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Original Article

Dynamics of natural infection by *Babesia bovis* and *Babesia bigemina* in dairy cattle from an enzootic instability area in Northeastern Brazil

Dinâmica da infecção natural por *Babesia bovis* e *Babesia bigemina* em bovinos leiteiros de uma área de instabilidade enzoótica no Nordeste do Brasil

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Abstract

This study aimed to determine the dynamics of natural infection in the transmission of *Babesia* spp. to cattle in an enzootic instability area in Northeastern Brazil. Blood samples were collected from 30 calves located on two dairy farms to determine the packed cell volume (PCV) and the timing of the primo-infection using polymerase chain reaction (PCR) and their association with climatic factors and management practices. On Farm A, the determination of primo-infection was observed on average at 249.4 (±24.42) days of age for *B. bigemina* and at 252.6 (±17.07) days of age for *B. bovis*; there was no significant difference between the times of infection (P> 0.05). The infection coincided with a period of high rainfall in the region. On Farm B, primo-infection infection was not observed. There was no infection by *Babesia* spp. on Farm B due to the intensive use of acaricides that led to an absence of ticks. There was no significant difference between the average PCV of animals from Farms A and B (P> 0.05). The management practices on the properties, in addition to the weather conditions influenced the exposure of the animals to disease vectors and may have contributed to the maintenance of this enzootic area in Northeastern Brazil.

Keywords: Tick, epidemiology, babesiosis, enzootic instability, tick-borne disease, polymerase chain reaction (PCR).

Resumo

Este estudo teve como objetivo determinar a dinâmica da infecção natural na transmissão de *Babesia* spp. em bovinos de uma área de instabilidade enzoótica no Nordeste do Brasil. Foram coletadas amostras de sangue de 30 bezerras, proveniente de duas propriedades leiteiras para determinação do volume globular e da primo-infecção por meio da reação em cadeia da polimerase associando aos fatores climáticos e medidas de manejo. Na fazenda A, o período médio da primo-infecção para *B. bigemina*, determinado por meio da PCR, foi de 249,4 (±24,42) dias de idade, enquanto que para *B. bovis* foi aos 252,6 (±17,07) dias de idade, não existindo diferença estatística. A infecção coincidiu com o período de alta precipitação pluviométrica na região. Não houve infecção por *Babesia* spp. na fazenda B, na qual o uso intensivo de acaricidas determinou ausência de carrapatos. Não houve diferença significativa entre médias de VG dos animais das fazendas A e B. O manejo adotado nas fazendas estudadas, associado às condições climáticas, interferem na exposição dos animais aos vetores, podendo favorecer a manutenção de uma área de instabilidade enzoótica no Nordeste do Brasil.

Palavras-chave: Carrapato, epidemiologia, babesiose, instabilidade enzoótica, doenças transmitidas por carrapatos, reação em cadeia da polimerase (PCR).

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Introduction

Bovine babesiosis is caused by *Babesia bovis* and *Babesia bigemina*, which are cattle parasites that exist in almost all Brazilian territories; the *Riphicephalus (Boophilus) microplus* tick is a vector for these parasites (ESTRADA-PENÃ et al., 2006; RÍOS-TOBÓN et al., 2014). In Brazil, the seroprevalence rates of babesiosis vary from 27.9% to 100%, and the epidemiological condition for this disease is related to the climatic conditions and management systems that directly affect the vector cycle (ALVES, 1987; SANTOS et al., 2001; RÍOS-TOBÓN et al., 2014). In this panorama, three different areas are observed: disease-free areas, areas of enzootic stability and areas of enzootic instability (GUIMARÃES et al., 2011). In the latter area, there is a risk of bovine babesiosis outbreaks due to high rates of adult bovine mortality; moreover, bovine babesiosis may occur because many animals in the area have not developed a specific immune response because they were not infected during their first months of life (GOFF et al., 2001; BERTO et al., 2008).

In some situations, enzootic instability may be related to unsatisfactory passive immunity, stress, nutritional status, time of year, management, type of pasture or high infestation rates of pastures and cattle by a vector (BARROS et al., 2005; AMORIM et al., 2014). The primo-infection usually occurs between four and six months of life, with the peak of the parasitemia coinciding with the fall of the packed cell volume; in old age, primo-infection is usually severe and can be fatal (CARRIQUE MAS et al., 2000; SMITH et al., 2000).

In Northeastern Brazil, predominantly endemic areas unstable for babesiosis include cities such as Garanhuns-PE (ALVES, 1987) and Uauá-BA (BARROS et al., 2005); the semi-arid region of Paraíba (COSTA et al., 2013); and the middle-north area of Brazil (SOUZA et al., 2013). In the latter area, babesiosis is certainly one of the main diseases that affect cattle, which may facilitate outbreaks (SOUZA et al., 2013).

Knowledge of the factors that favor enzootic instability is fundamental in developing efficient measures to control babesiosis in Brazil. Thus, the present research aims to determine the dynamics of primo-infection by *B. bovis* and *B. bigemina* and to examine the relationship between primo-infections and climatic factors and management in the cattle residing in an enzootic instability area in Northeastern Brazil.

Materials and Methods

Ethical principles

This study was conducted under the terms and conditions of the Ethics Committee for Animal Experimentation of the Universidade Federal do Piauí, Brazil, approved under number 028/13.

Location and climatic conditions

This study was conducted from June 2013 to June 2014 on two farms located 15 km apart; each farm had different management practices for dairy farming (Table 1); and the farms were located in the Litoral Piauiense micro region of Northeastern Brazil in an area of enzootic instability for babesiosis, as described by Souza et al. (2013). Farm A, which is in the municipality of Buriti dos Lopes-PI, is located at latitude 3° 6 '9 "S and longitude 41 ° 53' 38" W, has a total area of 850 ha, has a seroprevalence of 55.5% for *B.bigemina* and has a seroprevalence of 33.3% for *B. bovis*. Farm B is in the municipality of Parnaíba-PI, is located at latitude 2° 58 '56 "S and longitude 41° 47' 36" W, has an area of 95 ha, has a seroprevalence of 23.1% for *B. bigemina* and has a seroprevalence of 30.7% for *B. bovis* (SOUZA et al., 2013).

Monthly data, including the daily average rainfall, temperature and humidity, were obtained from the agrometeorological station of the Instituto Nacional de Meteorologia (INMET), located in the Embrapa Meio-Norte experimental area, Parnaíba-PI (Figure 1).

Collection procedures and laboratory tests

Fifteen Gir x Holstein crossbred females from each farm were sampled from birth (occurring during the months of June and July) until 12 months of age, totaling 30 heifers. The animals were naturally exposed to infestation by vectors under the specific management practices of each farm (Table 1).

Table 1. Characterization of the dairy farms participating in the study regarding the creation, management and control of bovine babesiosis and its vector in the Litoral Piauiense micro region, Northeastern Brazil.

	Farm A	Farm B
Type of breeding	Semi-intensive	Intensive
Type of heifer	Individual	Collective
Nº of animals	650	180
Cultivated species	*Panicum maximum* cv. *Tanzânia* *Hymenachne amplexicaulis*	*Hymenachne amplexicaulis Pennisetum purpureum*
Colostrum administration	5 days supplied in bucket + milk "in nature" up to 10 days of life	Suck directly on ceiling up to 50 day of life
Management at birth	Navel cure with iodine and application of ivermectin	Navel cure with iodine and application of doramectin
Tick control	Ivermectin - at 70 days and at 100, 200 and 310 kg/p.v	Doramectin – an application at birth and every 3 months until one year old. After this period every 6 months.
Control of babesiosis	Chemoprophylaxis with imidocarb at 70 days of age	Did not do

Every 15 days, individual clinical examinations were performed, including an inspection for ectoparasites as well as whole blood collection (with EDTA), to determine PCV using the microhematocrit technique and to extract DNA. DNA was extracted from aliquots of 300 µL of whole blood using a commercial kit (Wizard Genomic DNA Purification Kit, Promega, WI, USA) according to the manufacturer's instructions. The ectoparasites were placed in tubes containing 70% alcohol and were identified according to Aragão & Fonseca (1961).

The molecular detection of *B. bigemina* and *B. bovis* to determine primo-infection was performed by PCR using GAU7/GAU6 and GAU9/GAU10, respectively, which are primers previously described by Linhares et al. (2002) and are based on the sequence of the 18S rRNA gene. The mixture, which had a total volume of 25 µl, contained 0.4 µM of each primer (Integrated DNA Technologies, IA, USA), MasterMix [100 mM Tris-HCl pH 8.5, 500 mM KCl, 1.7 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphate (dNTPs), 1.5 U Taq DNA Polymerase (Ludwig Biotec, RS, Brazil)], and 200 ng of DNA sample. Sterile ultrapure water and DNA extracted from *B. bovis* and *B. bigemina* positive samples previously sequenced in the study of Souza et al. (2013), were used as negative and positive control, respectively, for each PCR reaction performed in this study.

PCR was performed under the following conditions: 94 °C for 2 min, followed by 35 repetitive cycles of denaturation for 30 sec at 94 °C, annealing for 30 sec at 60 °C (*B. bigemina*) or 58 °C (*B. bovis*), extension for 1 min at 72 °C, and a final extension at 72 °C for 5 min in a Gene Pro thermal cycler (Bioer Technology, ZH, China). Amplified products were subjected to electrophoresis in 1.5% agarose gel. PCR products had 690 bp for *B. bigemina* and 541 bp for *B. bovis*.

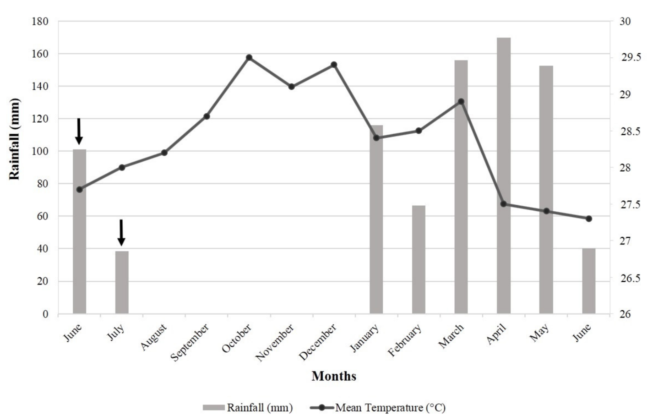

Figure 1. Meteorological data (mean temperature and rainfall) in the Litoral Piauiense microregion, Northeastern Brazil, from June 2013 to June 2014. The black arrows indicate the birth of calves period and the arrow heads indicate animal infestation by *R.* (*B.*) *microplus*. The onset of animal infestation was in September and the highest rate of infestation was in January. Source: INMET Conventional Agrometeorological Station, located in the Embrapa Meio-Norte/UEP Parnaíba.

Statistical analysis

All statistical analyses were performed using a commercial statistical package, GraphPad Prism, version 6.0 (GraphPad Software, CA, USA). A descriptive statistical analysis was performed for each variable. The monthly averages for temperature, humidity and rainfall, as well as for primo-infection, were compared between the months of the year using Student's t-tests (95% confidence). The mean and standard deviations were estimated for PCV values. Mean PCV values in the different age groups (0 to 60 days, 60 to 120 days, 120 to 180 days, 180 to 240 days and 240 to 300 days) of the heifers were compared between the farms using unpaired t-test with Welch's correction, with a significance level of 0.05.

Results and Discussion

The average period of primo-infection on Farm A, determined by PCR, was 249.4 (± 24.42) and 252.6 (±17.07) days of age for *B. bigemina* and *B. bovis*, respectively; there were no significant differences (P> 0.05). The highest percentage of infection was observed in animals between 240 and 300 days of age, but the first animals that emerged infected with *B. bigemina* and *B. bovis* were from 180 to 240 days of age, with a higher percentage of infected by *B. bigemina* (Figure 2). Only 20% (3/15) of the animals on Farm A did not become infected with *B. bovis*. All animals on this farm were diagnosed with *B. bigemina* until day 291; 13.3% (2/15) of the animals showed clinical signs of babesiosis, such as inappetence, anemia, anorexia and hemoglobinuria, all of which emerged in the third month after the onset of rains (Figure 1).

The highest rates of infection and clinical symptomatology on Farm A coincided with the highest rates of *R. (B.) microplus* infestations for the animals. The onset of animal infestation was observed in September, but the highest rate of infestation (46.6%) was observed in January, when the rainy season began; at that point, only the *R. (B.) microplus* species was found infesting the animals (Figure 1).

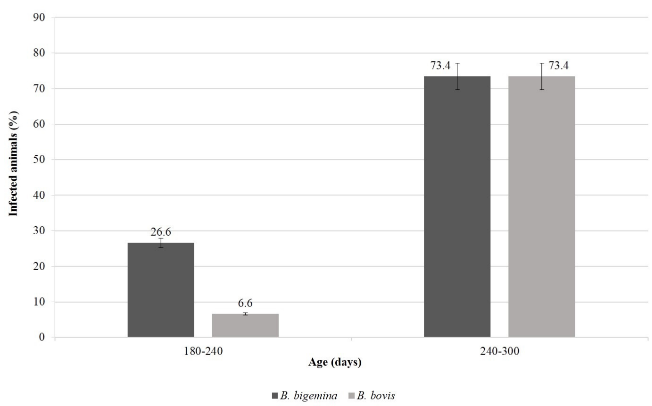

Figure 2. Mean age of *B. bigemina* and *B. bovis* primo-infection in calves born on Farm A in the Litoral Piauiense microregion, Northeastern Brazil. The columns of the graph shows the error bar and indicate the percentage of infected animals in different age groups from the age of primo-infection.

Little information is available on the epidemiology of the *R. (B.) microplus* tick in Northern and Northeastern Brazil. Although the presence of this tick in these regions was recorded, the regions were not considered preference areas due to their climatological and vegetation characteristics (ESTRADA-PEÑA et al., 2006; SANTOS et al., 2017). However, in other Brazilian regions, there is an increase in the *R. (B.) microplus* population during the rainy season (ROCHA et al., 2011; FERRAZ-DA-COSTA et al., 2014).

We consider that the late infection date of the animals may have been due to the dry period in this region being adverse to ticks, contributing to vector reduction in the environment and interfering with *Babesia* spp. infection in the animals. Thus, according to with Brown et al. (2006), Correia (2006) and Amorim et al. (2014) with the absence of the etiological agent, the production of antibodies is limited, leaving the animals susceptible to clinical disease and even to death.

On Farm B, no infection by *Babesia* spp. was observed due to breeding systems intensive, where the animals were confined 24 hours a day from birth, without access to pasture, consequently without contact with ticks. As well as due to the intensive and indiscriminate control of *R. (B.) microplus* through the use of doramectin every three months (Table 1) in all animals up to one year old. Then, after this period, the use of doramectin was every six months. All this practice kept the farm free of the *Babesia* spp. infection. This type of control is used by some producers in Brazil who are unaware of the factors that lead to enzootic instability, which ultimately hinders the implementation of control (AMARAL et al., 2011).

The absence of observed vectors on Farm B is directly related to health management measures. According to Santos-Júnior et al. (2000), Rocha et al. (2006) and Amaral et al. (2011), the lack of knowledge among the producers about the biology of the tick and the importance of the *Babesia* spp. infection in calves at an early age further aggravates the productivity losses caused by the enzootic instability. Moreover, the producers' lack of knowledge exposes the animals to a high risk because the babesiose agents do not circulate in the environment; therefore, the animals do not develop protective antibodies and the matrices cannot transmit the passive immunity to the calves (SANTOS-JÚNIOR et al., 2000; BROWN et al., 2006; AMORIM et al., 2014).

The primo-infection of the calves in the first six weeks of life was not observed on either property. According to Pereira et al. (2009) this is worrisome because the first six weeks of life would be the ideal time to contract the parasite and to develop antibodies since, at that stage, the clinical and hematological manifestations are less severe. This reduced severity is due to the increased erythropoietic activity of the bone marrow, the protective function of fetal hemoglobin and the rapid activity of innate immunity through macrophages and Natural Killer (NK) cells; macrophages process and present the *Babesia* spp. antigens for CD4 + cells, in addition to producing cytokines, such as INF-γ, TNF-α and interleukins 1 and 12 (BOCK et al., 2004; BENAVIDES et al., 2006; RÍOS-TOBÓN et al., 2014).

The average PCV in the different age groups (0 to 60 days, 60 to 120 days, 120 to 180 days, 180 to 240 days and 240 to 300 days) of the heifers ranged from 25.0% to 32.2% (29.5 ± 2.7%) on Farm A and ranged from 29.2% to 30.6% (29.2 ± 1.1%) on Farm B; there was no significant difference (P> 0.05) between the properties. Our results indicated that on Farm A, the PCV average had significantly declined to 240-300 days of age (25.0 ± 5.88%), coinciding with the primo-infection by *Babesia* spp. This reduction in the PCV indicates that anemia is proportional to parasitemia due to intravascular hemolysis as well as sequestration and erythrocyte lysis mechanisms (VIEIRA et al., 2001; BOCK et al., 2004).

The analysis of the climatological data showed that the region had a rainy season (January to June), not exceeding 180 mm/month, and a dry season (August to December), with average temperatures ranging between 28 to 30 °C, a maximum temperature of 35 °C and relative air humidity below 70% (Figure 1).

These conditions, with high temperatures, are not very favorable for the development of the biological cycle of *R. (B.) microplus*, influencing the mortality of larvae in the pastures due to caloric stress (SANTOS-JÚNIOR et al., 2000; GUIMARÃES et al., 2011) and the intensity of infection of these ticks by *Babesia* spp. (QUINTÃO-SILVA & RIBEIRO, 2003). In this region, an imbalance in the parasite/host relationship probably occurred, facilitating the appearance of new clinical cases and maintaining the region as an area of enzootic instability, which had already been observed in other studies (QUINTÃO-SILVA & RIBEIRO, 2003; SOUZA et al., 2013).

The dynamics of primo-infection by *B. bovis* and *B. bigemina* are directly related to the type of management adopted and the climate of Northeastern Brazil. The climate of this region considerably decreases the population of ticks during the dry period, causing infection by *Babesia* spp. to occur only during the rainy season. Thus, excessive use of acaricides and the climate of the region interfere with the tick population, facilitating the maintenance of an area of enzootic instability for *Babesia* spp. in Northeastern Brazil.

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References

Alves LC. *Prevalência da babesiose em gado leiteiro no município de Garanhuns, estado de Pernambuco* [Dissertação]. São Paulo: Universidade de São Paulo; 1987.

Amaral MAZ, Rocha CMBM, Faccini JL, Furlong J, Monteiro CMO, Prata MCA. Perceptions and attitudes among milk producers in Minas Gerais regarding cattle tick biology and control. *Rev Bras Parasitol Vet* 2011; 20(3): 194-201. PMid:21961747. http://dx.doi.org/10.1590/S1984-29612011000300003.

Amorim LS, Wenceslau AA, Carvalho FS, Carneiro PLS, Albuquerque GR. Bovine babesiosis and anaplasmosis complex: diagnosis and evaluation of the risk factors from Bahia, Brazil. *Rev Bras Parasitol Vet* 2014; 23(3): 328-336. PMid:25271452. http://dx.doi.org/10.1590/S1984-29612014064.

Aragão H, Fonseca F. Notas de ixodologia: VIII. Lista e chave para os representantes da fauna ixodológica brasileira: notas de ixolodologia. *Mem Inst Oswaldo Cruz* 1961; 59(2): 115-129. PMid:13861962. http://dx.doi.org/10.1590/S0074-02761961000200001.

Barros SL, Madruga CR, Araújo FR, Menk CF, Almeida MAO, Melo EP, et al. Serological survey of *Babesia bovis, Babesia bigemina*, and *Anaplasma marginale* antibodies in cattle from the semi-arid region of the state of Bahia, Brazil, by enzyme-linked immunosorbent assays. *Mem Inst Oswaldo Cruz* 2005; 100(6): 513-517. PMid:16302060. http://dx.doi.org/10.1590/S0074-02762005000600003.

Benavides MV, Sá GL, Sacco AMS. *Resposta imune dos bovinos frente à infecção por Babesia bovis.* Bagé: Embrapa Pecuária Sul; 2006.

Berto RS, Faustino MAG, Melo LEH, Alves LC, Madruga CR, Almeida MAO, et al. Frequência de anticorpos IgG anti - *Babesia bovis* e anti - *Babesia bigemina* em bovinos no Município do Paudalho, Zona da Mata do Estado de Pernambuco. *Med Vet* 2008; 2(3): 9-12.

Bock R, Jackson L, De Vos A, Jorgensen W. Babesiosis of cattle. *Parasitology* 2004;129(Suppl): 247-269. PMid:15938514. http://dx.doi.org/10.1017/S0031182004005190.

Brown WC, Norimine J, Knowles DP, Goff WL. Immune control of *Babesia bovis* infection. *Vet Parasitol* 2006; 138(1-2): 75-87. PMid:16510249. http://dx.doi.org/10.1016/j.vetpar.2006.01.041.

Carrique Mas JJ, Widdowson MA, Cuéllar AM, Ribera H, Walker AR. Risk of babesiosis and anaplasmosis in different ecological zones of Santa Cruz Departament, Bolivia. *Vet Parasitol* 2000; 93(1): 29-38. PMid:11027858. http://dx.doi.org/10.1016/S0304-4017(00)00328-9.

Correia TLC. *Frequência de anticorpos para Babesia spp. em bovinos da região de Encruzilhada do Sul, RS, Brasil e sua correlação com a infecção da hemolinfa de carrapatos Boophilus microplus* [Dissertação]. Rio Grande do Sul: Universidade Federal do Rio Grande do Sul; 2006.

Costa VMM, Ribeiro MFB, Duarte ALL, Mangueira JM, Pessoa AFA, Azevedo SS, et al. Seroprevalence and risk factors for cattle anaplasmosis, babesiosis and trypanosomiasis in a Brazilian semiarid region. *Rev Bras Parasitol Vet* 2013; 22(2): 207-213. PMid:23802235. http://dx.doi.org/10.1590/S1984-29612013005000022.

Estrada-Peña A, Bouattour A, Camicas JL, Guglielmone A, Horak I, Jongejan F, et al. The known distribution and ecological preferences of the tick subgenus *Boophilus* (Acari: Ixodidae) in Africa and Latin America. *Exp Appl Acarol* 2006; 38(2-3): 219-235. PMid:16596355. http://dx.doi.org/10.1007/s10493-006-0003-5.

Ferraz da Costa MS, Guimarães MP, Lima WS, Ferraz da Costa AJ, Facury Filho EJ, Araujo RN. Seasonal variation and frequency distribution of ectoparasites in Crossbreed cattle in Southeastern Brazil. *J Vet Med* 2014; 2014: 759854. PMid:26464941. http://dx.doi.org/10.1155/2014/759854.

Goff WL, Johnson WC, Parish SM, Barrington GM, Tuo W, Valdez RA. The age-related immunity in cattle to *Babesia bovis* infection involves the rapid induction of interleukin-12, interferon-gamma and inducible nitric oxide synthase mRNA expression in the spleen. *Parasite Immunol* 2001; 23(9): 463-471. PMid:11589775. http://dx.doi.org/10.1046/j.1365-3024.2001.00402.x.

Guimarães AM, Carvalho AHO, Daher DB, Hirsch C. Soroprevalência e fatores de risco para *Babesia bovis* em rebanhos leiteiros na região sul de Minas Gerais. *Cienc Agrotec* 2011; 35(4): 826-832. http://dx.doi.org/10.1590/S1413-70542011000400024.

Linhares GFC, Santana AP, Laueman LH, Madruga CR. Assessment of primers designed from the small ribosomal subunit RNA for specific discrimination between *Babesia bigemina* and *Babesia bovis* by PCR. *Cienc Anim Bras* 2002; 3(2): 27-32.

Pereira MA, Guimarães AM, Rocha CMBM. Efeito da estação de nascimento sobre a frequência de bezerras soropositivas para *Anaplasma marginale* e *Babesia bovis* na região sul de Minas Gerais, Brasil. *Cienc Anim Bras* 2009; 10(3): 975-983.

Quintão-Silva MG, Ribeiro MFB. Infection rate of *Babesia* spp. sporokinetes in engorged *Boophilus microplus* from an area of enzootic stability in the state of Minas Gerais, Brazil. *Mem Inst Oswaldo Cruz* 2003; 98(8): 999-1002. PMid:15049079. http://dx.doi.org/10.1590/S0074-02762003000800003.

Ríos-Tobón S, Gutiérrez-Builes LA, Ríos-Osorio LA. Assessing bovine babesiosis in *Rhipicephalus (Boophilus) microplus* ticks and 3 to 9-month-old cattle in the middle Magdalena region, Colombia. *Pesq Vet Bras* 2014; 34(4): 313-319. http://dx.doi.org/10.1590/S0100-736X2014000400002.

Rocha CMBM, Leite RC, Bruhn FR, Guimarães AM, Furlong J. Perceptions about the biology of *Rhipicephalus (Boophilus) microplus* among milk producers in Divinópolis, Minas Gerais. *Rev Bras Parasitol Vet* 2011; 20(4): 289-294. PMid:22166382. http://dx.doi.org/10.1590/S1984-29612011000400006.

Rocha CMBM, Oliveira PR, Leite RC, Cardoso DL, Calic SB, Furlong J. Percepção dos produtores de leite do município de Passos, MG, sobre o carrapato *Boophilus microplus* (Acari: Ixodidae), 2001. *Cienc Rural* 2006; 36(4): 1235-1242. http://dx.doi.org/10.1590/S0103-84782006000400029.

Santos GB, Gomes IMM, Silveira JAG, Pires LCSR, Azevedo SS, Antonelli AC, et al. Tristeza Parasitária em bovinos do semiárido pernambucano. *Pesq Vet Bras* 2017; 37(1): 1-7. http://dx.doi.org/10.1590/s0100-736x2017000100001.

Santos HQ, Linhares GFC, Madruga CR. Estudo da prevalência de anticorpos anti-*Babesia bovis* e anti-*Babesia bigemina* em bovinos de leite da microrregião de Goiânia determinada pelos testes de imunofluorescência indireta e Elisa. *Cienc Anim Bras* 2001; 2(2): 133-137.

Santos-Júnior JCB, Furlong J, Daemon E. Controle do carrapato *Boophilus microplus* (Acari: Ixodidae) em sistemas de produção de leite da Microrregião Fisiográfica Fluminense do Grande Rio – Rio de Janeiro. *Cienc Rural* 2000; 30(2): 305-311. http://dx.doi.org/10.1590/S0103-84782000000200018.

Smith RD, Evans DE, Martins JR, Cerésér VH, Correa BL, Petraccia C, et al. Babesiosis (*Babesia bovis*) stability in unstable environments. *Ann N Y Acad Sci* 2000; 916(5): 510-520. http://dx.doi.org/10.1111/j.1749-6632.2000.tb05330.x. PMid:11193666.

Souza FAL, Braga JFV, Pires LV, Carvalho CJS, Costa EA, Ribeiro MFB, et al. Babesiosis and anaplasmosis in dairy cattle in Northeastern Brazil. *Pesq Vet Bras* 2013; 33(9): 1057-1061. http://dx.doi.org/10.1590/S0100-736X2013000900002.

Vieira D, Mendonça CL, Kohayagawa A, Madruga CR, Schenki MA, Kessler R. Avaliações da parasitemia, do hematócrito e dos níveis bioquímicos séricos, de bezerros nelore (*Bos indicus*), inoculados com isolados de *Babesia bigemina* (Smith & Kilborne, 1893) das regiões Sul, Sudeste, Centro-Oeste, Nordeste e Norte do Brasil. *Cienc Anim Bras* 2001; 2(2): 101-109.