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Babesiosis prevalence in malaria-endemic regions of Colombia

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ABSTRACT

Background & objectives: The presence of *Babesia* spp in humans, bovine cattle and ticks (the transmitting vector) has not been well characterized in Colombia. Babesia infection in humans can be overlooked due to similarity of the disease symptoms with malaria specially in the regions where malaria is endemic. The aim of the present work was to study the frequency of *Babesia* infection in humans, bovines and ticks in a malaria endemic region of Colombia, and explore the possible relationship of infection with host and the environmental factors.

Methods: A cross-sectional study was carried out between August 2014 and March 2015 to determine the frequency of *B. bovis* and *B. bigemina* infection in a sample of 300 humans involved in cattle raising, in 202 bovines; and in 515 ticks obtained from these subjects, using molecular (PCR), microscopic and serological methods. In addition, the demographic, ecological and zootechnical factors associated with the presence of *Babesia*, were explored.

Results: In the bovine population, the prevalence of infection was 14.4% (29/202); the highest risk of infection was found in cattle under nine months of age (OR = 23.9, CI 8.10–94.30, $p = 0.0$). In humans, a prevalence of 2% (6/300) was found; four of these six cases were positive for *B. bovis*. Self-report of fever in the last seven days in the positive cases was found to be associated with *Babesia* infection (Incidence rate ratio = 9.08; CI 1.34–61.10, $p = 0.02$). The frequency of *B. bigemina* infection in the collected ticks was 18.5% (30/162).

Interpretation & conclusion: The study established the presence of *Babesia* spp in humans, bovines and ticks. The most prevalent species responsible for babesiosis in humans and bovines was *B. bovis*, while *B. bigemina* was the species most frequently found in the tick population. The results contribute to the knowledge of the epidemiology of babesiosis in the country and can provide guidelines for the epidemiological surveillance of this non-malarial febrile illness in humans as well as cattle.

Key words *Babesia bigemina*; *B. bovis*; babesiosis; bovine; Colombia; human; tick

INTRODUCTION

Babesiosis is a parasitic disease caused by a group of *Babesia* species that parasitize various hosts such as bovine cattle, buffaloes and other animal species; and are considered zoonotic[1–2]. The infected tick bite is the main route of transmission of the *Babesia*[3]. Epidemiological studies in humans and cattle have used different diagnostic methods, including PCR and microscopy for identification of parasites, and indirect immunofluorescence assay (IFA) and ELISA for seroprevalence[4–5].

In tropical countries bovine babesiosis is highly prevalent and has a high economic impact; the main causative agents reported include *Babesia bovis* and *B. bigemina*. In Latin America, several studies have reported about the presence of bovine *Babesia:* (i) in Northern Brazil the prevalence of infection detected by PCR was 33.2% for *B. bovis* and 52% for *B. bigemina*; (ii) in Southern Brazil, the seroprevalence for *B. bovis* was 96.1% by PCR, whereas it was 68.8 and 52.5% by IFA for *B. bovis* and *B. bigemina* respectively[6–7]; (iii) in Mexico, an ELISA seroprevalence of 36% for *B. bovis* and 45% for *B. bigemina* was estimated[8]; and (iv) in Colombia, in the region of Valley of the Magdalena River, the frequency of infection was 22.4% by microscopy, while by PCR it was 63.3% (59.9% by *B. bigemina* and 3.4% by mixed infection). Seroprevalence by IFA was 65.6% (57, 1% by *B. bovis* and 25.9% by *B. bigemina*)[9–10].

Human babesiosis is an emerging tick-borne infectious disease having worldwide distribution. In most cases, it is associated with the population that works on cattle ranches or in moist wooded areas, where the vector is generally observed. The cases are commonly reported in Europe and North America, where the main causal organisms are *B. microti*, *B. bovis*, *B. divergens* and *B. bigemina*[11–12]. This disease causes an acute febrile syndrome like malaria and can be misdiagnosed due to morphological similarities of *Babesia* with *Plasmodium* parasite[13]. In Colombia two studies have described babesiosis in humans. The study carried out in the Magda-

lena Medio region reported 0.5% samples positive by microscopy and 3.6% seropositive by IFA for *B. bovis* or *B. bigemina*[14]; while the another study from the Department of Cordoba reported 30.6% positivity by IFA for *B. microti*[15].

The prevalence of bovine babesiosis in other regions of Colombia is not known, because this disease does not require mandatory notification to the health authorities[9–10]. Also the prevalence of infection is unknown in people living in malaria-endemic areas (where livestock farming is an important economic activity), due to its clinical and parasitological similarity with malaria that may confuse the diagnosis and ignore its existence. Undiagnosed cases or misdiagnosis might lead to serious consequences for the patient. This prevents the epidemiological surveillance of babesiosis and generate gaps in the epidemiological characterization of this infection. The objective of this study was to characterize and establish the magnitude of the *Babesia* infection in bovine cattle and humans, and to identify the presence of *B. bovis* and *B. bigemina* species in ticks, in two towns where farmers practice bovine ranching and which are endemic for malaria in the Urabá-Colombia region.

MATERIAL & METHODS

Design and study site

A cross-sectional descriptive study was carried out between February 2014 and March 2015 in two Urabá towns: Turbo (8° 05' 42" N; 76° 44' 23" W) and Necoclí (8° 25' 39" N; 76° 46' 58" W) (Fig. 1). In both towns, cattle ranching represents the second most important economy activity[16]; Turbo predominates in dual-purpose of rearing livestock, *i.e.* for milk and meat production (92%), while in Necoclí it is aimed at meat production (63%). It has been estimated that these municipalities have an average risk of malaria transmission with annual parasite rates of 2.7 and 2.8 per 1000 inhabitants for Turbo and Necoclí, respectively; without any report of human or bovine babesiosis[17].

Sample size

Sample size (n) for bovine and human populations was estimated according to Lwanga *et al*[18] and using the Epidat program (version 4.1), on the basis of the following data/criteria. For bovines: Total population = 280,767 (records of the Department of Agriculture of the Department of Antioquia in 2014 for both towns[16]), prevalence of babesiosis = 13.65% (the median of frequencies reported in Colombia[9, 19]; and sampling error = 5%. For humans : People related to livestock activity[16] = 2559; prevalence of babesiosis in exposed humans[13] = 30.6%; and sampling error = 5%. The sample size calculated was 202 for bovines and 319 for humans.

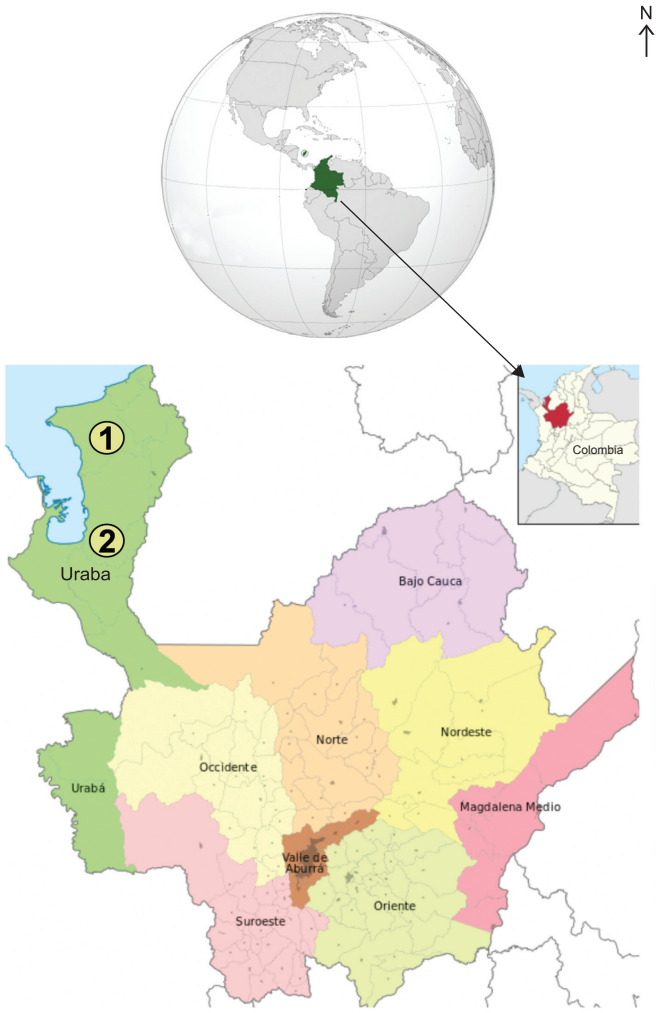

Fig. 1: Location of sampling sites in the Urabá region (green area), Antioquia, Colombia— ① Necocli; ② Turbo.

Sampling strategy and selection of the units for analysis

The selection of the study units (bovines and humans) were made from each productive unit (PU) in total 18 localities from both towns. The PUs were chosen for their homogenous production characteristics (cattle farms) and health status (vaccination against brucellosis and aphtose fever), and for their proximity to the municipal head; in total 379 farms fulfilled these characteristics, 164 in Turbo and 215 in Necoclí. The PUs that had implemented vaccination against *Babesia* and applied tick control insecticides in the last eight days of the visit, were excluded. The selection was made by proportional fixation sampling proposed by Silva *et al*[20] in 1993. Finally, 30 PUs located in 15 locations, eight in Turbo and seven in Necoclí, met the selection criteria (Fig. 1).

Selection of bovine and human subjects

The bovine sample was divided according to their proportion reported in each town: 60% (n = 121) for Turbo and 40% (n = 81) Necoclí. Each sub-sample was distributed among the PUs from each town, and in each PU, the bovines were selected through a list of random numbers.

The selected human subjects were town residents or working in a PU; when necessary, adjacent residents were included to complete the sampling of a PU. The selection of human subjects was based on the following inclusion criteria: Age over 18 yr, willingness to participate and sign the informed consent.

Data collection

Data recorded on a standardized form included information on:

Production units: The productive and sanitary characteristics investigated were production orientation, type of pasture, use of tick control insecticides, deworming and quarantines.

Cattle: Each animal was investigated for sex, race, age and presence of ticks; the clinical status (signs of infection) was evaluated by a veterinarian. The association between the presence of *Babesia* and factors of the herd, such as zootechnical orientation (dual purpose, meat, milk), pastures and availability of professional veterinarians were also explored.

Humans: Sociodemographic characteristics (sex, age, ethnic group), labour activities (cattle farming, housewife, student), housing conditions (wall, floor, ceiling material), presence of disease symptoms in the last seven days (headache, fever, arthralgia) and presence of clinical signs at the time of the survey (pallor, jaundice, fever and haemorrhages).

Ticks: The number of ticks was determined and registered for each bovine using the technique described by Álvarez *et al*[21] in 2003. From each bovine, 1 to 5 ticks were captured and stored for seven days, guaranteeing the development of the vector parasitic cycle.

Sampling and laboratory analysis

DNA extraction and PCR: For the diagnosis of babesiosis in both, humans and cattle, 5 ml of venous blood was taken; 400 µl were distributed in two tubes with heparin and then stored at –20°C until analyzed for molecular diagnosis. DNA was extracted using the DNeasy Tissue and Blood kit, following the manufacturer's instructions[22]. The primers reported by Figueroa *et al*[23] were used to amplify the *18S* gene by nested PCR, modified by Terkawi *et al*[24]; the PCR products were examined on a 2% TAE agarose gel by electrophoresis at 100 volts for 40 min. The final products were 291 bp for *B. bovis* and 178 bp for *B. bigemina*[23]. Quality control of the results was done with DNA samples sequenced by the Institute of Agricultural Technology of Argentina, INTA, Rafaela.

Microscopic diagnosis: The presence of active infection and morphological identification of the species was performed by peripheral blood smears stained with Giemsa[25] and read under a light microscope with a 100 × objective. A sample was considered negative when no parasites were identified in 300 fields. For quality control, two blind readings were performed on all the positive samples and on 10% of the negative samples.

Serological diagnosis: Bovine and human sera were centrifuged at 2500 rpm. The presence of antibodies was determined by ELISA using a suspension of purified merozoites obtained *in vitro* from *B. bovis* or *B. bigemina*; a bovine IgG1 heavy chain anti-chain monoclonal antibody conjugate (M 23ADRI-Canada) and human IgA multispecies (Pierce Biotechnology, Rockford, IL, USA) samples were also used. The 10% of the samples were analyzed by immunofluorescence (IFA), a technique in which the parasites (*B. bovis* and *B. bigemina*) were first cultured in leukocyte free red blood cells with equine serum, until 5–6% parasitaemia. After thin blood smears preparation, 1/100 *B. bovis* and 1/120 *B. bigemina* sera dilutions were made. Fluorescent reactions were observed with a Leitz microscope equipped for epi-illumination using a 50 W mercury vapour lamp. The ELISA and IFA were performed as described by de Echaide *et al*[26].

Statistical analysis

The analysis was carried out with the statistical program SPSS ver. 23 (IBM Corporation) licensed for the University of Antioquia. Descriptive analysis of quantitative variables was carried out using measures of central tendency (median or average) and dispersion [interquartile range–(IQR) or standard deviation (SD)]. Qualitative variables were analyzed by proportions; a bivariate analysis was performed for the bovine and human populations using as a dependent variable the PCR diagnosis of *Babesia* spp. Categorical variables were analyzed using the Chi-square test and the Fisher's exact test. Infection analysis in cattle was performed by logistic regression using the step-by-step method and infection in humans by Poisson regression. Applying the Hosmer Lemeshow (H–L) criterion ($p \leq 0.25$) the variables entered into the models, and according epidemiological importance and biological plausibility. p-value <0.05 was considered as statistically significant.

Ethical statement

The international ethical standards for biomedical research with human subjects established by the WHO and the ethical norms of the ministry of social protection of Colombia for human research (Resolution 8430 of 1993) and animal research (Law 84 of 1989) were followed. The collection of specimens were carried out in compliance with the regulations established by the Colombian government (National Environmental Licensing Authority Resolution ANLA 0524 of 2014). The procedures were approved by both the Bioethics Committee and the Ethical Committee for Animal Research of the University Research Headquarters of the University of Antioquia (Acts 13-32-436 of 2012 and 15-32-436 of 2015).

RESULTS

Babesia infection was diagnosed in bovine cattle, humans and ticks in five locations, namely El Tres, Alto Mulatos, Turbo, Mulatos, Las Changas, and Totumo) out of the 18 localities visited.

Cattle characteristics and infection status

Among the 202 bovines studied, majority (74.8%) were reared for dual purpose (meat and milk production), which were grazing on native pastures (63.9%) such as *Brachiaria decumbens*. The general characteristics of cattle are described in (Table 1). The majority were females (77.2%) corresponding to cross Cebu (*Bos indicus*); the median age was 48 months (IQR 9–84) with a high proportion of bovines over 48 months (44%). The bovines were mostly asymptomatic (83%) at the time of the study; 34 had a rectal temperature > 38.5°C without other clinical signs (Table 1). The prevalence of *Babesia* established by PCR in cattle was 14.4% (29/202); 19 infections were by *B. bovis* (65.5%), six by *B. bigemina* (20.7%) and four infections were due to both the species (13.8%). The prevalence of infection by microscopy was 4.5% (9/202); 77% for *B. bovis* (n = 7) and 33% for *B. bigemina* (n = 2) (Fig. 2). Antibodies against the *Babesia* species were found in 55.4% (112/202) population (by ELISA); 71.4% (80/112) for *B. bovis* and 73.2% (82/112) for *B. bigemina*.

The age of the bovines was categorized according to the median and the age at greater risk for the presence of *Babesia* (<9 months); the frequency of babesiosis by molecular diagnosis was as follows: 41.5% between 0–9 months, 6.7% between 10–48 months; and 3.4% for animals older than 48 months. The frequencies of serum antibodies for *Babesia* in these groups were 77.4, 55 and 42.7%, respectively.

Table 1. Characteristics of the bovine and human population

Characteristics	Categories	Number	
Bovine variables			
Town	Turbo	76	(37.6)
	Necoclí	126	(62.4)
Zootechnical orientation	Dual purpose	151	(74.8)
	Meat	40	(19.8)
	Milk	11	(5.4)
Pasture type	Native	129	(63.9)
	Other	73	(36.1)
Availability of veterinarians	Yes	177	(87.6)
	No	25	(12.4)
Sex	Male	46	(22.8)
	Female	156	(77.2)
Race	Holstein × Cebu	103	(51)
	Simmental	12	(5.9)
	Gyr	8	(4)
	Holstein	3	(1.5)
Fever	Yes	35	(17.3)
	No	167	(82.7)
Age	<9 months	53	(26.2)
	10–48 months	60	(29.7)
	>48 months	89	(44.1)
Human variables			
Town	Turbo	150	(50)
	Necoclí	150	(50)
Sex	Male	259	(86.3)
	Female	41	(13.7)
Ethnic group	Mestizo	287	(95.7)
	African descendant	9	(3)
	Indigenous	4	(1.3)
Domestic animals in the house	Yes	261	(87)
	No	39	(13)
Primary activity	Cattle farming	247	(82.3)
	Housewife	34	(11.3)
	Student	19	(6.3)
Tick bites	Yes	236	(68.3)
	No	64	(31.7)
Fever	Si	270	(90)
	No	30	(10)
Shaking chills	Yes	276	(92.0)
	No	24	(8)
Headache	Yes	173	(57.7)
	No	127	(42.3)

Figures in parentheses indicate percentages.

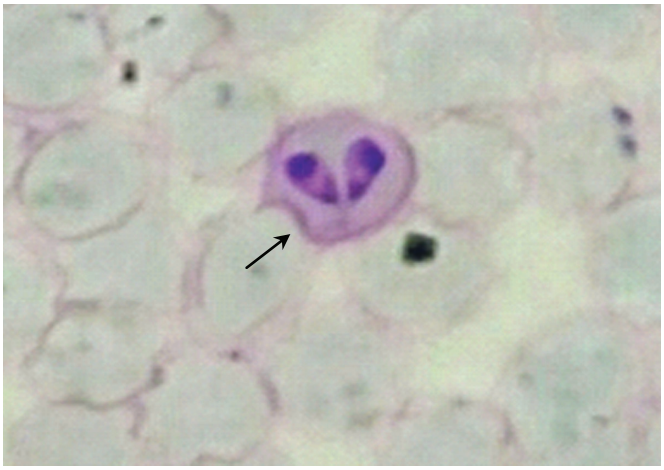

Fig. 2: Babesia bigemina in a Giemsa stained blood smear from a bovine (Urabá, Colombia). Pear-shaped *B. bigemina* inside a red blood cell (arrow).

Human subject characteristics and infection status

The study was carried out in 300 residents. The median age was 35 yr (Range, 25–48); 95.7% were recognized as a mestizo population (people of mixed European and Amerindian ancestry). The houses were characterized by having wooden walls (53.7%), earthen floors (39%) and zinc roofs (62%). Of all the subjects studied 87% had domestic animals in the house. The most common clinical symptoms during the seven days prior to the study were headache (42.3%) and fever (30%); though joint pain and sore throat were also reported. Sociodemographic and clinical data are summarized in Table 1.

The frequency of babesiosis in humans diagnosed by PCR was 2% (6/300); 66.6% (n = 4) for *B. bovis* and 33.3% (n = 2) for *B. bigemina*. By microscopy, *Babesia* spp was diagnosed in three cases (1%), two infections were due to *B. bovis* and one was due to *B. bigemina*. The agreement between both tests was 50%, with a Kappa index = 0.6. Seroprevalence in humans was 0.33% (1/300) with antibody titres in one subject for both species. Two positive subjects for *Babesia* presented fever and headache, one presented only headache and the other three were asymptomatic. The frequency of these symptoms does not differ with the subjects without infection (p >0.05, chi-square test).

Vector characteristics and infection status

Seventy percent (141 out of 202) of the bovines studied were parasitized by ticks, from which 515 specimens were collected and then divided into 162 sets. These sets were classified according to species, stage and sex. The frequency of *Babesia* infection in the tick subsets was 18.5% (30/162); 73.3% due to *B. bigemina* infection (22/30), 16.7% due to *B. bovis* infection (5/30) and 10% due to infection by both species (3/30).

Association between bovine and human babesiosis

The logistic regression analysis for the bovine population, with a goodness of fit of 0.921, showed that bovines < 9 months of age presented the highest probability of infection by *Babesia* (Table 2). Poisson regression for humans indicates that babesiosis was associated with subjective fever in the last seven days [incidence rate ratio (IRR) = 9.08; CI = 1.34–61.10] with a goodness of fit for the model of 0.780 (Table 3).

DISCUSSION

Since the first case of babesiosis reported in humans in 1957 in Yugoslavia[27], diverse studies have measured the frequency of *Babesia* in humans and cattle. To the best of our knowledge there are no studies investigating the presence of this infectious agent in the population of humans, bovines and vectors simultaneously. This study evaluated the prevalence of *Babesia* in these three populations, in a zone endemic for malaria, and favourable for

Table 2. Bivariate and multivariate analysis of *Babesia* infection and livestock variables

Variables	Crude OR	95% CI	OR	*p*-value	Adjusted* OR	95% CI	OR	**p*-value
Age (Months)								
<9	20.3	5.7	72.5	0.001	24	6.1	94.3	0.001
10–48	2.1	0.4	0.5	0.36	1.8	0.4	8.5	0.48
>48				Ref				Ref
Sex (Female)	0.3	0.1	0.6	0.001	0.9	0.3	2.5	0.845
Town	2.1	0.8	5.1	0.11	3.3	0.8	13.2	0.1
Adm. tick insecticide (spray)	2.6	0.8	9.1	0.13	0.3	0.1	1.2	0.08
Pasture rotation (days)	1	0.9	1	0.14	0.8	0.3	2.4	0.72
Presence of ticks								
Babesia Positive	0.7	0.2	2.5	0.61	0.5	0.1	2.5	0.41
Babesia Negative	0.5	0.2	1.3	0.16	0.5	0.2	1.5	0.21
Without ticks				Ref				Ref

*Adjusted for all other livestock variables; OR: Odds ratio; CI: Confidence interval.

Table 3. Bivariate and multivariate analysis of *Babesia* infection in humans and some individual variables

Variables	Crude IRR	95% CI	IRR	*p*-value	Adjusted IRR*	95% CI	IRR	*p*-value
Fever informed by the participant	4.5	0.8	24.7	0.08	9	1.3	61.1	0.024
Months dedicated to cattle ranching	1	0.9	1	0.3	0.1	0.9	1	0.97
Bovine with ticks (PCR positive for *Babesia* spp)	1.6	0.8	3.2	0.23	2.2	0.8	5.9	0.123
Working in cattle ranches	0.4	0.1	2.3	0.32	0.8	0.1	7	0.79
Bovines PCR positive for *Babesia*	0.7	0.2	2.1	0.54	0.4	0.2	1.9	0.54

*Adjusted for all other variables; IRR: Relative risk index (Hosmer Lemeshow criteria, p <0.25); CI: Confidence interval.

the presence of *Babesia* due to its eco-epidemiological conditions[28].

In the bovine population, this study found a higher frequency of infection for *B. bovis* (79.3%) compared to *B. bigemina* (34.5%), proportions that included coinfections. Although, both are transmitted by the same vectors, this could be explained by the fact that *B. bovis* infection can persist in hosts for 24 months or more compared to *B. bigemina* infection, which persists for 12 months[29]. Although, the frequency of bovine cases in this study is lower than that reported in other studies in Colombia, the species wise proportion coincides with that reported by Ríos *et al*[10, 34], who identified a higher frequency of *B. bovis* (57.1%) than of *B. bigemina* (25.9%). The seroprevalence was higher than the presence of active infection, this can be supported by the fact that a large proportion of the bovines (73%) were from meat breeds that are more resistant to *Babesia* infection indicating high proportion of asymptomatic bovines. This resistance of cattle to clinical signs is an important factor in the maintenance of enzootic stability because it aids sporadic babesiosis outbreaks when new animals enter these areas.

In cattle, a statistically significant association was found between the prevalence of *Babesia* infection by the PCR technique and the presence of antibodies by the ELISA diagnosis, with a higher risk in animals younger than nine months of age compared to adults. This is in agreement with earlier studies that reported greater susceptibility to infection at this age[6–7].

In the human subjects, infection prevalence of the disease was 2% by PCR and 1% by microscopy. Among the six positive subjects, three were positive by both methods and presented fever on the day of diagnosis, suggesting that they were in the acute phase of the disease and thus were potential transmitters of the infection[32]. By the serological technique (IFA), IgG antibodies were observed in one only person out of 300, which may be due to poor prior contact with the parasite or to the variability of these antibodies over time as evidenced by Gumber *et al*[29] who reported that in apes infected with *B. microti*, this immunoglobulin is detected in chronic phase of infection (56 days after contact with the parasite). Although, the frequency of *Babesia* infection in humans by microscopy and PCR was low for the both species studied, it predominated for *B. bovis*, similar to the findings reported by Ríos *et al*[14], who found a seroprevalence of 2.1% for *B. bovis* and 1.5% for *B. bigemina* in a cattle zone endemic for malaria in Colombia.

The exploration of sociodemographic and epidemiological variables in relation to the diagnosis of infection in humans showed no associations. Specifically, the sex and age variables were not related to an increased risk of infection. This is similar to what Hong *et al*[30] reported in their study, suggesting a similar exposure in women and men, though, an analysis was not performed according to their occupation.

The frequency of infestation in tick groups was 18.5%. However, the history of tick bites in people during the last year was not associated with a risk of infection. This situation can be explained by a low frequency of tick infection, or this might be due to memory bias[29].

When analyzing clinical variables in people, fever was observed to be the most persistent and common symptom associated with *Babesia* infection similar to other infections[31–33], and was present in up to 91% of the patients.

Microscopic diagnosis is suitable as a diagnostic alternative, in cases of symptomatic animals and in acute stages of the disease, and is the most common procedure used by veterinarians to screen for possible clinical cases. However, its diagnostic capacity is much lower than the molecular diagnosis in asymptomatic animals[28]. In this study the identification of seropositive animals by microscopy was 4% as opposed to 14.4% by PCR.

No association was found between *Babesia* infection in humans and bovines with the presence of the parasite in ticks. This may be due to the low prevalence of babesiosis in humans and the low parasitaemia in bovines, a condition that decreases the transmission capacity of the parasite[34]. The lower frequency of *B. bovis* in ticks corresponds to the fact that this species does not have vertical transmission, while *B. bigemina* has food, transovarial and vertical transmission[35]. This is in concordance with a study[9] carried out in Puerto Berrío-Antioquia, Colombia that showed a greater presence of *B. bigemina* (79.2%) compared to *B. bovis* (9.4%).

CONCLUSION

The study established the presence of *Babesia* parasite in bovine cattle, humans and its vectors inhabiting a region endemic for malaria in Colombia. The prevalence was low (2%) for *B. bovis* and *B. bigemina* infection in humans; however, the frequency in bovines and ticks were 14.4 and 18.5%, respectively. Since, it is not mandatory to notify Babesiosis in cattle in Colombia, the epidemiology of this disease is not well known, and therefore, it is not suspected as a cause of disease in the human population. The presence of Babesiosis in humans, represents an important problem for diagnosis. The results contribute to the knowledge of the epidemiology of babesiosis in the country and can provide guidelines for the epidemiologi-

cal surveillance of non-malarial febrile illness in people and febrile pathologies in cattle.

Conflict of interest

The authors of the article have no conflict of interests to declare.

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