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 8.10–61.10, 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 zoonotic1–2. The infected tick bite is the main route of transmission of the Babesia1. 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 seroprevalence4–5.

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 respectively6–7; (iii) in Mexico, an ELISA seroprevalence of 36% for B. bovis and 45% for B. bigemina was estimated8; 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. bigemina11–12. This disease causes an acute febrile syndrome like malaria and can be misdiagnosed due to morphological similarities of Babesia with Plasmodium parasite13. In Colombia two studies have described babesiosis in humans. The study carried out in the Magda-
González et al: Babesiosis in Colombia

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

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. 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; 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.

Sample size

Sample size (n) for bovine and human populations was estimated according to Lwanga et al 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), prevalence of babesiosis = 13.65% (the median of frequencies reported in Colombia and sampling error = 5%). For humans: People related to livestock activity = 2559; prevalence of babesiosis in exposed humans = 30.6%; and sampling error = 5%. The sample size calculated was 202 for bovines and 319 for humans.

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. 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. bigemina25. 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 Giemsa25 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 (mean or median) 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 ≤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, 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

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**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     | Ref    | Ref     | Ref          | Ref     | 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     | Ref    | Ref     | Ref          | Ref     | Ref    | Ref     |

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

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**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 conditions28.

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 were 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 months29. 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 Rios et al10, 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 age6–7.

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 infection32. 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 al28 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 Rios et al34, 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 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-

CONCLUSION

The presence of 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 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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**REFERENCES**

1. Adl SM, Simpson AG, Farmer MA, Andersen RA, Anderson OR, Barta JR, et al. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *J Eukaryot Microbiol* 2005; 52(5): 399–451.

2. Gomez JE. Protozoología médica: Protozoos parásitos en el contexto latinoamericano. ed. Bogota: Manual, Moderno 2010; p. 265.

3. Homer MJ, Aguilar-Delfin I, Telford SR, Krause PJ, Persing DH. Babesiosis. *Clin Microbiol Rev* 2000; 13(3): 451–69.

4. Mosqueda, Olvera-Raymírez J, Aguilar-Tipacama A. Current advances in detection and treatment of babesiosis. *Curr Med Chem* 2012; 19(10): 1504–18.

5. Babesiosis bovis. USA: The Center for Food Security and Public Health. Available from: http://www.cfsph.iastate.edu/DiseaseInfo/disease.php?name=bovine-babesiosis&lang=es (Accessed on July 30, 2017).

6. Souza F, Braga J, Pires L, Carvalho C, Costa E, Ribeiro M, et al. Babesiosis and anaplasmosis in dairy cattle in northeastern Brazil. *Pesq Vet Bras* 2013; 33(9): 1057–61.

7. Brito LG, Rocha RB, Barbieri FDS, Ribeiro ES, Vendrami FB, Souza GCR, et al. *Babesia bovis* infection in cattle in the southwestern Brazilian Amazon. *Ticks-Tick Borne Dis* 2013; 4(1–2): 78–82.

8. Lozano ME. Situacion sanitaria de la babesiosis y anaplasmosis en la ganaderia lechera en tres sistemas de produccion (Maestria). Queretaro: Universidad Autonoma de Queretaro, Facultad de Ciencias Naturales 2014; p. 64.

9. Rios S. Evaluación de indicadores de babesiosis bovina en garrafatas Rhipicephalus *Boophilus microplus* and bovinos de 3a9 meses de la zona del Magdalena Medio Colombiano. Colombia Universidad de Antioquia 2010.

10. Rios L, Zapata R, Reyes J, Mejía J. Enzootic stability of bovine babesiosis at Puerto Berrio region, Colombia. *Revista Científica* 2010; 29(5): 485–92.

11. Walsh MG. The relevance of forest fragmentation on the incidence of human babesiosis: Investigating the landscape epidemiology of an emerging tick-borne disease. *Vector Borne Zoonotic Dis* 2013; 13(4): 250–5.

12. Lempereur L, Shiels B, Heyman P, Moreau E, Saegerman C, Losson B, et al. A retrospective serological survey on human babesiosis in Belgium. *Clin Microbiol Infect* 2015; 21(1): 96 e1–7.

13. Montenegro-James S. Prevalence and control of babesiosis in the Americas. *Mem Inst Oswaldo Cruz* 1992; 87(Suppl 3): 27–36.

14. Rios L, Alvarez G, Blair S. Serological and parasitological study and report of the first case of human babesiosis in Colombia Estudio serológico e parasitológico e relato del primero caso de babesiose humana na Colômbia. *Rev Soc Bras Med Trop* 2003; 36(4): 493–8.

15. Buelvas F, Alvis N, Buelvas I, Miranda J. Alta Prevalencia de Anticuerpos contra Bartonella y *Babesia microti* en Poblaciones Rurales y Urbanas en dos Provincias de Córdoba, Colombia. *Rev Salud Publica* 2008; 10(1): 168–77.

16. Población de Bovinos-Antioquia. Medellín: Secretaría de Agricultura de Antioquia, Gobernación de Antioquia 2014.

17. Eventos de salud pública. Mexico: Secretaría de salud 2016. Available from: https://www.dssav.gob.mx/index.php/estadisticas/eventos-en-salud-publica (Accessed on July 30, 2017).

18. Lwanga SK, Lameshov S. Determinación del tamaño muestral en los estudios sanitarios: Manual práctico 2001. Geneva: World Health Organization; p. 80.

19. Herrera M, Soto A, Urrego V, Rivera G, Zapata M, Rios L. Freuenz de hemoparasitos in bovinos del Bajo Cauca y alto San Jorge, 2000–2005. *Rev MVZ Córdoba* 2008; 13(3): 1486–94.

20. Silva L. Muestreo para la investigación en ciencias de la salud. 1 edn. Díaz de Santos, editor. Madrid, Spain: Díaz de Santos 1993; p. 176.

21. Álvarez V, Bonilla R, Chacón I. Frecuencia relativa de *Boophilus microplus* (Acari Ixodidae) in Bovinos (*Bos taurus* y *B. indicus*) in ocho zona ecológicas de Costa Rica. *Rev Biol Trop* 2003; 51(2): 427–34.

22. DNeasy blood and tissue kit. The Netherlands: Quiagen 2013. [Internet]. available from: https://www.qiagen.com/es/shop/sample-technologies/dna/dna-preparation/dneasy-blood-and-tissue-kit/ (Accessed on July 30, 2017).

23. Figueroa JV, Chieves LP, Johnson GS, Bueing GM. Multiplex polymerase chain reaction based assay for the detection of *Babesia bigemina*, *Babesia bovis* and *Anaplasma marginale* DNA in bovine blood. *Vet Parasitol* 1993; 50(1–2): 69–81.

24. Terkawi MA, Alhasan H, Huyen NX, Sabagh A, Awier K, Cao S, et al. A retrospective serological survey on human babesiosis in Belgium. *Vet Parasitol* 2012; 187(1–2): 307–11.

25. Materlab Reactivos y materiales de laboratorio. Madrid. España 2013. Available from: http://www.materlab.com/documentacion/hematologia/colorantes/catalogo_general_tinciones.pdf. (Accessed on July 30, 2017).

26. de Chaide S, Chaide S, Gaido AB, Mangold AJ, Lugaresi CI, Vanzini VR, et al. Evaluation of an enzyme linked immunosorbent assay-kit for the detection of *Babesia bovis*-antibodies in cattle in Argentina. *Prev Vet Med* 1995; 24(4): 277–83.

27. Martinot M, Zadeh MM, Hansmann Y, Grauwy I, Christmann
D, Aguillon S, et al. Babesiosis in immunocompetent patients. Emerg Infect Dis 2011; 17(1): 114–6.
28. Montoya D. Zonas de vida y la valoracion del riesgo para la transmision de la babesiosis bovina en Colombia. [Trabajo de grado optar titulo Microbiologia]. Escuela de Microbiologia Medellin: Universidad de Antioquia 2008.
29. Gumber S, Nascimento FS, Rogers KA, Bishop HS, Rivera HN, Xayavong MV, et al. Experimental transfusion-induced Babesia microti infection: Dynamics of parasitaemia and immune responses in a rhesus macaque model. Transfusion 2016; 56(6 Pt 2): 1508–19.
30. Hong S, Anu D, Jeong Y, Ahmed D, Cho S, Lee W, et al. Molecular detection and seroprevalence of Babesia microti among stock farmers in Khutul City, Selenge Province, Mongolia. Korean J Parasitol 2014; 52(4): 443–7.
31. Babesiosis. Maryland, USA: US Army Public Heal Command 2010; Available from: https://phc.amedd.army.mil/PHC Resource Library/Babesiosis_FS_18-007-1115.pdf (Accessed on July 30, 2017).
32. Vannier E, Gewurz BE, Krause PJ. Human babesiosis. Infect Dis Clin North Am 2008; 22(3): 469–88.
33. Mylonakis E. When to suspect and how to monitor babesiosis? Am Fam Physician 2001; 63(10): 1969–74.
34. Callow, I. The infection of Boophilus microplus with Babesia bigemina. Parasitology 1968; 58(3): 663–70.
35. Melendez R. Revision integral de los factores epidemiológicos que inciden en la relación Boophilus microplus-Bovino-Babesia spp. Rev Cient 1998; 3(1): 25–34.

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