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Epidemiology of Ehrlichia canis: hematological and biochemical aspects, associated factors, and molecular features in healthy dogs in Porto Seguro, Bahia, Brazil

Epidemiologia de Ehrlichia canis: aspectos hematológicos, bioquímicos, fatores associados e moleculares em cães hígidos no município de Porto Seguro, Bahia, Brasil

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Highlights

High frequency of dogs infected with *E. canis* in Porto Seguro.
Thrombocytopenia was the main hematological alteration identified.
Dogs positive for *E. canis* are six times more likely to have thrombocytopenia.
Living in urban areas and the presence of ticks were identified as risk factors.
Living near deforested areas and being kept indoors were protective factors.

Abstract

This cross-sectional observational study aimed to investigate the frequency, associated factors, and assess the hematological and biochemical alterations of *E. canis* infection in 396 healthy, household dogs in Porto Seguro, Bahia. In addition to blood sample collection, further information on the dogs' intrinsic and extrinsic characteristics was obtained through semi-structured interviews with their owners to identify factors associated with infection. DNA extraction from blood samples and testing for *E. canis* were performed using the *nested* PCR technique. The frequency of *E. canis* found was

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30.8% (122/396). It was observed that thrombocytopenia (p<0.05) was the most evident hematological alteration among dogs infected with *E. canis.* It was also found that positive dogs are more likely to have thrombocytopenia, leukopenia, and anemia than negative animals (p<0.05). The logistic regression model revealed the presence of ticks (odds ratio [OR] = 1.66; confidence interval [CI]: 1.05 - 2.63; p-value = 0.03) and living in urban areas (OR = 1.90; CI: 1.19 - 3.04; p-value = 0.007) were risk factors for infection, while living near deforested areas (OR = 0.56; CI: 0.31 - 0.99; p-value = 0.05) and being kept indoors (OR = 0.51; CI: 0.31 - 0.85; p-value = 0.01) were identified as protective factors. The high frequency of *E. canis,* combined with thrombocytopenia and associated factors that signal the need for tick control measures and diagnostic testing for the infection, contribute to a better understanding of the local epidemiology of the infection.
Key words: Ticks. Tick-borne disease. Canine Monocytic Ehrlichiosis. Thrombocytopenia.

Resumo

Este estudo observacional transversal teve como objetivo investigar a frequência, fatores associados e avaliar as alterações hematológicas e bioquímicas da infecção por *E. canis* em 396 cães saudáveis e domiciliados do município de Porto Seguro, Bahia. Além da coleta de amostras de sangue, foram obtidas informações adicionais sobre características intrínsecas e extrínsecas dos cães por meio de entrevistas semiestruturadas com seus tutores, a fim de identificar os fatores associados à infecção. Foi realizada a extração de DNA das amostras de sangue e pesquisa de *E. canis* pela técnica de *nested* PCR. A frequência de *E. canis* encontrada foi de 30,8% (122/396). Observou-se que a trombocitopenia (p<0,05) foi a alteração hematológica mais evidente entre os cães infectados por *E. canis.* Verificou-se que cães positivos possuem maior probabilidade de terem trombocitopenia, leucopenia e anemia em comparação aos animais negativos (p<0,05). Por meio do modelo de regressão logística, identificou-se que a presença de carrapatos (odds ratio [OR] = 1,66; intervalo de confiança [IC]: 1,05 - 2,63; valor p = 0,03) e residir na zona urbana (OR = 1,90; IC: 1,19 - 3,04; valor p = 0,007) foram fatores de risco para a infecção, enquanto morar próximo a áreas desmatadas (OR = 0,56; IC: 0,31 - 0,99; valor p = 0,05) e ter hábito domiciliar (OR = 0,51; IC: 0,31 - 0,85; valor p = 0,01) foram identificados como fatores de proteção. A elevada frequência de *E. canis,* combinada com a trombocitopenia e fatores associados que sinalizam para adoção de medidas de controle de carrapatos e diagnóstico da infecção, contribuem para uma melhor compreensão da epidemiologia local da infecção.
Palavras-chave: Carrapatos. Doença transmitida por carrapatos. Erliquiose monocítica canina. Trombocitopenia.

Introduction

Ehrlichia canis is the main etiological agent of canine monocytic ehrlichiosis (CME) (Mylonakis & Theodorou, 2017; Sainz et al., 2015). Infection by this pathogen in dogs has been widely reported worldwide (Cordeiro et al., 2020; Díaz-Regañón et al., 2020; Dordio et al., 2021; Qiu et al., 2018; Rodríguez-Alarcón et al., 2020; Springer et al., 2019). In Brazil, *E. canis* infection has been identified in all five geographical regions (Guimarães et al., 2021;

Lopes et al., 2018; Paschoal et al., 2020; Paulino et al., 2018; Soares et al., 2017), with the tick *Rhipicephalus sanguineus* sensu lato (s.l.) recognized as the main biological vector of the disease (Gray et al., 2013; Moraes et al., 2011; Soares et al., 2020).

CME is a multisystemic disease characterized by nonspecific clinical signs (Harrus & Waner, 2011), which may overlap or vary depending on the disease stage. The most common clinical manifestations in both the acute and chronic phases include high fever, depression, lethargy, anorexia, lymphadenopathy, splenomegaly, bleeding, pale mucous membranes, ocular and neurological alterations (Aroch et al., 2018; Mylonakis et al., 2004; Parashar et al., 2016; Rungsipipat et al., 2009; Veloso et al., 2018). In the subclinical phase, dogs are asymptomatic but can be persistent carriers of the pathogen for years (Harrus et al., 1998; Nelson & Couto, 2015; Waner et al., 1997). At this stage, the infection may either be resolved or progress to the chronic phase, while in severe cases, high mortality rates occur due to septicemia, uremia, or severe bleeding (Mylonakis et al., 2004).

Thrombocytopenia is the most common hematological alteration in dogs infected by *E. canis,* reported in all stages of the disease (Bulla et al., 2004; Harrus et al., 1996b; Mylonakis et al., 2004; Waner et al., 1997). Anemia and leukopenia are also among the main laboratory findings, with neutropenia being the primary alteration in the specific leukocyte count (Castro et al., 2004; Mylonakis et al., 2004; Rungsipipat et al., 2009). Dogs with CME may also exhibit biochemical abnormalities, such as hyperproteinemia, hyperglobulinemia, hypoalbuminemia, hypergammaglobulinemia, and increased levels of alkaline phosphatase (AP) and alanine aminotransferase (ALT) (M. de P. Costa et al., 2015; Harrus et al., 1996a; Rungsipipat et al., 2009).

Intrinsic factors of the dog, such as age, gender, sex, and breed, and extrinsic factors, such as tick infestation, interaction with other dogs, access to the outdoors and habitat, have been investigated in epidemiological studies in various regions of Brazil and abroad (Avizeh et al., 2010; Cordeiro et al., 2020; Guedes et al., 2015; Harrus et al., 1997; Paulino et al., 2018). The study of factors associated with *E. canis* infection allows the understanding of the dynamics of CME and directs more effective measures in the control and prevention of this infection.

Infected dogs play a crucial role as reservoirs for this pathogen, and it can also impact other mammal species (Oliveira et al., 2020; Mazzotti et al., 2018; Perez et al., 2006). The confirmation that *E. canis* is a zoonotic agent highlights the need for public health policies due to the close interaction among humans, dogs, and vectors (Bouza-Mora et al., 2017; Bowser & Anderson, 2018; Perez et al., 2006). Diagnosing *E. canis* infection in dogs is essential to prevent complications, halt disease progression, promote One Health, and understand the pathogen's epidemiology. This study aimed to determine the frequency of *E. canis,* analyze the hematobiochemical profile, and identify factors associated with infection in dogs from the municipality of Porto Seguro-BA.

Material and Methods

Study location and sample population

An observational cross-sectional study was conducted in the city of Porto Seguro, located in the far southern region of Bahia, Brazil (Latitude: 16°27'04" S, Longitude: 39°03'53" W). The city is fully encompassed within the Atlantic Forest biome and has an estimated population of 167,955 people, covering a total area of 2,285.734 km², with a population density of 73.48 inhabitants per km² (Instituto Brasileiro de Geografia e Estatística [IBGE], 2023).

The sample was calculated using Epi Info 3.5.3™ software. A 95% confidence interval and a 50% prevalence rate were considered, with a margin of error of 5%. Furthermore, the canine population in Porto Seguro was estimated to be 10% of the human population in the municipality, as indicated by Escobar Cifuentes (1988). The collections were conducted for convenience and, through strata covering rural and urban areas, proportionally to the population of each district. This stratification was based on the number of dogs vaccinated against rabies during the campaign carried out in the municipality in 2016, using data from epidemiological surveillance. From March to November 2017, 225 blood samples were collected in Porto Seguro (main city), 63 in Trancoso, 46 in Arraial D'ajuda, 17 in Agrovila, 16 in Pindorama, 14 in Vera Cruz, nine in Imbiruçu, four in Vale Verde, and two in Caraíva. Porto Seguro (headquarters) is classified as an urban area, while the other locations are considered rural areas of the municipality.

This study was approved by the Ethics Committee for Animal Use (CEUA) of the State University of Santa Cruz located in the city of Ilhéus, Bahia, Brazil, under protocol numbers 022/16 and 004/21.

Epidemiological data collection

To identify potential factors associated with *E. canis* infection, information on dogs was gathered, including their profile (name, breed, gender, and age), residence characteristics, history regarding tick presence, use of ectoparasiticides, and veterinary follow-up. Aspects of the dogs' lifestyles, such as their access to the outdoors, being kept indoors, and whether they slept indoors, were analyzed. Additionally, potential contact with other animals was documented. This information was obtained through semi-structured interviews conducted with the dog owners by the same interviewer, who was a veterinarian.

Biological sample collection

Following physical restraint, 5 mL of blood were rawn from each dog through venipuncture of either the jugular or cephalic vein. Of the total volume collected, 3 mL were placed into tubes containing EDTA, while 2 mL were dispensed into tubes without the addition of an anticoagulant.

Hematological and serum biochemical analysis

The complete blood count was performed using the URIT 3000 PLUS

Veterinary™ automated analyzer (URIT Medical Electronic Co., Ltd., Guilin, China). Whole blood smears were prepared and stained with Rapid Panoptic® (Laborclin, Pinhais, Paraná, Brazil) for differential leukocyte counts, morphological observation of blood cells, and the detection of hemoparasites via optical microscopy. Total plasma protein (TPP) concentrations were determined using a manual clinical refractometer. The reference ranges applied for the CBC were those recommended by Jain (1993).

The serum was extracted from samples without anticoagulant for the determination of serum urea, creatinine concentrations, and the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). The serum biochemistry was performed on a semi-automatic biochemical analyzer BA88® (Bioclin, Belo Horizonte, Minas Gerais, Brazil) using commercial biochemical reagents (Bioclin®, Belo Horizonte, Minas Gerais, Brazil). The reference ranges for the biochemical tests were those recommended by Kaneko et al. (1997).

Extraction, quantification, and purity of genomic DNA

Genomic DNA was extracted from the leukocyte layer using the commercial reagent PureLink™ Genomic DNA Mini Kit (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts, United States), following the manufacturer's recommendations. The DNA samples were subjected to quantification and purity analysis (260/280nm ratio) using a NanoDrop™ 2000/2000c spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, United States), with ultrapure water used as the blank.

Nested PCR for the detection of Ehrlichia canis DNA

The nested PCR technique was used for the amplification of *E. canis* DNA. In the first reaction, the primer oligonucleotides ECC (5'-AGAACGAACGCTGGCGGCAAGC-3') and ECB (5'-CGTATTACCGCGGCTGCTGGCA-3') were used to amplify a part of the 16S rRNA gene from *Ehrlichia* spp. In the second reaction, the primer oligonucleotides ECAN (5'-CAATTATTTATAGCCTCTGGCTATAGGA-3') and HE3 (5'TATAGGTACCGTCATTATCT TCCCTAT-3') were used to amplify a product of 396 base pairs from *E. canis,* following the methodology described by Murphy et al. (1998).

Each reaction mixture was prepared to a final volume of 25 µL and contained 0.2 mM of each dNTP (Invitrogen™, Carlsbad, USA), 10x Buffer, 5.0 mM $MgCl_2$, 40 pmol of each primer in the first reaction and 30 pmol of each primer in the second reaction, 1.25 U of Platinum Taq DNA Polymerase (Invitrogen™, California, USA). In the first reaction, 5 µL of the sample DNA was added, and 3 µL of the amplification product from this reaction was used in the second reaction. Sterile ultrapure water was used to complete the final volume of both reactions. As a positive control, a sample from the study by Ferraz (2021) was used, and sterile ultrapure water was used as a negative control.

The amplification protocol for the initial reaction consisted of an initial denaturation for 3 minutes at 94°C, followed

by 35 cycles of denaturation at 94°C for 1 minute, annealing at 68°C for 2 minutes, extension at 72°C for 2 minutes, and a final extension at 72°C for 7 minutes. The thermocycling conditions for the second reaction included an initial denaturation for 3 minutes at 94°C, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 58°C for 2 minutes, extension at 72°C for 1.5 minutes, and a final extension at 72°C for 7 minutes (Cordeiro et al., 2020).

GAPDH PCR

After performing a nested PCR to detect *E. canis* DNA, samples that tested negative were then subjected to a conventional PCR to amplify the gene for the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The purpose of this step was to assess the DNA integrity and to minimize the likelihood of false negatives due to the presence of PCR inhibitors. The primers used were GAPDH-F (5′-CCTTCATTGACCTCAACTACAT-3′) and GAPDH-R (5′-CCAAAGTTGTCATGG ATGACC-3′), which correspond to conserved sequences found in all mammals that anneal to a specific sequence of the GAPDH gene, resulting in the production of an amplified product of 400 base pairs (Birkenheuer et al., 2003). The preparation of the reagent mix and the programming of the thermocycler were adapted and carried out according to the protocol previously described by Lacerda et al. (2020). The PCR was performed with 2.5 µL of DNA sample in 25 µL of reaction mix, which included 0.2 mM of each dNTP, 10X Buffer, 2.0 mM $MgCl_2$, 1.0 µM of each primer, 1.25U of Platinum Taq DNA Polymerase (Invitrogen™, Carlsbad, California, USA) and ultrapure water to complete the final volume. The thermocycling conditions consisted of an initial denaturation for 5 minutes at 95°C, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 1 minute, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes.

DNA agarose gel electrophoresis

The obtained amplicons were subjected to agarose gel electrophoresis at 2% concentration, using SYBR™ Safe DNA Gel Stain (Invitrogen, California, USA) for visualization. A 1 Kb Plus DNA Ladder™ (Invitrogen, California, USA) served as the molecular weight marker to determine the size of the amplified products. Imaging was conducted using the L-PIX EX electrophoresis gel photo-documentation system (Loccus®, São Paulo, Brazil), and samples were deemed positive if they showed a band at the same height as the positive control.

Sequencing

The amplicons from four samples, contained in the reaction tubes, were purified using the PureLink™ PCR Purification kit (Invitrogen, California, USA) and subsequently sent for sequencing. The technique used was capillary electrophoresis (modified Sanger sequencing) performed on an ABI 3500XL sequencer (Applied Biosystems™, California, USA), using both oligonucleotide primers used in the nested PCR. Chromatogram analysis was conducted using FinchTV software version 1.5.0. The consensus sequences were obtained through the

QIAGEN CLC Genomics Workbench software version 21.0 and compared with sequence data available in GenBank using BLAST. The sequences were deposited in GenBank under accession numbers OR978368, OR978369, OR978370, and OR978371. The identity of the detected *E. canis* species was determined based on the closest match in BLAST, considering a minimum similarity value of 98-100% for GenBank entries.

Statistical analysis

The data regarding the complete blood count and serum biochemistry of animals testing positive and negative for *E. canis* were subjected to the Kolmogorov-Smirnov test to confirm normality. In cases of homoscedasticity, the Student's t-test was conducted, while the Mann-Whitney test was used for heteroscedasticity. The presence or absence of anemia, thrombocytopenia, and leukopenia were also evaluated in relation to *E. canis* infection using the chi-square test with Yates' correction. The results of the nested PCR for *E. canis* and the data collected from the semi-structured interviews were tabulated and analyzed using the EPI INFO statistical software, version 3.5.2. The odds ratio (OR) from the bivariate analysis was calculated along with measures of association and a 95% confidence interval. Biologically plausible variables were assessed for collinearity using the Spearman correlation (p<0.8) in the Bioestat 5.0 statistical program, and then included in the preliminary model for unconditional logistic regression. The final model was constructed through the backward elimination of variables.

Results and Discussion

Molecular diagnosis

In this study, a prevalence of 30.8% (122/396 dogs) of *E. canis* infection was observed in the city of Porto Seguro, using the nested PCR technique. All samples negative for *E. canis* tested positive for the GAPDH gene. This high frequency aligns with previous studies that also used molecular diagnostics, the same dog population type (healthy, household, and living in both urban and rural areas), and similar climatic conditions (contributing to the vector's maintenance) (Cordeiro et al., 2020; R. L. da Costa et al., 2019; Guedes et al., 2015; Paulino et al., 2018).

In general, frequencies higher than those observed were found in blood samples from dogs treated in hospitals (Ramos et al., 2010; Santos et al., 2009; Soares et al., 2017), which were clinically suspected of diseases transmitted by ticks, and in samples from stray dogs captured by the Center for Zoonoses Control (CZC) (Brandão et al., 2019), which are more exposed to tick parasitism compared to household dogs, thus overestimating the positivity of the results.

Laboratory findings

Ehrlichia spp. morulae were observed in only 0.5% (2/396) of the blood smears, which is consistent with previous research that also reported the low sensitivity of this technique (Cirino et al., 2021; Rotondano et al., 2015).

The blood count parameters for dogs naturally infected and not infected by *E. canis* are presented in Table 1. A significant reduction in the average values of erythrocytes ($p<0.001$), hemoglobin (Hb) ($p<0.001$), packed cell volume (PCV) ($p<0.001$), mean corpuscular volume (MCV) ($p < 0.001$), and platelets ($p<0.001$) was observed in infected dogs compared to those not infected. There was also a significant increase in the mean corpuscular hemoglobin concentration (MCHC) ($p<0.001$), total plasma proteins (TPP) ($p<0.001$), and lymphocytes (p value <0.006) in infected animals compared to the results of the non-infected. It is important to note that in infected dogs, the averages for platelets and PCV were below the established reference range for the species, while the average for TPP was above the range. All other parameters in infected dogs, which were statistically significant compared to non-infected dogs, remained within the reference range. Moreover, it was identified that dogs positive for *E. canis* were 6 times more likely to have thrombocytopenia, 5.5 times more likely to have leukopenia, and 4 times more likely to have anemia compared to dogs negative for this pathogen (Table 2).

Table 1
Average values and standard deviation of hematological parameters in dogs naturally infected with *Ehrlichia canis* compared to non-infected dogs in Porto Seguro, BA

Parameters	Infected Animals (n=122)	Non-Infected Animals (n=274)	Reference Ranges[1]	*p*-value
Erythrocytes (x 10^6/µL)	5.70 (± 1.16)	6.66 (± 1.24)	5.5 – 8.5	< 0.001
Hemoglobin (g/dL)	12.52 (± 2.72)	14.79 (± 2.89)	12 – 18	< 0.001
Hematocrit (%)	36.77 (± 8.39)	44.34 (± 9.89)	37 – 55	< 0.001
MCV (fL)	64.22 (± 3.86)	65.98 (± 4.68)	60 – 77	< 0.001
MCHC (%)	34.15 (± 1.85)	33.51 (± 2.26)	32 – 36	< 0.001
TPP (g/dL)	8.24 (± 1.46)	7.30 (± 3.67)	6.0 – 8.0	< 0.001
Platelets (/µL)	131,273 (±100,504)	218,580 (± 117,923)	200,000 – 500,000	< 0.001
Leukocytes (/µL)	13,991.8 (± 5,071.2)	13,575.9 (± 5,236.8)	6,000 – 17,000	0.532
Segmented Neutrophils (/µL)	7,632.2 (± 3,669)	8,042.5 (± 3,529.6)	3,000 – 11,500	0.32
Lymphocytes (/µL)	4,301.9 (±3,146.4)	3,302.9 (± 2,132.4)	1,000 – 4,800	0.006
Monocytes (/µL)	734.38 (± 542.44)	752.67 (± 613.03)	150 – 1,350	0.902
Eosinophils (/µL)	1,326.4 (± 1206.9)	1,443.7 (± 1,369.3)	100 – 1,250	0.462

[1]Reference Ranges: Jain (1993).

Table 2
Bivariate analysis of the variables "Has Anemia", "Has Leukopenia", and "Has Thrombocytopenia" associated with *Ehrlichia canis* infection in naturally infected dogs in the municipality of Porto Seguro, BA

VARIABLES	*Ehrlichia canis*				*Odds ratio*	
	POSITIVE		NEGATIVE		95% CI	p-value
	N	%	N	%		
Has Anemia						
Yes	61	53.04	54	46.96%	4.1 (2.56-6.47)	<0.0001
No	61	21.70%	220	78.30%		
Has Leukopenia						
Yes	7	70.00%	3	30.00%	5.5 (1.40-21.64)	0.017
No	115	29.79%	271	70.21%		
Has Thrombocytopenia						
Yes	99	46.48%	114	53.52%	6.0 (3.61-10.09)	<0.0001
No	23	12.57%	160	87.43%		

Thrombocytopenia was the most common hematological alteration observed in dogs positive for *E. canis* in this study, aligning with findings from other studies (Harrus et al., 1998; Waner et al., 1997). Waner et al. (1997) suggest that the mild and persistent thrombocytopenia at this phase can be attributed to the continuous production of anti-platelet antibodies in response to the presence and proliferation of the ehrlichial organism. It is worth noting that, in our study, 114 dogs that tested negative for *E. canis* also presented with thrombocytopenia. Therefore, although thrombocytopenia is a common alteration at all stages of CME (Bulla et al., 2004; Harrus et al., 1996b; Mylonakis et al., 2004; Waner et al., 1997), it is not sufficient and should not be used to confirm the diagnosis of Ehrlichiosis in dogs.

The total white blood cell count of the infected animals was within the reference ranges for the species. This observation supports previous findings (Harrus et al., 1998; Waner et al., 1997). Only ten dogs in the study were leukopenic, which limits the scope of the discussion. Leukopenia is a consistent finding in dogs during both the acute and chronic phases of CME (Castro et al., 2004; Gianopoulos et al., 2016; Harrus et al., 1996a; Mylonakis et al., 2004; Rungsipipat et al., 2009), but not in the subclinical phase (Harrus et al., 1998; Waner et al., 1997).

Anemia was identified in half of the dogs that tested positive, however, only the PCV levels were below the reference ranges, contrasting with previous studies (Harrus et al., 1998; Waner et al., 1997). Waner et al. (1997) reported that none of the dogs in the subclinical phase of CME were explicitly anemic, yet they observed declines in erythrocyte parameters that were detected inconsistently and concluded that these parameters are not reliable indicators for

CME. Anemia is often identified in dogs with CME during both acute and chronic phases (Borin et al., 2009; Mylonakis et al., 2004; Parashar et al., 2016; Waner et al., 1997). The anemia caused by *E. canis* is associated with the removal of erythrocytes from circulation by the mononuclear phagocyte system (MPS) and lysis by the complement system. In addition, there is also suppression of erythropoiesis in the bone marrow, primarily during the chronic phase of CME, and bleeding in dogs with marked thrombocytopenia (Moreira et al., 2003; Mylonakis et al., 2010).

The TPP were found to be above the reference ranges in infected animals (Table 1). The observed hyperproteinemia is consistent with other studies that have reported that dogs infected with *E. canis* have high total protein content (Gould et al., 2000; Harrus et al., 1996a).

The results of the biochemical analyses are described in Table 3. There was no statistically significant difference between the biochemical findings of the positive and negative animals, except for ALT. However, the values remained within the reference range for both groups.

Table 3
Average values and standard deviation of biochemical analyses in dogs naturally infected with *Ehrlichia canis* compared to non-infected dogs in Porto Seguro, Bahia

Parameters	Infected Animals (n=122)	Non-Infected Animals (n=274)	Reference Ranges[1]	*p*-value
Urea (mg/dL)	35.55 (± 15.37)	36.69 (± 14.87)	21.4 – 59.9	0.49
Creatinine (mg/dL)	1.01 (± 0.23)	1.04 (± 0.28)	0.5 – 1.5	0.3786
ALT (UI/L)	37.29 (± 28.73)	45.97 (± 52.74)	21 – 73	0.0225
AST (UI/L)	36.68 (± 38.16)	37.46 (± 31.76)	21 – 45	0.3984
FA (UI/L)	54.68 (± 37.86)	67.88 (± 104.46)	20 – 156	0.3337

[1] Reference ranges: Kaneko et al. (1997).

Associated factors

Supplementary Material 1 presents the bivariate analyses conducted between the exposure variables (biologically plausible) and the outcome (positive/negative to *E. canis*). It was found that none of the exposure variables showed collinearity, allowing for their inclusion in the preliminary model of the non-conditional logistic regression (Supplementary Material 2). In the final model (Table 4), the variables "defined breed," "Lives near a deforested area," and "Is kept indoors" were identified as protective factors, while "Ticks" and "Lives in an urban area" were considered risk factors for *E. canis* infection.

Table 4
Final model of the unconditional logistic regression for factors associated with *Ehrlichia canis* infection in dogs in Porto Seguro, BA

Variables	Odds ratio	CI (95%)	*p*-Value
Lives near deforested area	0.56	0.31 – 0.99	0.05
Presence of ticks	1.66	1.05 – 2.63	0.03
Is kept indoors	0.51	0.31 – 0.85	0.01
Purebred	0.33	0.18 – 0.59	0.0002
Lives in urban area	1.90	1.19 – 3.04	0.007

p<0.001 Likelihood = 44.43

These findings support the research of Carlos et al. (2011), Guedes et al. (2015), and Paulino et al. (2018), which demonstrated a positive association between tick infestation and infection by *E. canis* in dogs. The climatic conditions in the Extreme South region of Bahia play a significant role in the population dynamics of ticks. The high temperature and humidity of the tropical climate provide a conducive environment for the development of the *R. sanguineus* from the tropical clade, known to be an efficient vector for the transmission of *E. canis* (Cicuttin et al., 2015; Moraes et al., 2015). This finding underscores the importance of preventive tick control measures in dogs to reduce the risk of *E. canis* infection and other tick-borne diseases.

This study found that dogs living in urban areas are more prone to infection with *E. canis* compared to dogs from rural areas. This finding supports the results of Guedes et al. (2015), who reported that dogs from rural areas were less likely to be afflicted with the disease when compared to their urban counterparts. *R. sanguineus* is the tick species most frequently identified in studies involving dogs that live in urban areas (Dantas-Torres et al., 2004; Labruna & Pereira, 2001; Rotondano et al., 2015; Soares et al., 2020; Souza et al., 2010).

Keeping dogs indoors has been characterized as a protective factor against infection by *E. canis,* according to this study. This is likely due to the reduced exposure of these dogs to other animals infested with ticks, thereby lowering the chances of being bitten by infected ticks. These results agree with the findings of Paulino et al. (2018), who reported that dogs with access to the outdoors are more likely to be infected by *E. canis.* It is important to note that the protection offered by keeping dogs indoors may vary according to the geographic region and the environmental conditions in which the animal lives (Figueredo et al., 2017; Guedes et al., 2015).

This study identified that having a defined breed acts as a protective factor against *E. canis* infection. However, it is important to note that this variable may have been influenced by bias due to unconsidered factors, such as the higher number of mixed-breed animals in the study and the financial

situation of the dog owners, which was not addressed in the semi-structured interview. Further research is necessary to better understand this association and to consider other potential confounding factors that could influence the results.

It was observed that living near deforested areas acts as a protective factor against infection by *E. canis.* This finding can be explained by the fact that the lifecycle of the *R. sanguineus* tick thrives in urban environments (Labruna & Pereira, 2001). Moreover, it is possible that in deforested areas, tick dispersion may occur more intensely, leading to a reduced exposure of dogs to infected ticks.

Conclusions

The high frequency observed in asymptomatic animals in this study highlights an insidious course in most cases of CME and reinforces the need for monitoring the disease during routine clinical evaluations of the animals. Additionally, it emphasizes the necessity of adopting effective measures for vector control in both the environment and among the animals, given that the municipality has excellent climatic characteristics for the vector's development.

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