

RESEARCH ARTICLE

Epidemiological, Molecular, and Hematological Characteristics of Infection by *Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp., and *Hepatozoon* spp., in Shelter Dogs from Northeastern Colombia

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Abstract: Tick-borne pathogens represent a significant health problem for dogs, particularly in shelters, where overcrowding and limited veterinary care can facilitate transmission. Although these agents are widely distributed throughout Colombia, epidemiological studies in shelter populations remain scarce. This study evaluated 112 dogs from nine shelters in the Metropolitan Area of Bucaramanga (MAB), Santander, to determine the frequency of infections caused by tick-borne pathogens of the genera *Anaplasma*, *Babesia*, *Ehrlichia*, and *Hepatozoon*. Overall, 5.35% (6/112; 95% CI: 0.20–11.3%) of dogs were positive by blood smear, whereas 42.85% (48/112; 95% CI: 33.5–52.6%) tested positive by conventional PCR (cPCR). The κ index between blood smear and cPCR indicated slight agreement ($\kappa = 0.077$; 95% CI: -0.159 to 0.005). *Ehrlichia canis* was the most frequent infection (27.67%; 95% CI: 19.6–36.9%), followed by *Hepatozoon canis* (8.92%; 95% CI: 4.4–15.8%), *Babesia vogeli* (7.14%; 95% CI: 3.1–13.6%), and *Anaplasma platys* (6.25%; 95% CI: 2.5–12.5%), respectively. Four coinfection patterns (*E. canis* + *B. vogeli*, *E. canis* + *H. canis*, *B. vogeli* + *H. canis*, *E. canis* + *A. platys*) were identified in 7.14% (8/112; 95% CI: 3.1–13.6%) of the dogs. Hematological analysis revealed thrombocytopenia, reduced mean corpuscular hemoglobin, and increased monocyte and basophil counts in dogs infected with *E. canis*, while *A. platys* infection was associated with basophilia ($P < 0.05$). Interestingly, coinfecting dogs exhibited more pronounced alterations, including decreased red blood cell count, hematocrit, hemoglobin concentration, and platelet count ($P < 0.05$). In the epidemiological analyses, shelter of origin, sex, municipality, and the presence of tick infestation were associated with infection by *E. canis* and *B. vogeli* ($P < 0.05$). This study provides the first molecular surveillance of multiple tick-borne pathogens in shelter dogs from northeastern Colombia, revealing a high frequency of infections and coinfections, as well as the main hematological alterations associated with each pathogen. These findings contribute to a better understanding of the clinical and epidemiological characteristics of tick-borne diseases in this population.

Keywords: Tick-Borne Disease, Shelter Dogs, Bucaramanga, One Health

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Introduction

Tick-borne pathogens represent an emerging global health concern with significant impacts [1, 2]. In dogs, these pathogens primarily include bacteria such as *Ehrlichia canis* and *Anaplasma platys*, followed by *Rickettsia* spp., *Anaplasma phagocytophilum*, *Mycoplasma haemocanis*, and *Bartonella* spp. [3-5]. Tick-borne protozoa, including *Babesia vogeli*, *Hepatozoon canis*, and *Babesia gibsoni*, also contribute substantially to canine disease, several of which have zoonotic importance [6-8]. These pathogens are transmitted mainly by ticks of the genera *Rhipicephalus*, *Ixodes*, and *Amblyomma*, with *Rhipicephalus sanguineus* s.l (the brown dog tick) recognized as the principal vector of several canine tick-borne pathogens [9-11]. This species complex is common in urban environments affected by poverty, overcrowding, poor sanitation, and environmental change [2, 12].

In dogs, diseases caused by tick-borne pathogens exhibit variable clinical manifestations that depend on the infecting pathogen, host immune status, and the presence of coinfections [6, 13]. Common signs include pyrexia, anorexia, lethargy, lymphadenopathy, and splenomegaly, which can lead to mortality rates of up to 60% in severe infections caused by *Ehrlichia* and *Babesia* species [14-16]. Coinfections, resulting from exposure to multiple pathogens through repeated contact with infected ticks and from host immune system failures, often lead to increased disease severity, higher mortality rates, diagnostic difficulties, and reduced treatment efficacy [13, 17]. Studies conducted in urban areas have reported a higher frequency of coinfections in shelter dogs compared with stray or owned dogs, underscoring the role of these environments in amplifying pathogen transmission and disease complexity [18-20]. Nevertheless, the clinical and epidemiological factors driving this phenomenon remain poorly understood.

Free-roaming dogs constitute a substantial component of the global canine population, and many live under conditions of abandonment or receive limited human care [21]. These animals are often exposed to malnutrition, trauma, and infectious diseases, including vector-borne pathogens, making their management an important public health challenge [22, 23]. In Colombia, the problem is particularly evident in large urban centers such as Bogotá, Medellín, Cali, and Barranquilla, as well as in the Metropolitan Area of Bucaramanga (MAB), where thousands of dogs live on the streets under conditions that increase their vulnerability to infectious diseases [24]. To address this problem, municipal governments and non-governmental organizations have established animal shelters to rescue, rehabilitate, and facilitate the adoption of these animals [25, 26]. It is estimated that approximately 35% of shelter dogs are currently awaiting adoption in these places; however, many animals are admitted without prior screening for infectious agents that could affect resident canine populations or, more concerningly, carry zoonotic pathogens [27-29].

In Colombia, molecular studies in owned dogs have reported *E. canis* infection rates of up to 13% in Bucaramanga and Medellín [30]. In contrast, seroprevalence studies have found frequencies of 74% in Pereira and 80% in Bucaramanga [31, 32]. Infection rates for *A. platys* vary widely, ranging from about 2% in Bogotá, Villavicencio, and Bucaramanga to 20% in Santa Marta [18, 33, 34]. For *B. vogeli*, molecular studies in Bucaramanga reported an infection rate of 1%, while serological studies in Villavicencio, Bogotá, Bucaramanga, and Pereira found seroprevalence values of up to 51% [31, 35]. Molecular surveys of *H. canis* in Magdalena and Norte de Santander reported infection rates around 8% [34-36]. Despite the documented circulation of these pathogens in Colombia, information on their occurrence in shelter dog populations remains scarce. Therefore, this study aimed to determine the frequency of *Anaplasma*, *Babesia*, *Ehrlichia*, and *Hepatozoon* infections in shelter dogs from the MAB and to evaluate their associated hematological and epidemiological characteristics.

Materials and Methods

Study Area and Sample Collection

Between January and August 2025, a cross-sectional study was conducted in nine canine shelters (A–I) across the MAB, located in Santander Department, northeastern Colombia (Fig. 1). The MAB comprises the municipalities of Bucaramanga, Floridablanca, Girón, Piedecuesta, and Lebrija, the latter located approximately 17 km west of Bucaramanga. Together, these municipalities form the fifth-largest metropolitan area in Colombia. The region is situated on the eastern Andean plateau at a

mean altitude of 959 m and is characterized by annual temperatures of 23–28°C and an average yearly precipitation of approximately 1,041 mm. According to international ecological classifications, the area corresponds mainly to a tropical dry forest ecosystem, characterized by a heterogeneous landscape that includes flat, undulating, and mountainous terrain [37]. Although the exact number of canine shelters in MAB is unknown, approximately six are legally registered, and at least 21 operate informally, managed by non-profit organizations, volunteer groups, rescue networks, and the Municipal Animal Welfare Unit [24].

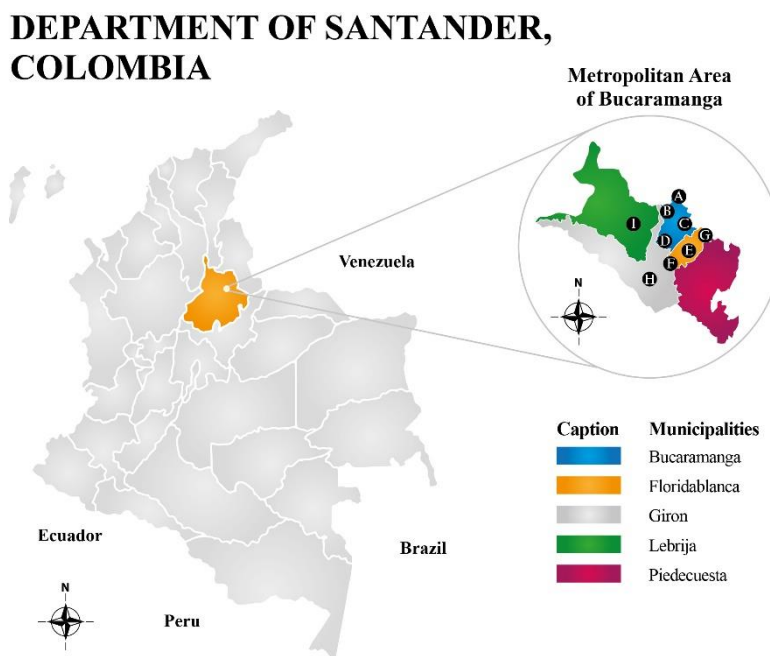


Fig. 1: Map of the Metropolitan Area of Bucaramanga showing the municipalities and the locations of the nine shelters (A–I) included in the study

Legend: The location of dog shelters in each municipality is indicated by the letters A–I in black circles.

Sample size estimation was performed assuming a population of 630 dogs across the nine participating shelters, an expected tick-borne pathogen infection frequency of 12% derived from previous molecular studies in the MAB [30], and a 95% confidence level, resulting in a minimum sample of 130 dogs. Animals were randomly selected using age-stratified sampling to ensure representation of different age groups. Informed consent was obtained from the director or legal representative of each participating shelter before sampling.

Peripheral blood (3 mL) was obtained from the cephalic vein of each dog. Of this volume, 2 mL was transferred to BD Vacutainer™ K3-EDTA tubes for molecular and hematological analyses, whereas the remaining 1 mL was collected in additive-free BD Vacutainer™ tubes for serum biochemical analyses. Whole-blood samples collected in EDTA tubes were stored at –80°C until molecular testing.

Hematological and Biochemical Analysis

EDTA-anticoagulated blood samples were first processed for hematological evaluation using a VH-50 VET 5-part differential hematology analyzer (Genrui Biotech Inc., China). The analysis included erythrocyte indices [Red Blood Cell (RBC) Count, Hematocrit (HCT), Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC)], Platelet Count (PLT), total leukocyte count (WBC), differential leukocyte percentages (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), and absolute leukocyte counts. Serum biochemical analyses were subsequently performed using a DRI-CHEM NX600 automatic dry chemistry analyzer (FUJIFILM Europe GmbH) to determine Serum Glutamic-Pyruvic Transaminase (SGPT) and Creatine Phosphokinase (CPK) concentrations.

Blood Smear Examination and Molecular Diagnostics of Tick-Borne Pathogens

For the blood smear examination, 5 µL of EDTA-anticoagulated blood was applied to a slide, dried, and fixed by immersion in absolute methanol for 2 seconds. The smears were then stained with 5% Giemsa solution for 20 minutes at 20 °C, as previously reported [38, 39]. At least 50 microscopic fields were examined for each sample before it was reported as negative for tick-borne pathogens of the genera *Anaplasma*, *Babesia*, *Ehrlichia*, and *Hepatozoon*.

Genomic DNA was isolated from 200 µL of whole blood using the Corpogen® Genomic DNA Purification Kit (Corpogen, Bogotá, Colombia) following the manufacturer's protocol. DNA quality and concentration were assessed by spectrophotometry (NanoDrop™, Thermo Fisher Scientific, USA) using the A260/A280 absorbance ratio. Molecular screening was performed by conventional PCR (cPCR) targeting *Babesia* spp., *E. canis*, *H. canis*, and *A. platys*. The primers used for each assay are listed in Table 1. Each amplification reaction was prepared in a final volume of 25 µL containing nuclease-free water, 1× PCR buffer, 1.6 mM dNTPs, 3 mM MgCl₂, 0.4 µM of each primer, 0.1 U/µL Taq DNA polymerase (Corpogen, Bogotá, Colombia), and 100 ng of genomic DNA. Cycling conditions followed previously published protocols specific for each target [40–43]. Positive controls consisted of well-characterized clinical samples previously confirmed for the presence of *A. platys*, *B. vogeli*, *E. canis*, and *H. canis* by molecular methods; these samples were sourced from BioVet Veterinary Clinical Laboratory located in Bucaramanga. Negative controls consisted of DNA derived from dogs previously confirmed to be molecularly negative for these infections. All PCR batches included both positive and negative controls. The PCR products were analyzed by electrophoresis on 2% agarose gels stained with EZ-Vision® (Amresco, Solon, OH, USA) and visualized under UV light using a gel documentation system (Cleaver Scientific, Cambridge, UK).

Table 1: Primers used for the molecular detection of tick-borne pathogens in shelter dogs from the Metropolitan Area of Bucaramanga

Organisms	Target DNA	Primer name	Oligonucleotide sequence (5'→3')	pb	Tm °C	Reference
<i>E. canis</i>	dsb	dsb-330	GATGATGTCTGAAGATATGAA	409	54,4	[40]
		dsb-728	CTGCTCGTCTATTTTACTTCT		55,5	
<i>Babesia</i> spp	18s	Pio-A	AATACCCAATCCTGACACAGGG	400–450	62,4	[41]
		Piro-B	TAAATACGAATGCCCCAAC		57,6	
<i>H. canis</i>	18s	HepF	ATACATGAGCAAAATCTCAAC	666	52,21	[43]
		HepR	CTTATTATTCCATGCTGCAG		52,09	
<i>A. platys</i>	groESL	PLA-HS475F	AAGGCGAAAGAAGCAGTCTTA	700	58	[44]
		PLAT-HS1198R	CATAGTCTGAAGTGGAGGAC		48	

To verify PCR specificity, two to three representative amplicons from each target pathogen were randomly selected for sequence confirmation. PCR products were purified and subjected to Sanger sequencing by Macrogen Inc. (Seoul, South Korea). The resulting sequences were assembled and edited using BioEdit, and their identities were confirmed by comparison with reference sequences available in the GenBank database using the BLASTn algorithm.

Statistical Analyses

The frequency of infection for each pathogen was estimated as the percentage of positive animals, together with its corresponding 95% confidence interval (95% CI). The level of agreement between blood smear microscopy and conventional PCR (cPCR) was quantified using Cohen's kappa statistic (κ). Agreement was classified as poor ($\kappa < 0$), slight ($\kappa = 0.00–0.20$), fair ($\kappa = 0.21–0.40$), moderate ($\kappa = 0.41–0.60$), substantial ($\kappa = 0.61–0.80$), or almost perfect ($\kappa = 0.81–1.00$), according to [45].

To compare biochemical and hematological parameters between animals positive and negative for tick-borne pathogens in the molecular analysis, the Levene test was first used to assess variance homogeneity. This result informed the selection of the appropriate T-test. To identify clinical epidemiological variables associated with infection, a bivariate chi-square (χ^2) analysis was performed using 12 independent variables: 5 demographic factors (gender, age, breed, municipality, and dog shelter) and 7 individual factors (body condition, fever, color of the mucous membranes, lymphadenitis, tick infestation, flea infestation, and insecticide or acaricide treatment). For each hemotropic pathogen, the association between explanatory variables and molecular infection status (positive/negative) was first explored using bivariate analyses. Variables with $P < 0.25$ were considered eligible for multivariable analysis according to the approach described by [46]. Multivariable models were fitted using Generalized Estimating Equations (GEE) with a binomial distribution and log link function, specifying an

exchangeable correlation structure and shelter as the clustering variable. Variable selection was performed using a stepwise procedure.

Multivariable GEE models were fitted only for pathogens with frequencies of at least 5%; for pathogens with lower frequencies, only bivariate chi-square analyses were performed. Results from the GEE models were expressed as Prevalence Ratios (PR) with 95% CI. Associations were considered statistically significant at $P < 0.05$.

Results

Study Population

A total of 112 dogs from nine MAB shelters were analyzed, of which 47.3% (53/112), 30.4% (34/112), 13.4% (15/112), and 8.9% (10/112) were from Bucaramanga, Floridablanca, Girón, and Lebrija, respectively (Fig. 1). The study population consisted of 54.5% (61/112) females and 45.5% (51/112) males, with a mean age of 4.48 years (range: 1 month–17 years). Additionally, 83.0% (93/112) were adults (>1 year old) and 79.5% (89/112) were mixed-breed dogs. On clinical examination, flea and tick infestations were observed in 37.5% (42/112) and 16.1% (18/112) of the dogs, respectively. Pale mucous membranes were observed in 17.0% (19/112), while lymphadenitis was detected in 44.6% (50/112). Regarding the use of insecticide or acaricide treatments, 77.7% (87/112) had not received these treatments. The final sample size was slightly lower than the initial target ($n = 130$), primarily because some shelter owners refused to provide blood samples from randomly selected animals.

Blood Smear Examination and Molecular Diagnosis

Of the 112 dogs analyzed by blood smear, 5.35% (6/112; 95% CI: 0.20–11.3%) were positive for tick-borne pathogens. Among these, 3.57% (4/112; 95% CI: 1.0–8.9%) were positive for *Hepatozoon* spp., and 0.89% (1/112; 95% CI: 0.02–4.9%) for *Ehrlichia* spp. and *Babesia* spp., respectively. No *Anaplasma* spp. infections were detected using this technique. In contrast, cPCR detected tick-borne pathogens in 42.85% of samples (48/112; 95% CI: 33.5–52.6%). At the species level, 27.67% (31/112; 95% CI: 19.6–36.9%) were positive for *E. canis*, 8.92% (10/112; 95% CI: 4.4–15.8%) for *H. canis*, 7.14% (8/112; 95% CI: 3.1–13.6%) for *B. vogeli*, and 6.25% (7/112; 95% CI: 2.5–12.5%) for *A. platys*. The κ index between blood smear and cPCR indicated slight agreement ($\kappa = 0.077$; 95% CI: -0.159 to 0.005). At the genus level, *Hepatozoon* showed fair agreement ($\kappa = 0.247$; 95% CI: -0.159 to 0.005), similar to that of *Babesia* ($\kappa = 0.210$; 95% CI: -0.138 to 0.557). In contrast, *Ehrlichia* displayed only slight agreement ($\kappa = 0.046$; 95% CI: -0.042 to 0.134) (Table 2). Four distinct coinfection patterns were identified by cPCR in 7.14% of the animals (8/112; 95% CI: 3.1–13.6%). Coinfections with *E. canis* + *B. vogeli* and *E. canis* + *H. canis* were each detected in 2.67% (3/112; 95% CI: 0.6–7.6%) of the dogs, respectively. Additionally, *B. vogeli* + *H. canis* and *E. canis* + *A. platys* were each identified in 0.89% (1/112; 95% CI: 0.02–4.9%) of the animals, respectively (Fig. 2).

Table 2: Agreement between conventional PCR (cPCR) and Peripheral Blood Smear (PBS) for the detection of tick-borne pathogens in shelter dogs from the Metropolitan Area of Bucaramanga

Test	n observed agreements	Cohen's kappa coefficient (κ)	CI 95%
cPCR <i>E. canis</i> vs PBS <i>Ehrlichia</i> spp.	82 (73.21%)	0.046	-0.042 - 0.134
cPCR <i>B. vogeli</i> vs PBS <i>Babesia</i> spp.	105 (93.75%)	0.210	-0.138 - 0.557
cPCR <i>H. canis</i> vs PBS <i>Hepatozoon</i> spp.	102 (91.07%)	0.247	-0.065 - 0.560
cPCR Vs PBS	44 (39.29%)	0.077	-0.159 - 0.005

Sequencing analysis revealed high nucleotide identity with reference sequences. The *Ehrlichia* isolates obtained in this study (GenBank accession number PX983997) showed 100% identity with an *E. canis* isolate from Santa Marta, Colombia (MK783025.1). The *Hepatozoon* isolate (PX980412) showed 99.5% identity with an *H. canis* isolate from Italy (GU376453.1). Similarly, the *Babesia* isolate (PX986054) exhibited 99% identity with a *B. vogeli* isolate from Mexico (MG430179.1), while the *Anaplasma* isolate (PX987263) showed 100% identity with an *A. platys* isolate from Argentina (KC525895.1).

Hematological and Biochemical Alterations Associated With the Infection

The evaluation of hematological and biochemical parameters between positive and negative animals for tick-borne pathogens revealed significant differences for *E. canis*, *A. platys*, and coinfecting animals ($P < 0.05$). In dogs positive for *E.*

canis, four parameters were significantly altered. Platelet counts and MCH values were significantly lower in animals positive for *E. canis* compared to the negative group, whereas monocyte percentage and basophil percentage were significantly higher ($P<0.05$) in positive animals. In *A. platys*, two parameters (basophil count and basophil percentage) were significantly higher in the positive group compared to the negative ones ($P<0.05$). Coinfected animals exhibited alterations in six parameters. RBC count, HCT, Hb concentration, and platelet count were significantly lower in coinfecting dogs, while monocyte percentage and basophil count were significantly higher in coinfecting animals compared with negative ones ($P<0.05$) (Table 3). No significant differences were detected in the hematological or biochemical parameters of dogs positive for *H. canis* or *B. vogeli*.

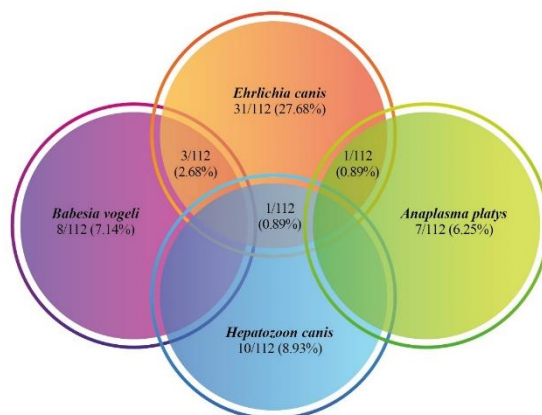


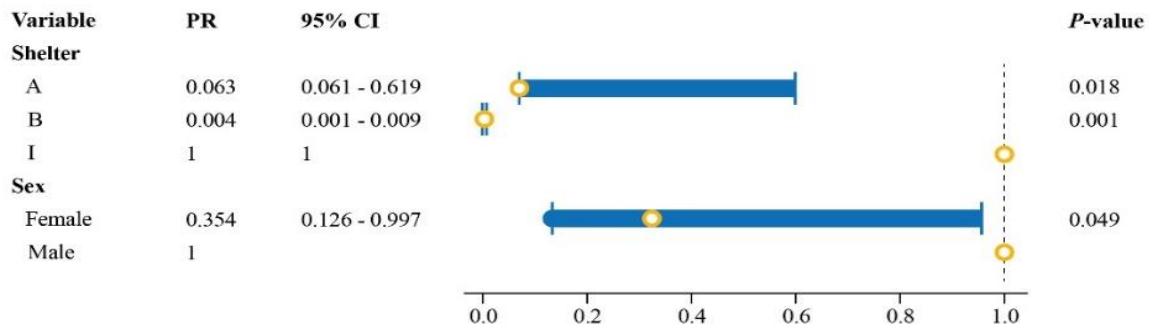
Fig. 2: Venn diagram showing the frequency of infections by tick-borne pathogens of the genera *Anaplasma platys*, *Babesia vogeli*, *Ehrlichia canis*, and *Hepatozoon canis* in shelter dogs from the Metropolitan Area of Bucaramanga

Table 3: Comparison of hematological and biochemical parameters between cPCR-negative and cPCR-positive dogs for *E. canis*, *H. canis*, *B. vogeli*, *A. platys*, and coinfections, during the surveillance in shelters dogs from the Metropolitan Area of Bucaramanga

Parameter	Unit	Normal values*	Negative cPCR N = 64 Median $\pm$ SD	<i>E. canis</i> cPCR+ N = 31 Median $\pm$ SD	p<0,05	<i>H. canis</i> cPCR+ N = 10 Median $\pm$ SD	p<0,05	<i>B. vogeli</i> cPCR+ N = 8 Median $\pm$ SD	p<0,05	<i>A. platys</i> cPCR+ N = 7 Median $\pm$ SD	p<0,05	Coinfect ed N = 8 Median $\pm$ SD	p<0,05
RBC	$\times 10^{12}/L$	5,0-10,0	4.66 $\pm$ 1.22	4.38 $\pm$ 0.93	0.376	4.83 $\pm$ 1.01	0.584	4.48 $\pm$ 0.72	0.810	4.41 $\pm$ 0.88	0.715	3.95 $\pm$ 0.65	0.039*
HCT	$\times 10^{-2} L/l$	35-57	31.51 $\pm$ 8.07	28.90 $\pm$ 6.34	0.159	32.40 $\pm$ 6.17	0.666	29.50 $\pm$ 4.82	0.600	30.06 $\pm$ 5.91	0.746	26.56 $\pm$ 4.53	0.032*
Hb	$\times 10 g/l$	9,8-15,4	11.94 $\pm$ 3.32	10.79 $\pm$ 2.34	0.103	12.40 $\pm$ 2.83	0.607	11.46 $\pm$ 2.35	0.796	11.04 $\pm$ 2.02	0.582	10.07 $\pm$ 1.67	0.033*
MCV	fL	66-77	67.76 $\pm$ 5.66	65.33 $\pm$ 6.61	0.058	67.29 $\pm$ 2.59	0.738	65.84 $\pm$ 2.57	0.332	68.36 $\pm$ 5.64	0.860	67.17 $\pm$ 1.88	0.453
MCH	pg	21-26,2	25.68 $\pm$ 2.32	24.66 $\pm$ 2.03	0.035*	25.64 $\pm$ 1.35	0.902	25.56 $\pm$ 2.23	0.846	25.17 $\pm$ 1.69	0.550	25.52 $\pm$ 1.04	0.808
MCHC	g/dL	32-36,3	37.97 $\pm$ 2.70	37.65 $\pm$ 1.48	0.169	38.16 $\pm$ 2.57	0.822	38.74 $\pm$ 2.11	0.428	36.93 $\pm$ 2.60	0.335	37.98 $\pm$ 1.13	0.953
PLT	$\times 10^9/L$	211-621	204.81 $\pm$ 104.39	136.60 $\pm$ 108.50	0.002*	165.10 $\pm$ 109.65	0.203	168.00 $\pm$ 82.49	0.267	149.57 $\pm$ 77.41	0.141	133.75 $\pm$ 107.7	0.047*
WBC	$\times 10^9/L$	5-14,1	12.44 $\pm$ 5.25	12.37 $\pm$ 4.61	0.929	13.67 $\pm$ 4.15	0.526	11.17 $\pm$ 2.92	0.526	13.29 $\pm$ 4.41	0.714	13.73 $\pm$ 5.06	0.553
Neu%	%	58-85	58.93 $\pm$ 13.85	54.53 $\pm$ 15.49	0.083	53.53 $\pm$ 16.32	0.181	52.58 $\pm$ 12.67	0.152	57.39 $\pm$ 16.01	0.635	53.62 $\pm$ 18.57	0.238
Lym%	%	8-21	22.16 $\pm$ 13.30	24.68 $\pm$ 15.14	0.373	27.25 $\pm$ 16.09	0.269	29.20 $\pm$ 12.32	0.150	17.21 $\pm$ 7.02	0.392	28.76 $\pm$ 18.23	0.207
Mon%	%	2-10	9.36 $\pm$ 4.81	11.67 $\pm$ 4.70	0.009*	10.37 $\pm$ 4.31	0.375	10.74 $\pm$ 4.87	0.312	13.14 $\pm$ 8.09	0.231	12.72 $\pm$ 4.54	0.029*
Eos%	%	0-9	9.29 $\pm$ 6.66	7.90 $\pm$ 6.98	0.520	8.16 $\pm$ 7.04	0.768	7.16 $\pm$ 4.77	0.488	11.70 $\pm$ 7.71	0.296	4.53 $\pm$ 4.22	0.079

Bas%	%	0-1	0.26±0.32	0.36±0.25	0.03 0*	0.28±0.10	0.59 3	0.33±0.25	0.39 3	0.56±0.36	0.01 0*	0.35±0.1 4	0.27 3
# Neu	$\times 10^9/L$	2,9- 12	7.14±3.86	6.42±3.01	0.32 9	7.13±3.13	0.93 8	5.67±1.21	0.29 0	7.97±4.05	0.65 8	7.13±3.7 4	0.94 7
# Lym	$\times 10^9/L$	0,4- 2,9	2.92±3.10	3.32±2.91	0.58 3	3.87±3.36	0.42 3	3.45±2.33	0.67 9	2.05±0.43	0.49 5	4.05±3.7 7	0.38 7
# Mon	$\times 10^9/L$	0,1- 1,4	1.13±0.83	1.36±0.64	0.11 5	1.43±0.79	0.23 6	1.19±0.71	0.76 7	1.77±1.12	0.05 6	1.78±1.0 1	0.09 9
# Eos	$\times 10^9/L$	0-1,3	1.11±0.93	1.07±1.20	0.88 4	1.21±1.06	0.62 5	0.83±0.63	0.53 5	1.44±0.95	0.30 0	0.70±0.9 0	0.34 0
# Bas	$\times 10^9/L$	0- 0,14	0.03±0.03	0.07±0.18	0.12 1	0.04±0.02	0.06 2	0.03±0.02	0.31 7	0.07±0.05	0.00 0*	0.04±0.0 2	0.00 7*
SGPT	U/l	13- 15	74.74±121 .69	64.08±58. 55	0.33 2	55.87±40. 47	0.52 5	43.33±20. 72	0.40 6	41.20±17. 92	0.41 3	60.67±33 .00	0.63 7
CPK	U/l	52- 368	302.45±26 7.28	329.25±20 5.43	0.90 3	293.56±13 4.31	0.84 5	299.38±31 2.75	0.89 3	253.45±14 7.38	0.39 5	354±290	0.83 0

A.



B.

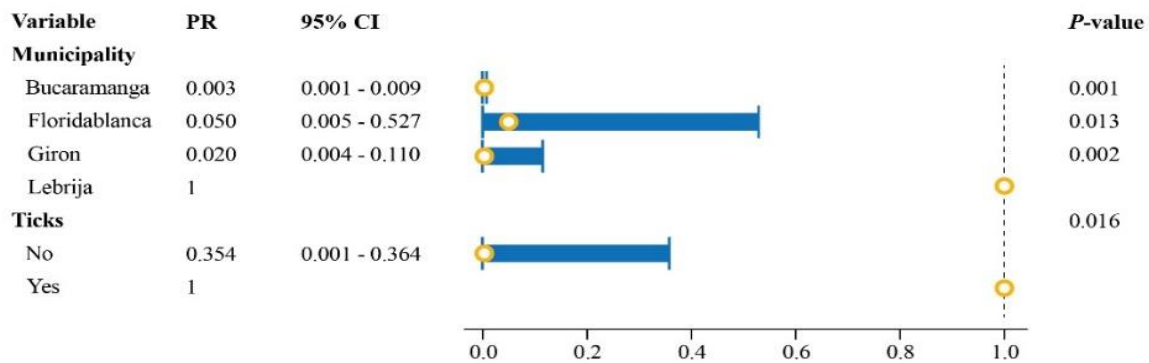


Fig. 3: Forest plot of the Prevalence Ratios (PR) obtained from the multivariate GEE model for infections with *E. canis* (A) and *B. vogeli* (B) in dogs from the Metropolitan Area of Bucaramanga

Epidemiological Factors Associated With the Infection

The bivariate analysis of epidemiological variables associated with the frequency of tick-borne pathogens showed that municipality, shelter of origin, and tick infestation were significantly associated with *E. canis* frequency ($P<0.05$) (Table 4).

However, in the multivariable model, only shelter origin and sex remained significantly associated with this infection. The model indicated that dogs housed in shelters A and B had a lower PR than those observed in shelter I. Additionally, females exhibited a lower PR compared with males ($P<0.05$) (Fig. 3). For *B. vogeli*, the bivariate analysis revealed that municipality, shelter of origin, and flea infestation were significantly associated with infection ($P<0.05$) (Table 4). However, in the multivariable analysis, only municipality and tick infestation remained significantly associated with *B. vogeli* infection ($P<0.05$). The model indicated that dogs from Bucaramanga, Floridablanca, and Giron had a lower PR than those observed in Lebrija. Additionally, dogs without tick infestation exhibited a lower PR than those infested ($P<0.05$) (Fig. 3). No significant epidemiological associations were identified for *H. canis* and *A. platys*.

Table 4: Bivariate analysis of epidemiological variables associated with the frequency of tick-borne pathogens in shelter dogs from the Metropolitan Area of Bucaramanga

Variable	N° of samples	<i>Ehrlichia canis</i>				<i>Hepatozoon canis</i>				<i>Babesia vogeli</i>				<i>Aanaplasma platys</i>			
		N° positive	Prevalence %	χ^2	p-value	N° positive	Prevalence %	χ^2	p-value	N° positive	Prevalence %	χ^2	p-value	N° positive	Prevalence %	χ^2	p-value
Gender																	
Male	51	19	37.25	3.456	0.063	4	7.84	0.001	0.972	3	5.88	0.011	0.916	3	5.88	0.000	1.000
Female	61	12	19.67			6	9.84			5	8.20			4	6.56		
Age																	
Young (<1 year old)	19	5	26.32	0.000	1.000	2	10.53	0.000	1.000	0	0.00	0.702	0.402	2	10.53	0.106	0.745
Adult (≥ 1 year old)	93	26	27.96			8	8.60			8	8.60			5	5.38		
Breed																	
Pedigree dogs	23	5	21.74	0.205	0.651	1	4.35	0.206	0.649	0	0.00	2.138	0.144	0	0.00	0.821	0.365
Mixed-breed dogs	89	26	29.21			9	10.11			8	8.99			7	7.53		
Body condition																	
Normal (>3/5)	64	17	26.56	0.008	0.928	5	7.81	0.020	0.886	6	9.37	0.474	0.491	3	4.68	0.217	0.642
Abnormal (1-3/5)	48	14	29.16			5	10.41			2	4.16			4	8.33		
Fever																	
No	87	23	26.43	0.168	0.682	8	9.17	0.000	1.000	5	5.74	0.472	0.492	6	6.89	0.000	0.898
Yes	24	8	33.33			2	8.33			3	12.5			1	4.16		
Lymphadenitis																	
No	62	14	22.5	1.278	0.258	5	8.06	0.000	0.986	5	8.06	0.002	0.958	6	9.67	1.628	0.202
Yes	50	17	34.0			5	10.0			3	6.0			1	2.0		
Municipality																	
Bucaramanga	53	9	16.98	20.26	1.5 × 10 ^{-5*}	3	5.66	2.200	0.532	0	0.00	20.504	0.001*	3	5.66	1.990	0.574
Floridablanca	34	10	29.41			5	14.71			3	8.82			2	5.88		
Giron	15	11	73.33			1	6.67			1	6.67			2	13.33		

Lebrija	10	1	10.00			1	10.00			4	40.00			0	0.00		
Dog shelter																	
A	13	1	7.69			0	0.00			0	0.00			0	0.00		
B	10	0	0.00			1	10.00			0	0.00			0	0.00		
C	10	1	10.00			1	10.00			4	40.00			0	0.00		
D	11	3	27.27	34	3.8	3	27.27	6.	0.	1	9.09	22.	0.0	0	0.00	8.	0.
E	15	11	73.33	.1	10	1	6.67	38	60	1	6.67	52	04	2	13.33	48	38
F	15	6	40.00	4	-5*	1	6.67	8	4	0	0.00	3	*	0	0.00	5	8
G	8	1	12.50			1	12.50			0	0.00			1	12.50		
H	17	1	5.88			1	5.88			0	0.00			2	11.76		
I	13	7	53.85			1	7.69			2	15.38			2	15.38		
Ticks																	
Yes	18	9	36.00	4.	0.0	2	8.00	0.	1.	3	12.00	1.4	0.2	2	8.00	0.	1.
No	94	22	25.29	09	43*	8	9.20	00	00	5	5.75	72	25	5	5.75	00	00
Fleas																	
Yes	42	15	60.00	1.	0.2	4	16.00	0.	1.	7	28.00	7.0	0.0	4	16.00	0.	0.
No	70	16	18.39	57	09	6	6.90	0	0	1	1.15	36	*	3	3.45	49	48
																8	0

Discussion

Tick-borne diseases are widely distributed in tropical and subtropical regions, where favorable environmental conditions sustain tick populations and increase the risk of pathogen transmission to animal hosts [47]. In animal shelters, the epidemiology of these infections becomes especially complex due to overcrowding [25], poor sanitation, limited access to preventive veterinary care, and the presence of animals with heterogeneous immunological and health statuses [12, 48, 49]. This study found a high frequency of tick-borne pathogens in shelter dogs from the MAB, along with a significant rate of coinfections. Notably, coinfections were associated with more pronounced hematological alterations than single infections, emphasizing their clinical importance. These findings underscore the impact of tick-borne pathogens on the health of shelter dogs in the MAB and reinforce the need for vector control strategies and systematic surveillance to improve treatment and prevention protocols.

In this study, *E. canis* was identified as the most common pathogen, consistent with earlier findings from tropical regions. [50, 51]. These findings reinforce the role of *E. canis* as a major health threat to canine populations in areas where *R. sanguineus* s.l is endemic, especially in shelters where conditions favor tick infestation and pathogen transmission [18, 30, 51]. Notably, the molecular frequency of *E. canis* in our study was higher than that recently reported in domiciled dogs from the MAB (13%) using the same methods [30]. It was also markedly higher than frequencies reported in shelter dogs from Medellín (2.2%) and in dogs attending veterinary hospitals in that city (18%) [18, 50]. Furthermore, our frequency exceeded the 15.3% reported in dogs from northern Colombia [53]. These comparisons reveal a marked difference between MAB shelter and national frequencies, suggesting that the MAB shelters may represent a hyperendemic focus for *E. canis*. This hyperendemicity likely results from the convergence of multiple shelter-related risk factors: high dog density, warm and humid environmental conditions that favor vector proliferation, heavy tick burdens, irregular access to acaricides, and limited veterinary oversight [1, 6, 54, 55].

Regarding *H. canis*, the molecular frequency found in this study was higher than previous reports from Colombia, including 7.1% on the Caribbean coast and 4.5% in Ibagué. However, it was lower than the frequencies reported in the Brazilian Amazon (66.5%) and in anemic dogs from Paraíba, Brazil (67.3%) [33, 34, 51, 56]. The relatively high frequency of *H. canis* in MAB shelters may be explained by its oral transmission route through the ingestion of infected ticks or paratenic hosts, which is likely favored in overcrowded shelter environments [57]. In contrast, the higher frequencies reported in Brazil may reflect differences in eco-epidemiological conditions or greater vector competence of ticks in those regions [23, 58, 59]. For *B. vogeli* (7.1%), the frequency found in this study was within the range reported in owned and stray dogs from Brazil, Colombia, and Peru (1.4%–10.9%) [60, 61] and higher than that reported in domiciled dogs from the MAB (1.09%) and Argentina (0.02%)

[30, 62]. However, it was lower than the 45.3% reported in anemic dogs from Brazil [56]. These findings suggest that the frequency of *B. vogeli* may be more closely related to the dogs' clinical condition than to shelter housing itself, although this still requires further evaluation.

For *A. platys*, the frequency observed here was lower than the frequencies previously reported in northern Colombia [36, 63] and substantially lower than the frequency observed in stray dogs from Brazil (21.5–32.9%) [53, 58, 60, 64]. Several non-mutually exclusive factors may explain these differences, including variations in the ecological vector competence of local *R. sanguineus* populations [65], possible ecological inhibition associated with the high prevalence of *E. canis* (27.6%) [17], the intermittent and short-lived parasitemia of *A. platys*, which may reduce PCR detection in cross-sectional studies [66], and differences in prior acaricide use among shelters [67]. Despite sharing the same vector and host population, these pathogens exhibited markedly different frequencies, suggesting important epidemiological differences. Further studies, including vector competence assays and longitudinal sampling, are needed to better understand the mechanisms underlying these patterns.

The coinfection rate in our study was lower than those reported in Brazil and Peru [58, 68]. In Brazil, dogs from the Caatinga biome showed a coinfection rate of 38.5%, with 19.2% infected by three pathogens and 5.8% by four [58]. Other studies from Belém and Paraíba reported coinfection rates of 13.4% and 16.8%, respectively, in dogs treated at veterinary hospitals [56, 63]. In Peru, 55% of clinically symptomatic dogs were coinfecting [69]. The lower coinfection frequency observed in MAB shelters may be partly explained by differences in vector capacity to transmit multiple pathogens and by ecological interactions among them. However, because our random sampling did not focus on clinically symptomatic animals, higher coinfection rates may have been underestimated.

The predominance of *E. canis* in coinfection patterns is consistent with regional reports and may be related to its high prevalence and shared transmission cycles [70–72]. Moreover, experimental canine ehrlichiosis has shown that *E. canis* induces lymphocyte apoptosis and suppresses Th1 responses [73], creating a permissive environment for concurrent pathogens [16, 57]. The clinical relevance of these coinfections is supported by our hematological findings. Dogs infected only with *E. canis* showed thrombocytopenia, reduced MCH, and increased monocyte and basophil counts. In contrast, coinfecting dogs exhibited more pronounced alterations, including lower red blood cell, hematocrit, hemoglobin, and platelet counts. Clinically, moderate thrombocytopenia can increase the risk of bleeding, while anemia may lead to weakness and lethargy, affecting animal welfare and reducing adoptability, especially in shelters with limited veterinary care. [18, 72, 74]. These results suggest that while *E. canis* plays an important epidemiological role, coinfections are associated with greater hematological impairment, affecting erythroid and platelet parameters, and underscore the need for continuous surveillance and appropriate therapeutic management [54, 75].

The frequency of *E. canis* infection in MAB shelters was associated with epidemiological factors. Females had a lower PR than males, unlike previous studies that reported a higher risk in females [76]. This difference could be related to lower sterilization rates in males, as sterilization campaigns in the MAB usually focus on females. Expanding sterilization programs to males could reduce contact with infected animals during mating and potentially decrease transmission [29, 75]. The shelter of origin was also associated with *E. canis* infection. Shelter I, which had no chemical vector control for three months, dirt floors, high animal density, and no isolation of sick animals, showed a higher PR, highlighting the need for improved sanitary measures and training for shelter owners. For *B. vogeli*, municipality was significantly associated with infection. Dogs from Lebrija had a higher PR than those from Bucaramanga, Floridablanca, and Girón, possibly due to differences in microclimate, vector density, and local control practices. Tick infestation was also associated with *B. vogeli* infection, supporting the role of *R. sanguineus* s.l. as the main vector in Colombia [9, 10]. No significant associations were detected for *H. canis* or *A. platys*, suggesting these pathogens may be widely distributed without clear clustering. However, the low number of positive cases for these agents limited statistical power, and these non-significant findings should be interpreted with caution.

Some tick-borne pathogens detected in this study, such as *E. canis* and *A. platys*, have zoonotic potential, with human cases reported in neighboring countries such as Venezuela [77–79]. Although human cases have not been formally documented in Colombia, shelter owners and workers may be at risk of exposure [67, 80]. Within a One Health approach that emphasizes the links between animal, human, and environmental health, community strategies in the MAB should focus on managing stray dog populations through sterilization efforts, encouraging responsible pet ownership with regular veterinary attention, and using long-lasting chemical treatments for ectoparasite prevention. Additionally, government agencies should support surveillance of tick-borne diseases and other zoonotic infections among shelter staff. These actions could help lower the risk of zoonotic transmission both in shelters and neighboring communities [25, 77].

Conclusion

This study provides molecular evidence of the circulation of multiple tick-borne pathogens among shelter dogs in the MAB, with *E. canis* identified as the predominant species, followed by *H. canis*, *B. vogeli*, and *A. platys*. Infection frequencies exceeded those reported in most previous molecular studies conducted in Colombia, highlighting the epidemiological importance of shelter dog populations. Coinfected animals exhibited more pronounced hematological alterations, particularly reduced erythrocyte, hematocrit, hemoglobin, and platelet values, suggesting a greater impact on hematological health than single infections. To our knowledge, this is among the first molecular surveys to simultaneously investigate multiple canine tick-borne pathogens in shelter dogs from northeastern Colombia. These findings support the implementation of integrated tick control strategies, routine molecular surveillance, and population management programs in animal shelters. Nonetheless, since the study covered only nine shelters in the MAB, the findings may not fully represent other shelter populations.

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Author's Contributions

Maria Victoria Esteban-Mendoza: Conceptualization, methodology, investigation, formal analysis, write original draft, writer review and edited.

Ian Sebastián Murcia-Cueto, Sirly Catherine Esteban-Villegas, Susan Sofía Cabeza-Paredes and Iván Santiago Delgado-Fuentes: Investigation, validation.

Angela Patricia Jiménez: Methodology, Investigation, formal analysis, reviewing and edited.

Jeiczon Jaimes-Dueñez: Conceptualization, Investigation, project administration, writer review nad edited.

Ethics

All procedures involving animals were reviewed and approved by the Ethics Committee of Universidad Cooperativa de Colombia (Approval No. 007–2021; Act No. 001 of 2021) and were conducted in accordance with Colombian legislation governing the care and use of animals in research (Law 84 of 1989 and Law 576 of 2000). The study was classified as research involving minimal risk.

Conflict of Interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the conduct or reporting of this study.

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