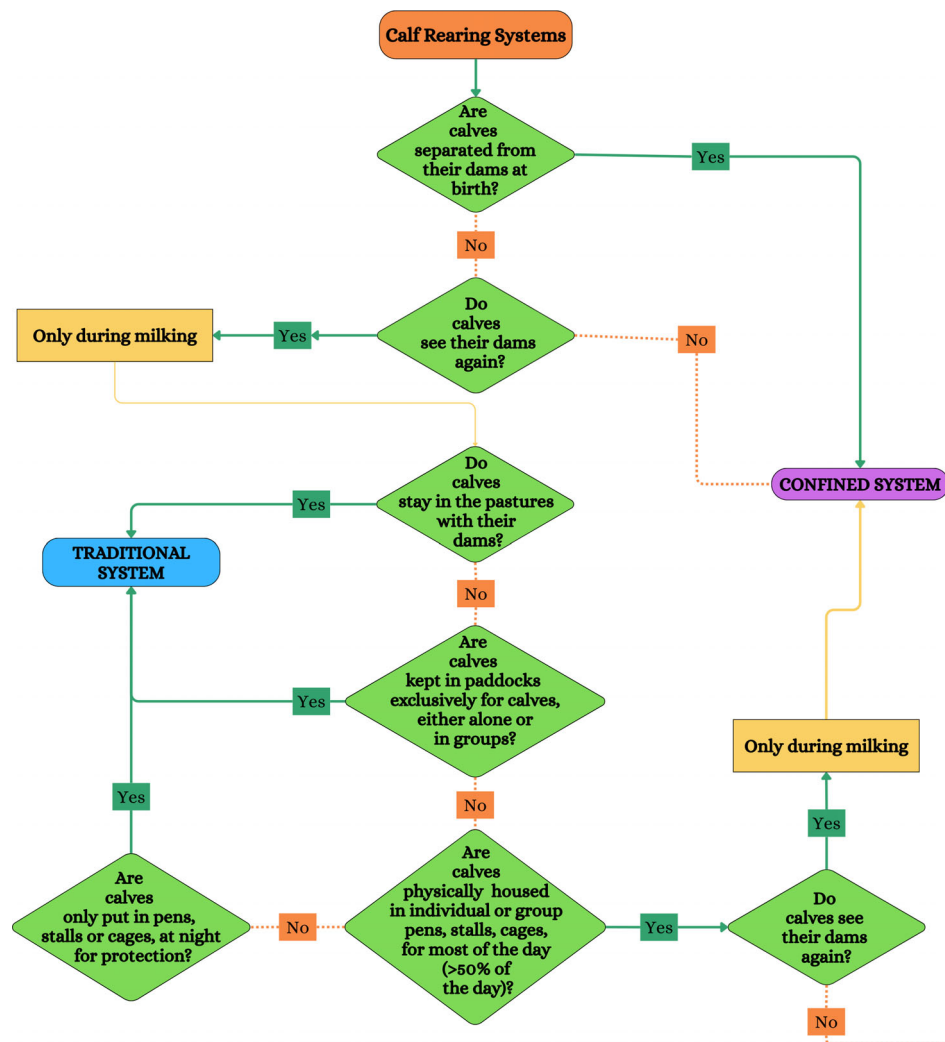


FIGURE 1 Decision flowchart used to classify calf-rearing systems on farms as either traditional or confined systems.

the farm manager. The data were collected using Epicollect-5 mobile application (Epicollect5, 2021).

Serological tests

Sera were tested for *Anaplasma marginale* antibodies using a competitive ELISA that targets the major surface protein 5 (MSP5), following the protocol of the commercial kit Anaplasma Antibody Test Kit cELISA v2 (Veterinary Medical Research and Development; VMRD, Inc.), following the manufacturer's instructions, catalogue number 283-2. For the interpretation of results, the optimal cut-off point was 35.76, with a high sensitivity and specificity of 99%–100%, respectively. These values were calculated based on laboratory data and adjusted cut-off analysis; these values were consistent with those reported by the manufacturer, which indicate a sensitivity of 99.7 % and a specificity of 100 %.

Babesia bovis antigens present in the serum were identified using the sandwich ELISA, in accordance with the protocol of the Bovine *Babesia bovis* (B. bovis) ELISA Kit (AFG Bioscience), following the

manufacturer's instructions (catalogue number EK760604). The optimal cut-off was 0.226 with high sensitivity and specificity, 90%–100%, calculated based on laboratory data and adjusted cut-off analysis.

Statistical analysis

Data were entered, organized and cleaned in Microsoft® Excel® for the Microsoft 365 spreadsheet, and the data were analysed using R studio version 4.2.0 statistical software (R Core Team, 2022). The seroprevalence of bovine anaplasmosis and babesiosis was determined as a proportion of positive animals out of the total animals sampled.

Optical density (OD) values obtained from ELISA assays were used to compare calf rearing systems. A mixed-effects model was fitted, with rearing system included as a fixed effect and farm included as a random effect. The model was implemented using the *lme4* (Bates et al., 2015) and *lmerTest* (Kuznetsova et al., 2017) packages in R to evaluate differences in antibody and antigen levels among calf

TABLE 1 *Anaplasma marginale* antibodies seroprevalence in calves (competitive Elisa).

Rearing system	Seronegative			Seropositive			Total
	N	% (95% CI) ^a	OD (95% CI)	N	% (95% CI) ^a	OD (95% CI)	OD (95% CI)
Traditional	1	2 (0–9)	0.65 (0.65–0.65)	66	98 (91–100)	0.14 (0.06–0.39)	0.15 (0.06–0.65)
Confined	39	68 (55–78)	0.66 (0.40–0.93)	18	32 (20–45)	0.20 (0.06–0.39)	0.52 (0.06–0.93)
Total	40	32 (24–41)	0.66 (0.40–0.93)	84	68 (59–76)	0.15 (0.06–0.39)	0.32 (0.06–0.93)

Note: The bold values indicates only to present the overall totals rather than the individual groups.

Abbreviations: N, Number of animals; OD, optical density.

^aPercentages calculated within each rearing system.

rearing systems. Spearman correlation coefficient from the *stats* package (R Core Team, 2022) was employed to assess the relationship between maternal and calf ODs. A scatterplot was used to visualize antibody levels in dams and their calves, with a smooth trend line fitted to illustrate the pattern. The data were visualized using a scatter plot created in the *ggplot2* package. The correlation was termed ‘very weak’ (0.0–0.20), ‘weak’ (0.21–0.40), ‘moderate’ (0.41–0.60), ‘strong’ (0.61–0.80) and ‘very strong’ (0.81–1.0) (Prion & Haerling, 2014). *p*-values < 0.05 were considered statistically significant.

RESULTS

Of the 53 farms sampled, 30 (56.6%) used the traditional rearing system for calves and 23 (43.4%) used the confined system. Sampled farms were also categorized according to farm and herd size as small, medium or large, comprising 10 small, 31 medium and 12 large farms, respectively.

Tick presence

The predominant tick species identified in the herds was *Rhipicephalus microplus*, which was detected in 92.5% of the farms. No ticks were detected in 4 of the 53 farms (8%). Among the remaining farms, 31 (58%) exhibited low tick infestation levels, 14 (26%) showed moderate infestation levels and 4 (8%) presented high infestation levels of this cattle tick species. Additional tick species were detected at low frequencies, including *Ixodes boliviensis*, identified as a single specimen in one farm, and *Amblyomma mixtum*, with two specimens collected from one farm. In farms where these species were detected, they co-occurred with *R. microplus*.

Antibody and antigen seroprevalence in calves

The total seroprevalence of antibodies (Ab) for *A. marginale* in calves was 68% (84/124) (95% CI: 59–76) at the animal level and 72% (38/53) (95% CI: 57–83) at the farm level. Seroprevalence at the animal level in calves raised under a traditional system was 98% (66/67) (95% CI: 91–100), and it was higher in comparison to those reared under the confined system, which had a lower seroprevalence of 32% (18/57) (95% CI: 20–45). (Table 1); this difference was statistically significant (*p*-value < 0.001). For dams, the seroprevalence at the animal

level of antibodies for *A. marginale* was 75% (93/124) (95% CI: 66–82) and 93% (47/53) (95% CI: 89–95) at the farm level (Table S1).

Calves 84% (84/100) and dams 92% (92/100) were recorded with antibodies against *A. marginale* at altitudes under 2400 m.a.s.l (Table S2). Above this altitude, the calves did not present antibodies against *A. marginale*, and in the case of the dams, just one cow (1/24) presented antibodies against *A. marginale*.

Babesia antigen seroprevalence in calves

There were no dams that presented antigens (Ag) for *B. bovis*. In the case of calves, the sero-antigen prevalence at the animal level for *B. bovis* was <1% (95% CI: 0–5) (Table 2), corresponding to 1 farm seroprevalence at the farm level 2% (1/53) (95% CI: 0–12). The only positive calf was raised under the traditional system at an altitude of 1074 m.a.s.l. (Table S1).

Comparison between two different calf rearing systems

Optical Densities (OD) were used to determine the amount of an antibody or antigen present in the sample analysed, and they varied according to the rearing system (Table 3). For *A. marginale* (measured via competitive ELISA), a significant difference (*p*-value < 0.001) in OD values was observed between the two rearing systems. Calves raised under traditional management exhibited lower OD values (indicating higher antibody levels) compared to those reared in a confined system (Figure 2). This difference suggests a stronger immune response in calves managed under traditional systems. (Table 1).

In the case of *B. bovis* (measured via sandwich ELISA), calves from the traditional system showed higher OD values (implying a higher antigen presence) compared to those from the confined system (Figure 3); although this difference was not statistically significant (*p*-value = 0.12).

Relationship in antibody and antigen responses between mother and calves under the different rearing systems for calves

Correlation between the ODs of *A. marginale* antibodies in dams and their calves (Figure 4) revealed a positive association in both

TABLE 2 *Babesia bovis* antigens seroprevalence in calves.

Rearing system	Seronegative			Seropositive			Total
	N	% (95% CI) ^a	OD (95% CI)	N	% (95% CI) ^a	OD (95% CI)	OD (95% CI)
Traditional	65	98 (91–100)	0.10 (0.06–0.21)	1	2 (0–9)	0.26 (0.26–0.26)	0.10 (0.06–0.26)
Confined	56	100 (92–100)	0.09 (0.06–0.15)	0	0 (0–8)	-	0.09 (0.06–0.15)
Total	121	99 (95–100)	0.09 (0.06–0.21)	1	1 (0–5)	0.26 (0.26–0.26)	0.10 (0.06–0.26)

Note: The bold values indicates only to present the overall totals rather than the individual groups.

Abbreviations: N, Number of animals; OD, optical density; –, no cases.

^aPercentages calculated within each rearing system.

TABLE 3 Comparison of Optical Densities (OD) between two different calf rearing systems.

Rearing systems	N	<i>Anaplasma marginale</i>		N	<i>Babesia bovis</i>	
		OD (95% CI)	p-value		OD (95% CI)	p-value
Traditional	67	0.15 (0.13–0.17)	<0.001**	66	0.10 (0.09–0.11)	0.12
Confined	57	0.52 (0.46–0.58)		56	0.09 (0.08–0.10)	

Abbreviation: N, Number of animals.

***p* < 0.01.

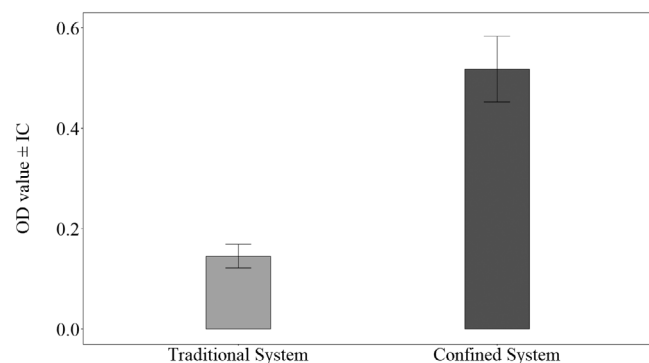


FIGURE 2 Antibody levels against *A. marginale* in calves with different rearing systems.

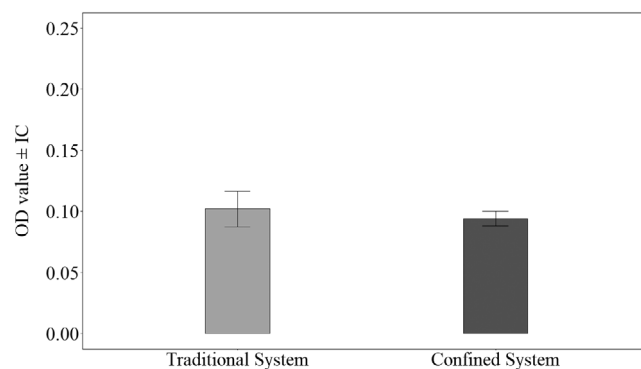


FIGURE 3 Antigen levels of *B. bovis* in calves with different rearing systems.

rearing systems. In the traditional system, the correlation was weak ($\rho = 0.28$) but statistically significant (p -value = 0.02), while in the confined rearing system, the correlation was stronger ($\rho = 0.65$) and statistically significant (p -value < 0.001). The smooth trend line illustrates the relationship between maternal and calf antibody levels. The results suggest that in confined systems, maternal antibody levels are associated with antibody levels in calves. In contrast, in traditional systems, calf-antibody levels tend to be higher than those of calves in confined systems, and less dependent on maternal levels.

A positive correlation was observed between the OD of antigens of *B. bovis* in dams and their calves in both rearing systems (Figure 5). The correlation was weak in both systems ($\rho = 0.36$ in the traditional system (p -value = 0.01) and $\rho = 0.08$ in the confined system (p -value = 0.57)). The trend in antigen levels suggests a potential independence between dams and calves, as the test measures antigen levels, which are not transferred from dam to offspring.

DISCUSSION

Ecuador is a tropical country where over 75% of cattle herds are located in areas that are either infested with or suitable for the development of cattle ticks (Gioia et al., 2018; Rodríguez-Hidalgo et al., 2017). Similarly, climate change and global warming may expand tick habitats, as Ecuador's average temperature has risen by 1.4°C since 1960, potentially enabling ticks to thrive at higher altitudes (Commission-European & GRID-Geneva, 2024). As a result, many farmers have reported observing symptoms in their animals related to the presence of anaplasmosis (*A. marginale*) and babesiosis (*B. bovis* and *B. bigemina*), which are the tick-borne diseases (TBDs) reported in cattle in Ecuador (Chávez-Larrea et al., 2021; Paucar-Quishpe, 2024).

In Ecuador, numerous studies have been conducted to investigate the presence of TBDs through methods such as blood smears, ELISA and PCR. This study utilized ELISA assays to detect the presence of

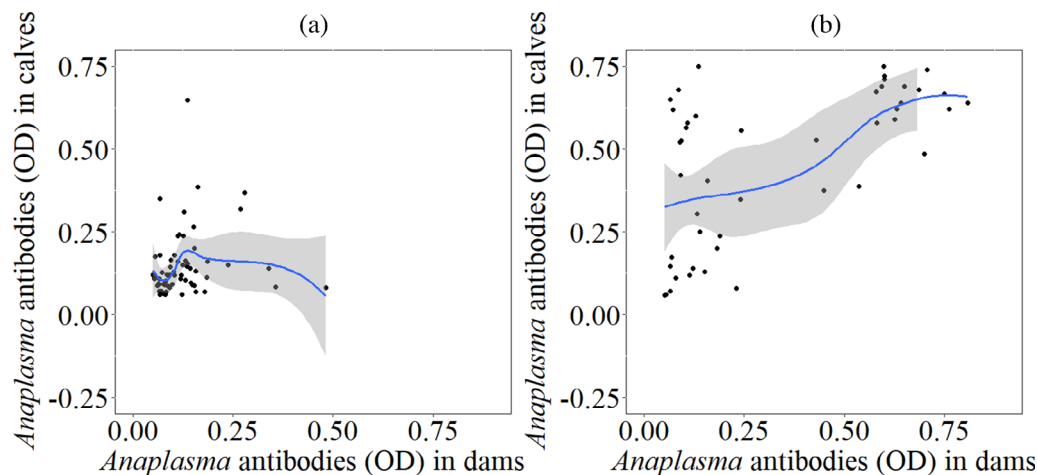


FIGURE 4 Relationship between antibodies against *A. marginale* of dams and calves. Legend: Figure A represents the traditional rearing system, and Figure B represents the confined system. Each black dot represents the coupled value of *A. marginale* antibody levels (measured in OD) in dams (x-axis) and their offspring (y-axis). The blue line represents the fitted smooth trend line of ODs, and the grey shaded area represents the confidence interval of the fitted line.

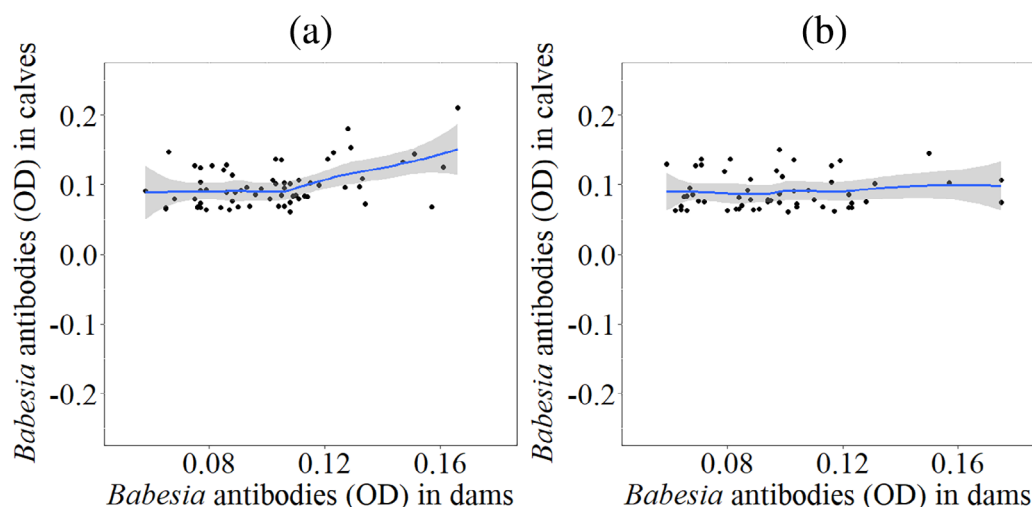


FIGURE 5 Relationship between antigens against *B. bovis* of dams and calves. Legend: Figure A represents the traditional rearing system, and Figure B represents the confined system. Each black dot represents the coupled value of *B. bovis* antigens= levels (measured in OD) in dams (x-axis) and their offspring (y-axis). The blue line represents the fitted smooth trend line of ODs, and the grey shaded area represents the confidence interval of the fitted line.

antibodies against *A. marginale*, revealing a seroprevalence of 75 % (95 % CI: 66–82) in dams and 68 % (95 % CI: 59–76) in calves. These findings are consistent with other ELISA studies, which report seroprevalences of 86 % in the Ecuadorian Coast (Fernandez, 2018) and 66 % in the Ecuadorian Amazon (Medina et al., 2017). In addition, in a study conducted by Guarnizo et al. (2020) in the Ecuadorian Amazon, using nPCR to determine the presence of *A. marginale*, a prevalence at farm level of 100 % and at animal level of 63.8 % (95 % CI: 56.3%–70.6%) was reported highlighting that none of the infected animals showed clinical symptoms of hemolytic diseases. In this study, most of the animals (dams and their calves) in tropical and subtropical areas presented antibodies against *A. marginale*, suggesting that the disease has achieved endemic stability in these areas. In such environments,

grazing cattle experience early and continuous exposure to ticks promoting the development of a subclinical immune response, enabling animals to acquire lifelong immunity as carrier animals (Kessler et al., 1983; Paucar-Quishpe et al., 2025; Tabor, 2022).

Conversely, no animals located above 2700 m.a.s.l. demonstrated the presence of antibodies for this disease. In regions around 2400 m. a.s.l., only 17% of the dams reared in traditional systems showed antibodies for *A. marginale*, and none of the calves had protective antibodies against the disease. This observation is not the first record of a tick-borne disease (TBD) at that altitude; Chávez Larrea et al. in (2021) reported another tick-borne pathogen (*Babesia* spp.) at a similar elevation of 2469 m.a.s.l. in similar areas. In terms of tick ecology, there is a positive correlation between temperature and daily rates of

interstadial development. Ticks require high temperatures to favour oviposition and high humidity to prolong female survival during this process (Pfäffle et al., 2013; Sales et al., 2024). Lower average temperatures, such as those found at higher altitudes, have also been shown to delay tick development (Burri et al., 2007). Therefore, the lower temperatures at altitudes above 2700 m.a.s.l. could contribute to a delay or even a halt in tick development, limiting their presence and consequently the transmission of *A. marginale*. However, despite its altitude, Ecuador has regions with a favourable climate for tick development (Bendix et al., 2008; Pourrut et al., 1983). The combination of tropical temperatures, even at higher elevations, and high humidity in some areas creates an environment conducive to the survival and reproduction of ticks and TBD.

In addition to *A. marginale*, this study also focused on *Babesia bovis*. The use of ELISA revealed that only 1 % of the calves tested positive for *B. bovis* antigens, suggesting a low prevalence of the disease in the studied population. This is the first time ELISA has been used to detect *B. bovis* antigens in Ecuador. Other studies, such as those using PCR, have reported a higher prevalence of *B. bovis* on the Ecuadorian Coast (14.77 %, Chávez-Larrea et al., 2021), but a complete absence of *Babesia* spp. in the Ecuadorian Amazon (Medina et al., 2017).

In this study, a significantly higher prevalence of antibodies against *A. marginale* was observed compared to *B. bovis* antigens. Several intrinsic characteristics of these agents may help explain the differences in prevalence observed between them. Tick-borne pathogen transmission is a dynamic process that varies according to tick species and pathogen biology. For instance, in hard ticks, transmission may require several hours, as observed for *Borrelia burgdorferi* transmitted by *Ixodes* spp., whereas in soft ticks transmission can occur within minutes, such as in relapsing fever borreliosis (CDC, 2024; Cook, 2014; Oregon Health, 2023). Likewise, *A. marginale* has a broader range of vectors, including various tick species such as *R. microplus*, *Dermacentor nitens*, as well as other arthropods like horse flies (*Tabanus* spp.), mosquitoes (*Psorophora* spp.), stable flies (*Stomoxys calcitrans*) and *Haematobia irritans*. Reports have documented the presence of *A. marginale* DNA in field-collected *Amblyomma* ticks; however, experimental evidence demonstrating vector competence is lacking. Thus, their role as biological vectors remains unconfirmed. These vector species are present in Ecuador (Cárdenas et al., 2009; Cardenas & Vieira, 2005; Cárdenas-Amaya et al., 2025; da Silva et al., 2015; Enríquez et al., 2020; Paucar et al., 2022, 2024; Paucar-Quishpe, 2024; Pazmiño-Palomino et al., 2024; Rodríguez-Hidalgo et al., 2017), which may enhance the ability of *A. marginale* to infect cattle herds (Ferreira et al., 2022; Kocan et al., 2010; Khaluhi, 2021). In contrast, for *B. bovis*, the principal known and probably the only vector is the tick *R. microplus* (Guglielmone, 1995), which would potentially reduce the prevalence of Babesiosis in the region.

Moreover, diagnostic techniques also play a critical role in prevalence assessments. For anaplasmosis, the cELISA test detects antibodies, indicating previous exposure to the bacterium and the development of immunity (carrier animals); this does not necessarily imply an 'active' infection (Paucar-Quishpe et al., 2025). In contrast,

the ELISA test for babesiosis detected antigens, reflecting an active infection. Therefore, the low prevalence of *B. bovis* observed in this study could suggest limited circulation of the protozoan among the sampled cattle and in ticks. However, it is important to note that low parasitemia during the acute phase of infection may underestimate prevalence rates (Canever et al., 2014; Ferreira et al., 2022).

Despite the low parasitemia observed in carrier or challenge-immunized animals, the transmission of *B. bovis* to ticks remains possible (Canever et al., 2014; Knowles et al., 1996; Kocan et al., 2010). Therefore, future studies should assess the antibody levels against *Babesia* spp. in the study areas to better understand the dynamics of infection. Currently, these measurements are not possible, as serological-antibody tests against *B. bovis* are unavailable in Ecuador. While challenge-immunized animals contribute to maintaining the stability of endemic areas, they also represent a potential source for introducing and disseminating the disease in non-endemic areas, particularly in the presence of competent vectors (Canever et al., 2014).

When comparing the OD between the two calf rearing systems, it was observed that calves raised in a traditional rearing system have a higher amount of antibodies against *A. marginale* compared to calves raised in an intensive system (confined). This suggests that calves in a controlled environment, with fewer vectors present, experience reduced exposure to ticks and TBDs. As a result, they might develop a weak immune response. Consequently, when these animals are later moved to pasture as adults, they may be at risk of experiencing the clinical and severe phases of the disease. In confined systems, the relationship between dam and calf antibody levels was strong, whereas in traditional systems, calves developed their own antibodies due to early exposure to the pathogen, making them less dependent on maternal antibody protection. Kocan et al. (2003) noted that, as long as dams are immunized to a given disease, calves receive protection from colostrum, which lasts for approximately 3 months. In areas endemic to ticks and TBDs, exposure of calves to continuous challenge of infected ticks throughout the year will allow them to develop immunity that could last a lifetime (Garry, 2008; Kocan et al., 2000; Medley et al., 1993). This constant exposure to infected ticks throughout the year, starting at an early age, gradually stimulates the production of antibodies, ensuring immunity that lasts throughout their life, independent of maternal antibodies (Benavides Ortiz et al., 2016; Kocan et al., 2000; Medley et al., 1993).

The clinical severity of diseases such as anaplasmosis and babesiosis is age-dependent, with more severe manifestations observed as animals increase their age (Saegerman et al., 2003). In adult animals, disease severity increases substantially and may result in severe clinical presentations and mortality rates ranging from 29% to 49% in animals older than 2 years. (Aubry & Geale, 2011; Richey, 1991). Therefore, in areas where tick-borne diseases are endemic, and where tick eradication is not feasible, early exposure to ticks is crucial to mitigate economic losses associated with these diseases.

In areas endemic to tick-borne diseases (TBDs), the maintenance of endemic stability relies on a balanced interaction between the vector, the host and the infectious agent. Consequently, hosts in endemic areas are typically exposed to the pathogen through repeated bites

from infective ticks rather than a single transmission event. This sustained exposure is required to stimulate and maintain an effective immune response in the host, thereby contributing to the persistence of endemic stability. Previous studies on the Ecuadorian coast have reported a 27% prevalence of *Anaplasma marginale* at the vector level, specifically in *Rhipicephalus microplus* ticks collected from cattle, while *Babesia* spp. was not detected in tick samples (Maya-Delgado et al., 2020). The absence of *Babesia* spp., in tick samples has been consistently observed in subsequent research, suggesting that the transmission potential of *Babesia* spp. within the tick population is extremely low. As a result, the occurrence of the disease remains unpredictable, emphasising the complexity of its epidemiology.

Properly managed confined systems provide calves with lower mortality rates, better control over colostrum intake, adequate nutrition and a reduced likelihood of infectious diseases by minimising exposure to extreme climates (Roland et al., 2016; Wójcik et al., 2013). However, in tropical areas, strict tick control measures, while effective in reducing tick infestations, can unintentionally increase the number of susceptible adult cattle to Babesiosis, heightening the risk of outbreaks, economic losses and mortality. Relying solely on tick elimination rather than controlled exposure may result in herds with little resistance to tick fever. A balanced approach integrating tick control with planned exposure is crucial for maintaining herd immunity and ensuring sustainable cattle production. The prevalence of *Babesia* spp. in *Rhipicephalus* ticks collected from cattle in Ecuador is generally low and inconsistently detected across studies, with prevalence values ranging from absence to occasional detection and likely influenced by environmental factors (Maya-Delgado et al., 2020). This pattern suggests that *Babesia* spp. is relatively rare in the tick populations studied. In addition, although population immunity to babesiosis is known to be highly variable and dependent on tick challenge intensity, cattle genotype and acaricide use, the nature of the immune response results in unpredictable outcomes under low or irregular exposure scenarios (Jonsson et al., 2008). Given this, continuous monitoring of infection rates and immunity levels in cattle is essential to prevent unexpected outbreaks and to design effective long-term disease control strategies.

Limitations

Several limitations should be considered when interpreting the findings of this study. First, because the study employed a cross-sectional design, pathogen exposure and immune status were assessed at a single time point, preventing evaluation of temporal dynamics and limiting causal inference between management system, tick exposure and immune response development. Second, the use of convenience sampling may have reduced the representativeness of the study population and limited extrapolation of the findings to other dairy systems in subtropical regions. However, inclusion criteria requiring simultaneous evaluation of dam-calf pairs restricted the number of eligible farms available for sampling. Third, although ELISA-based assays were used, antibody responses may have been influenced by several biological and management-related factors,

including variability in colostrum intake and passive transfer, calf and dam age, maternal health status, tick challenge intensity, acaricide use, grazing management and animal genetic background. These factors may have contributed to variation in humoral immune responses among calves independent of management system alone. Fourth, the low detection frequency of *Babesia bovis*, as reported in similar endemic settings, was assessed solely through antigen detection, where positive results are indicative of active infection. Consequently, the absence of complementary serological or molecular assays may have limited detection of prior exposure or sub-clinical carrier animals, and low-level endemic circulation within the study area cannot be excluded. Collectively, these limitations highlight the need for longitudinal studies integrating serological, antigenic and molecular diagnostics to better characterize immune development and the epidemiology of tick-borne diseases in subtropical dairy production systems.

CONCLUSION

This study highlights the potential importance of balancing tick control with the development of immunity in tropical calf-rearing systems to reduce the impact of tick-borne diseases (TBDs) in adult cattle. Our findings suggest that calf's rearing systems may influence calf immunity, with traditional systems being associated with higher levels of *A. marginale* antibodies compared to confined systems, where calves rely more on maternal immunity. This difference supports the possible role of early exposure in developing long-term resistance in herds. The low prevalence of *B. bovis* antigens in the study area indicates its limited circulation, though ongoing surveillance is essential. Sustainable cattle management in tropical regions should integrate controlled tick exposure with targeted control measures, ensuring both disease prevention and long-term herd resilience. A strategic approach that considers endemic stability will help reduce economic losses caused by ticks and tick-borne diseases (TBDs), enhance animal health and improve productivity, ultimately promoting sustainable livestock production.

AUTHOR CONTRIBUTIONS

Daniela Balseca: Investigation; methodology; visualization; writing – review and editing; formal analysis; data curation; writing – original draft. **Valeria Paucar-Quishpe:** Investigation; writing – original draft; writing – review and editing; formal analysis; software; data curation. **Richar Rodríguez-Hidalgo:** Investigation; funding acquisition; project administration; supervision; resources; writing – review and editing. **Ximena Pérez-Otáñez:** Validation; writing – review and editing; data curation; resources. **Darío Cepeda-Bastidas:** Investigation; funding acquisition; writing – review and editing; supervision. **Cecilia Pérez-Escalante:** Investigation; funding acquisition; writing – review and editing. **María-Augusta Chávez-Larrea:** Investigation; writing – review and editing; validation. **Sandra Enríquez:** Investigation; funding acquisition; writing – review and editing; methodology; resources. **Jorge Grijalva:** Writing – review and editing; validation; formal analysis; resources; supervision. **Sophie O. Vanwambeke:** Funding acquisition; writing – review and editing; validation; project administration; supervision; resources. **Claude Saegerman:** Funding

acquisition; conceptualization; writing – review and editing; validation; formal analysis; project administration; resources; supervision.
Lenin Ron-Garrido: Conceptualization; funding acquisition; writing – original draft; methodology; validation; visualization; writing – review and editing; software; formal analysis; project administration; supervision.

ACKNOWLEDGEMENTS

Our thanks to the Instituto de Investigación en Zoonosis (CIZ), Universidad Central del Ecuador (UCE), UCLouvain and the University of Liège for their collaboration in this project, as well as to the students and professionals who assisted with the fieldwork, and to all the farmers who participated.

FUNDING INFORMATION

Academy of Research and Higher Education (ARES) – Belgium.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

All ELISA test results obtained during this study have been deposited in public repositories and are publicly available. The data that support the findings of this study are openly available in Dryad (Paucar Quishpe, et al., 2026). The data are available via Dryad at <https://doi.org/10.5061/dryad.2rbnzs843>.

ETHICS STATEMENT

This study was approved by the Research Committee of the Faculty of Veterinary Medicine and Zootechnics (COIF-FMVZ) at the Universidad Central del Ecuador (approval code: UCE-FMVZ-DEC2023-0631-O). All animals were handled with care, and blood samples were collected following standard farm management practices. As the procedure involved routine blood sampling, no anaesthesia or analgesia was required. Sample collection was performed by licensed veterinarians, ensuring that no animals were subjected to mistreatment and that animal welfare was fully respected. Furthermore, farmers received detailed information about the study and provided written informed consent prior to sample collection. To maintain confidentiality, each participating farm was assigned a unique alphanumeric code indicating the specific farm and location.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Seroprevalence at the farm level by locality against *Anaplasma marginale* and *Babesia bovis*.

Table S2. Seroprevalence at the animal level by locality against *Anaplasma marginale* and *Babesia bovis*.

How to cite this article: Balseca, D., Paucar-Quishpe, V., Rodríguez-Hidalgo, R., Pérez-Otáñez, X., Cepeda-Bastidas, D., Pérez-Escalante, C. et al. (2026) Tick-borne diseases in subtropical dairy farming: The role of calf rearing strategies in antibody and antigen prevalence. *Medical and Veterinary Entomology*, 1–12. Available from: <https://doi.org/10.1111/mve.70103>