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HEMATOLOGICAL PARAMETERS AND SEROPREVALENCE OF *Ehrlichia canis* AND *Babesia vogeli* IN DOGS

PARÂMETROS HEMATOLÓGICOS E SOROPREVALÊNCIA DE Ehrlichia canis E Babesia vogeli EM CÃES

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Abstract
The aim of this study was to determine the prevalence of anti-*Ehrlichia canis* and anti-*Babesia vogeli* IgG antibodies in dogs and correlate this prevalence with risk factors to evaluate the relation of serological status to hematological findings. Blood samples of dogs attended from September 2011 to March 2012 at the veterinary hospital of the Federal University of Lavras, Brazil, were analyzed using an indirect fluorescence antibody test (IFAT). Of the total 160 dog serum samples, 23.7% (38 dogs; CI_{95} 17.7% – 30.7%) were seropositive for *E. canis*, 40.0% (64 dogs; CI_{95} 40.0% – 59.2%) for *B. vogeli*, and 5.6% (9 dogs) for both hemoparasites. None of the epidemiological variables showed a significant association (P>0.05) with seropositivity to *E. canis* and *B. vogeli*. Dogs seropositive for *E. canis* showed lower values for hematocrit (P<0.05). However, for the erythrogram, the platelet count, the leukogram and clinical signs, no significant difference (P>0.05) was observed between dogs that were seropositive and seronegative for *E. canis* or for *B. vogeli*. Serological results suggest that infection with *E. canis* and *B. vogeli* is endemic in the canine population in question, with a prevalence of the subclinical phase (asymptomatic) in dogs that are seropositive for ehrlichiosis or babesiosis.
Keywords: babesiosis; canine; ehrlichiosis; IFAT; laboratory findings.

Resumo
O objetivo deste estudo foi determinar a prevalência de anticorpos IgG anti- *Ehrlichia canis* e anti-*Babesia vogeli* em cães e correlacionar com a prevalência e fatores de risco para avaliar a relação do estado sorológico com os achados hematológicos. Amostras de sangue de cães coletadas de setembro de 2011 a março de 2012, no Hospital Veterinário da Universidade Federal de Lavras, Brasil, foram analisadas usando o teste de reação de imunofluorescência indireta (RIFI). Do total de amostras de soro de 160 cães, 23,7% (38 cães; IC95 17,7% - 30,7%) foram soropositivos para *E. canis*, 40,0% (64 cães; IC95 40,0% - 59,2%) para *B. vogeli*, e 5,6% (9 cães) para os dois hemoparasitas. Nenhuma das variáveis epidemiológicas mostrou associação significativa (P> 0,05) com a soropositividade para *E. canis* e *B. vogeli*. Cães soropositivos para *E. canis* mostraram valores médios mais baixos para hematócrito (P<0,05). No entanto, para o eritrograma, a contagem de plaquetas, o leucograma e os sinais clínicos, nenhuma diferença significativa (P> 0,05) foi observada entre cães soropositivos e soronegativos para *E. canis* ou para *B. vogeli.* Os resultados sorológicos deste estudo sugerem que a

infecção por *E. canis* e *B. vogeli* é endêmica na população canina em questão, com uma prevalência da fase subclínica (assintomática) em cães soropositivos para erliquiose ou babesiose.
Palavras chave: babesiose; cão; erliquiose; IFAT; parâmetros laboratoriais.

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Introduction

Canine monocytic ehrlichiosis (CME) is a disease caused by *Ehrlichia canis* (Rickettsiales: Anaplasmataceae), a Gram-negative, obligate intracellular bacteria transmitted by the tick vector *Rhipicephalus sanguineus*, commonly known as the "brown dog tick"[1]. Some dogs are asymptomatic, and for others, the clinical manifestations are nonspecific and often involve different systems. Hematological changes during infection are highlighted by thrombocytopenia and leukopenia[2,3]. Considered a cosmopolitan disease, CME is particularly prevalent in tropical and subtropical regions, with seroprevalence between 0.7 and 86.2%[4]. In Brazil, the occurrence of canine ehrlichiosis has been increasing significantly in different regions and coincides with the distribution of *R. sanguineus*, which is widely distributed in many urban areas in the country[5].

Canine babesiosis, which is caused by *Babesia vogeli* (Piroplasmorida: Babesiidae) and transmitted by the tick *R. sanguineus*, is a common disease in tropical areas[1]. The disease usually causes fever, lethargy, anorexia, and jaundice, and clinicopathological abnormalities typically found include regenerative immune-mediated hemolytic anemia, nonregenerative anemia, leukocytosis, leucopenia, and thrombocytopenia[6]. The seroprevalence of *B. vogeli,* a cosmopolitan species, ranges from 18.8% to 73.3%[7-10].

Knowledge of the prevalence and clinicopathological aspects of vector-borne pathogens that infect dogs at the local and regional level are of epidemiologic interest to veterinary practitioners because it facilitates a prompt diagnosis and appropriate therapy[6]. A study on parasitic ectofauna in a canine population in the city of Lavras, Minas Gerais, showed that *R. sanguineus* is the main ectoparasite in urban, indoor dogs[11], and this tick is the vector of *E. canis* and *B. vogeli*[1]. However, despite the importance of ehrlichiosis and babesiosis to veterinary clinical practice, there is no information on the seroprevalence of *E. canis* and *B. vogeli* in the population of dogs that are attended at the veterinary hospital in Lavras.

Therefore, this study aimed to determine the prevalence of these canine hemoparasites in dogs (from Minas Gerais State, Brazil) that were attended at the veterinary hospital and correlate the serological status of the dogs with epidemiological variables and hematological findings.

Material and methods

This study was conducted in Lavras (21° 14' 43 S 44° 59' 59 W), a town located in the southern part of the state of Minas Gerais, from September 2011 to March 2012, and received the approval of Ethics Committee on Animal Use - UFLA (Nº 018/2011).

A total of 160 dogs of both sexes and different ages and breeds were attended at the Veterinary Hospital of the Federal University of Lavras (UFLA) and blood samples were collected for convenience, regardless of what motivated the consultation, between September 2011 and March 2012. A tube of blood with anticoagulant (EDTA, 1%) was collected for a complete hemogram (erythrogram and leucogram) and platelet count, by the automated method, and another tube without anticoagulant for serology. After coagulation, the blood samples to be used for serology were centrifuged at 500 x g for 10 minutes, and the sera were individually identified and kept frozen in cryotubes at -20 °C until undergoing an indirect fluorescent antibody test (IFAT).

After a dog had undergone clinical examination, the owner responded to a questionnaire about the dog and provided the following data: name, sex, age, breed, origin, type of residence, frequency of acaricide treatments, and current or previous infestation by ticks as noticed by the owner. This information composed the individual record of the animal.

Seroprevalence was determined by the indirect fluorescent antibody test (IFAT), using commercially available IFAT slides that had *E. canis* (Laboratório Imunodot, Jaboticabal/SP/ Brazil) and *B. vogeli*--infected dog erythrocytes fixed on them, obtained from a splenectomized dog experimentally infected with an isolate from Lavras/MG, Brazil[12]. The antigen of B. vogeli was produced following the methodology described by IICA[13]. The IFAT was performed according to Ristic et al.[14] with fluorescein isothiocyanate-conjugated rabbit anti-dog antibody (Sigma-Aldrich, St. Louis, Missouri, USA) diluted at 1:32. Each IFAT slide included a positive and negative control. The evaluation was performed with a fluorescence microscope, and at a dilution of 1:80 (cut-off) was considered sero-reactive when there was complete fluorescence of intracellular organisms of *E. canis* and *B. vogeli*.

To calculate the true prevalence, and the 95% confidence interval, we considered the sensitivity (SE) and specificity (SP) of the IFAT was considered for *E. canis* (SE = 100%, SP = 100%)[15] and *B. vogeli* (SE = 79.2%, SP = 100%)[7] using the *EpiTools* epidemiological calculators[16]. Confidence limits for true prevalence were calculated as described by Reiczigel et al.[17].

To identify the epidemiological factors associated with *E. canis* and *B. vogeli* infections in dogs, serological results obtained by IFAT served as the dependent variable, and the variables collected in the interviews were the independent variables in the statistical analysis. Thus, the dependent variable was transformed into binary values (0-negative, 1-positive), and univariate analysis using the chi-square (x^2) test was performed. The variables that showed an association ($P<0.2$) according to the x^2 test or Fisher's Exact test (fewer than five observations were available in at least one cell of the contingency table) were selected for the construction of a logistic multiple regression model. Risk was estimated using an adjusted odds ratio (OR) and a confidence interval of 95% for the one variable that was significantly associated ($P<0.05$) in the logistic regression. The existence of differences in the averages of the results of laboratory tests (complete blood count) between seropositive and seronegative dogs for *E. canis* and for *B. vogeli* was verified by parametric Student t-tests.

Results and discussion

Most of the dogs that were sampled were male (93/160; 58.4%), mixed breed (86/160; 54.0%), adult (122/160; 76.4% were > 1 year of age), and from households in the urban area of the city of Lavras (138/160; 86.3%). Of the canine serum samples submitted to IFAT, 23.7% (38/160; IC_{95}: 17.7% – 30.7%) had anti-*E. canis* antibodies. A similar result was obtained by Trapp et al.[7] in a canine population at a veterinary hospital in Londrina (PR), with 23% (87/381) of the dogs being seropositive for *E. canis* (IFAT ≥80). The results for the city of Lavras (MG) is within the range reported by other studies conducted in Brazil, which have shown that between 14% and 45% of the dogs that are attended in veterinary hospitals and clinics are seropositive[18]. The frequency of *E. canis* in the serum of dogs in Lavras (MG) was lower than the results observed by Silva et al.[19] in Cuiaba (MT), 42.5% (108/254; IFAT ≥40), and by Souza et al.[20] in Salvador (BA), 35.6% (168/472; IFAT ≥80). However, Saito et al.[21] observed a prevalence of 4.8% (19/389; IFAT ≥80) in Rio Grande do Sul, which was low compared to that obtained in the present study. Importantly, high prevalence rates reported in some studies may be explained by the use of a sample population with clinical suspicion for CME, increasing the possibility of finding dogs with anti-*E. canis* antibodies.

The true prevalence of seropositive dogs for *B. vogeli* was 40.0% (64/160; IC_{95} 40.0% – 59.2%), lower than those previously reported for São Paulo (42.4%), Belo Horizonte (66.9%) and Paraná State (46.4%)[4,22,23]. Lower seroprevalence data (35.7%) were obtained from a population of dogs from a veterinary hospital in the same study area[7]. Thus, the results observed in the city of Lavras (MG) for *E. canis* and *B. vogeli* are consistent because it is an epidemiologic study where the samples

were collected for convenience, regardless of what motivated the consultation.

No significant association was observed (P>0.05) between the serological status of the dogs for *E. canis* and *B. vogeli* and any of the epidemiological variables evaluated. Age and gender was not associated with the risk of seropositivity among the canine population, a finding that is in agreement with most epidemiological studies[23-26]. However, in studies with dogs older than one or two years of age, the dogs have a higher frequency of anti-*B. vogeli* antibodies[7] and clinical disease[27]. A study evaluating the mode of infection revealed that dogs with only indoor access have a lower frequency (P<0.05) of infection by *E. canis*[17], most likely due to the close interaction with their owners. Such close interaction generates a greater zeal for ectoparasite control, and therefore lower parasitism of the dogs by the tick vector[5]. At the same time, the presence of infected dogs is not required for *B. vogeli* to remain in the tick population[28], a potential explanation for the higher prevalence of babesiosis. The presence of infected dogs is necessary for the *E. canis* to remain in a tick population because there is no vertical transmission in ticks[29]. *B. vogeli*, however, can be transmitted transovarially and be passed to the next tick generation even in the absence of infected dogs[28].

Regarding the laboratory findings, for the dogs seropositive for *E. canis* and *B. vogeli,* there was no significant difference (P>0.05) in erythrogram and platelet count parameters (Table 1), except that dogs seropositive for *E. canis* showed lower values for hematocrit (P<0.016) when compared with seronegative dogs. The frequency of dogs with anemia showed no significant association (P>0.05) with serological status, of the 66 (41.2%) animals with anemia, 18 (27.3%) were seropositive for *E. canis*, 24 (36.4%) for *B. vogeli*, and 2 (3.0%) for both hemoparasites. This is likely due to the studied canine population consisting predominantly of asymptomatic dogs, i.e., apparently normal and with no clinical signs suggestive of CME or babesiosis. Thus, the hematological findings were nonspecific, with both anemia and thrombocytopenia being identified in some dogs, but with most values of the erythrogram and the platelet count falling within the normal range for canines. Similarly, 30 dogs examined at the Veterinary Teaching Hospital of the São Paulo State University (UNESP), in Jaboticabal city, Brazil showed no association between the hematological signs (trombocytopenia and anemia; n= 13) and serological (IFAT) positive (n= 7) or negative (n= 6) results[30] . In 70 dogs suspected of *E. canis* infection attended at the Veterinary Hospital of UNESP in Botucatu city, Brazil, 25 anemic PCR-positive dogs did not reveal statistical significance (P>0.05) when compared with PCR-negative dogs [3].

Table 1 - Mean and standard deviation of the erythrogram and platelet count parameters according to the serologic status of *Ehrlichia canis* and *Babesia vogeli* in dogs (n=160) attended at the veterinary hospital of the Federal University of Lavras in Minas Gerais State, Brazil

Hematological parameters*	*Ehrlichia canis*			*Babesia vogeli*			Reference values ***
	Positive (n=38)	Negative (n=122)	*p*-value**	Positive (n=64)	Negative (n=96)	*p*-value**	
Red blood cells (million /mm³)	5.23±1.55	5.80±1.51	0.055	6.80±1.39	5.58±1.61	0.400	5.5 - 8.5
Hematocrit (%)	35.09±11.20	39.97±10.63	0.016	40.12±10.34	38.06±1.23	0.247	37 - 55
Hemoglobin (g/dL)	18.24±20.99	20.12±21.64	0.638	18.40±18.87	20.60±23.13	0.534	12 - 18
MCV (fL)	70.15±6.43	69.87±6.96	0.827	69.79±6.10	70.16±7.26	0.743	60 - 77
MCH (pg)	28.47±17.70	28.96±18.63	0.884	27.52±16.21	29.80±19.77	0.451	19 - 24
MCHC (%)	38.33±14.78	39.28±16.11	0.747	37.53±13.52	40.08±17.16	0.300	31 - 36
Platelets (mil/mm³)	180.66±107.66	201.38±132.43	0.381	196.67±119.91	199.22±131.78	0.865	200 - 500

MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, * Lack of data, normality by Kolgomorov-Smirnov test, **Mann-Whitney Test, *** [31].

The mechanisms for thrombocytopenia are multi-factorial, representing increased consumption or destruction of platelets and platelet sequestration in the spleen[32]. In this study, of the 79 (49.1%) dogs with thrombocytopenia, 20 (25.3%) had anti-*E. canis* antibodies, 35 (44.3%) had anti-*B. vogeli* antibodies, and 4 (5.1%) had antibodies for both hemoparasites, indicating that, although it is a characteristic finding, thrombocytopenia is not exclusive to canine ehrlichiosis and may be present in different diseases[18]. Thus, those dogs seronegative to *E. canis* and *B. vogeli*, but with values for hematological parameters and/or the platelet count that are low for canines, are likely infected by other hemoparasites, such as *Anaplasma platys* and *Hepatozoon canis*, which are also transmitted by the tick vector *R. sanguineus*[33,34].

Many studies have looked at the prevalence of thrombocytopenia and anemia in dogs with ehrlichiosis and babesiosis. This information is helpful, but from a clinical perspective, it is more important to know how many dogs with anemia or thrombocytopenia have these diseases. Dogs infected with Brazilian strains of *E. canis* consistently develop anemia[35,36], but they may[36] or may not develop thrombocytopenia[35].

Thrombocytopenia is a common finding in dogs with ehrlichiosis[37]. In the United States, thrombocytopenia was found in 47 of 61 (77%) dogs with ehrlichiosis in a retrospective serologic study[38] and in all 30 dogs in a case series[39]. In Israel, where ehrlichiosis is more prevalent than in the US, thrombocytopenia was present in 17 of 63 (27%) dogs in a serosurvey[40]. In a serologic survey in Switzerland, 20 of 75 (26.7%) thrombocytopenic dogs seroreacted to *E. canis*[41]. In 49 dogs from Thailand presenting anemia (31 dogs), thrombocytopenia (27 dogs), or fever (5 dogs), 38 (75%) had antibodies against *E. canis*[42]. Differences in prevalence may reflect diversity in strain pathogenicity or be a bias of selection, as thrombocytopenic dogs are more likely to be tested for ehrlichiosis and babesiosis.

We did not evaluate the prevalence of thrombocytopenia in dogs with ehrlichiosis; we verified that approximately 50% of thrombocytopenic dogs had ehrlichiosis. There is a widespread perception among veterinary practitioners that ehrlichiosis is the main cause of thrombocytopenia in dogs in Brazil. However, Trapp et al.[7] only implicated ehrlichiosis as the potential cause for the thrombocytopenia in one in five dogs, and thrombocytopenia was not more prevalent in dogs seropositive to *E. canis* than in seronegative dogs[43].

In the current study, there was no significant difference ($P>0.05$) in the leukogram parameters of dogs with or without anti-*E. canis* and anti-*B. vogeli* antibodies, with values within the normal range for the canine species. The leukopenia (26.6%) and leukocytosis (26.0%) frequencies were similar to those frequencies seen in the serological results for these dogs. Importantly, most studies, including the present, one evaluated dogs that were naturally infected by *E. canis* and *B. vogeli* without knowing the stage of the disease and disregarding the influence of stress caused by the parasite in the development of secondary changes in the leucogram, which complicates a comparison of the results of the different studies.

According to the study, approximately 25% of the dogs (40/160) that underwent a clinical examination were infested by ticks at the consultation. Importantly, the rate of tick infestation may be underestimated because clinical veterinarians normally prioritize the viewing of adult forms, neglecting to look for the presence of larvae and nymphs, which are smaller parasitic stages, and consequently, hader to see[11].

Although ticks were not identified in this study, the vector is very likely to be *R. sanguineus*, which is considered the main species observed in dogs bred in urban areas in Brazil[4,5,44]. This suspicion is supported because, in this survey in Lavras (MG), there was a predominance of urban indoor dogs (138/160; 86%). According to Guimarães et al.[11], in a study of parasitic ectofauna in 67 urban indoor dogs that were attended at private veterinary clinics in Lavras (MG), *R. sanguineus* was the only tick species ever observed on animals during clinical examinations.

By this research, there was no significant association ($P>0.05$) between ticks being present on an animal during the clinical examination and the serological status of the dog. Most likely, the relatively small number of dogs, in association with the population profile (randomly sampled), explains this

result. However, it is important to note that 84.2% (32/38) of the dogs that were seropositive for *E. canis* in Lavras (MG) had contact with ticks in the previous 12 months, according to reports from the owners.

Currently, due to changes in lifestyle, dogs are increasingly in contact with humans. In this study, 51.0% (82/160) of owners reported that they dedicate up to seven hours per week of leisure to their dogs, and 21.0% (34/160) more than seven hours a week, thus allowing for exposure to and a potential risk of infestation by the tick *R. sanguineus*. Fourteen owners (35.0%), whose dogs had ticks at the consultation time (14/40), also reported having observed ticks attached to their own skin. Among the infested dogs, seven (7/14; 50.0%) animals showed clinical suspicion for CME at the consultation, and four dogs (4/7; 57.0%) were seropositive for *E. canis*. Despite being considered rare, human infestation with brown dog tick varies regionally, and *R. sanguineus* feeds on humans much more commonly than previously thought[45]. In Brazil, there are few studies of parasitism by *R. sanguineus* on humans. Dantas-Torres et al.[46], in the metropolitan area of Recife (PE), described four cases of parasitism on humans by the tick *R. sanguineus*. Louly et al.[47], in Goiania (GO), also reported parasitism of humans by this tick. This fact may suggest that the tick is becoming more anthropophilic or that a more human-adapted population of *R. sanguineus* has been introduced.

Canine monocytic ehrlichiosis (CME) is a major infectious disease that affects dogs and is also of interest in public health due to the recent discoveries of infection by *E. canis* in humans in Venezuela, where the agent was likely transmitted by the tick vector *R. sanguineus*[48,49]. Thus, taking into consideration the concern about the zoonotic potential of *E. canis* and other hemoparasites, further studies on the parasitism of humans by the *R. sanguineus* are necessary.

Conclusion

In summary, our serological results support the hypothesis that natural infections of *E. canis* and *B. vogeli* in dogs attended at a veterinary hospital in the city of Lavras is common. Positive serologic results in dogs only prove exposure to *E. canis* and *B. vogeli*, but not a clinical disease, suggesting that, in the canine population in question, there was a prevalence of subclinical infection (asymptomatic). However, diagnosis of subclinical infections is of great epidemiological importance because infected dogs can serve as reservoirs of infection for other hosts, including humans. Detailed epidemiological surveys using more sensitive and specific methods (e.g., PCR) are needed for the confirmation of infection with *E. canis* and *B. vogeli* and for the determination of co-infection with other tick-borne diseases, whose vector is also the tick *R. sanguineus*.

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