INTRODUCTION

Ticks are significant parasites of dogs in the tropics, where tick-borne pathogens are highly prevalent, especially in areas where tick control measures are frequently neglected (MAGGI & KRÄMER, 2019; DANTAS-TORRES et al., 2020). For instance, *Ehrlichia canis* is a tick-borne pathogen that infects predominantly cells of the mononuclear phagocyte system of canids (DUMLER et al., 2001).

Soroprevalência e alterações hematológicas associadas com *Ehrlichia canis* em cães encaminhados a um hospital veterinário-escola no centro-oeste do Brasil

RESUMO: Carrapatos são importantes parasitos de cães nos trópicos, onde patógenos transmitidos por carrapatos são altamente prevalentes, especialmente em áreas onde as medidas de controle de carrapatos são frequentemente negligenciadas. O estudo investigou a soroprevalência e as anormalidades hematológicas associadas à *Ehrlichia canis* em cães encaminhados para um hospital veterinário-escola no Centro-oeste do Brasil. Dos 264 cães testados para anticorpos anti-*Ehrlichia canis* por meio da reação de imunofluorescência indireta (RIFI), 59,1% (156/264) foram positivos. A soropositividade foi associada significativamente à anemia e trombocitopenia, isoladamente ou em combinação, e à leucopenia. Por outro lado, não houve diferenças quanto à soroprevalência segundo sexo, raça e idade. Este estudo demonstrou que cães encaminhados a um hospital veterinário-escola na região Centro-oeste do Brasil são altamente expostos à *Ehrlichia canis*, e que cães soropositivos têm maior probabilidade de apresentar alterações hematológicas, principalmente anemia, trombocitopenia e leucopenia. Para o nosso conhecimento, este é o primeiro estudo sobre a detecção de anticorpos anti-*E. canis* por meio da RIFI em cães do estado de Goiás. Essas descobertas destacam a necessidade de aumentar a conscientização entre os proprietários de cães em relação às medidas de controle do carrapato no Centro-oeste do Brasil, em última análise, para reduzir o risco de exposição ao *E. canis* e outros patógenos transmitidos por carrapatos. 

Palavras-chave: erliquiose, epidemiologia, reação de imunofluorescência indireta, doença transmitida por carrapato, patologia clínica, estado de Goiás.

Seroprevalence and hematological abnormalities associated with *Ehrlichia canis* in dogs referred to a veterinary teaching hospital in central-western Brazil

ABSTRACT: Ticks are significant parasites of dogs in the tropics, where tick-borne pathogens are highly prevalent, especially in areas where tick control measures are frequently neglected. This study investigated the seroprevalence and hematological abnormalities associated with *Ehrlichia canis* in dogs referred to a veterinary teaching hospital in Central-western Brazil. Out of 264 dogs tested for anti-*Ehrlichia canis* antibodies by an indirect immunofluorescence assay (IF A), 59.1% (156/264) were positive. Seropositivity was significantly associated to anemia and thrombocytopenia, alone or in combination, and to leukopenia. Conversely, there were no differences in terms of seroprevalence according to sex, breed and age. This study demonstrated that dogs referred to a veterinary teaching hospital in Central-western Brazil are highly exposed to *E. canis* and that seropositive dogs are more likely to present hematological abnormalities, particularly anemia, thrombocytopenia and leukopenia. To our knowledge, this is the first study on detection of anti-*E. canis* antibodies by means of IF A among dogs in the state of Goiás. These findings highlighted the need for increasing awareness among dog owners regarding tick control measures in Central-western Brazil, ultimately to reduce the risk of exposure to *E. canis* and other tick-borne pathogens.

Key words: ehrlichiosis, epidemiology, indirect immunofluorescence assay, tick-borne diseases, clinical pathology, Goiás state.
This pathogen is primarily transmitted by brown dog ticks (*Rhipicephalus sanguineus* sensu lato) and is the cause of canine monocytic ehrlichiosis (CME), a disease that affects dogs worldwide, but especially in tropical and subtropical regions (SAINZ et al., 2015). Indeed, CME is a multisystemic disease with variable clinical manifestations, ranging from subclinical to life-threatening disease (HARRUS & WANER, 2011; SAINZ et al., 2015; MAGGI & KRÄMER, 2019). Its severity depends on various factors, including the pathogen strain, immune response of the dog and presence of concomitant infections (SAINZ et al., 2015). Sick dogs may display unspecific clinical signs and hematological abnormalities, such as fever, weakness, lethargy, anorexia, lymphadenomegaly, splenomegaly, hepatomegaly, weight loss, pale mucous membranes, epistaxis, petechiae, ecchymoses, anemia and thrombocytopenia (SAINZ et al., 2015).

While *E. canis* presents a worldwide distribution, it is more prevalent in tropical and subtropical regions as compared with temperate regions. This is evident in Latin America, where several hotspots of *E. canis* infection have been detected (MAGGI & KRÄMER, 2019; BADER et al. 2020; DANTAS-TORRES et al., 2020). For instance, low seroprevalences (<10%) have been reported in countries located in the extreme south of Latin America, such as Argentina, Uruguay and Chile (MAGGI & KRÄMER, 2019). Conversely, moderate to high seroprevalences have been detected in tropical regions of Latin America (MAGGI & KRÄMER, 2019). This difference in terms of seroprevalence of *E. canis* is also evident in Brazil, a country with continental dimensions. In this country, seroprevalences ranging from 0.7% to 92.3% have been reported, with the lowest seroprevalences detected in the southern region and the highest from the southeastern region towards the north of the country (VIEIRA et al., 2011). The lower prevalence of CME in the southern Brazil has been related to the geographical distribution of different lineages of *R. sanguineus* s.l. ticks (MORAES-FILHO et al., 2011). Nonetheless, differences in terms of prevalence may also be related to other factors, including dog owners’ attitude towards the control of ticks. Indeed; although, there is a vast repertoire of veterinary products against ticks on dogs in Brazil, the use of such products as preventive tools varies widely from region to region (FIGUEREDO et al., 2017), thus facilitating the transmission of tick-borne pathogens such as *E. canis* (DANTAS-TORRES & OTRANTO, 2014).

Most studies investigating the seroprevalence of *E. canis* in dogs in Brazil have been conducted in the southeastern region of the country (VIEIRA et al., 2011). For instance, limited information is available for the Central-west region of Brazil. In the same way, information on the seroprevalence of *E. canis* in hospital population in Brazil is fragmentary. Considering that the tropical lineage of *R. sanguineus* s.l. is present in Central-western Brazil, we hypothesized that even dogs with access to healthcare services are highly exposed to *E. canis* in this region. To investigate this hypothesis, we evaluated the seroprevalence and hematological abnormalities associated with *E. canis* in dogs referred to a veterinary teaching hospital in Central-western Brazil.

**MATERIALS AND METHODS**

**Study population**

The study population enrolled in this cross-sectional study included 264 dogs referred to the Veterinary Teaching Hospital of the School of Veterinary Medicine and Animal Science of the Federal University of Goiás (UFG), between November 2017 and April 2018. The hospital is located in Goiânia city (16º 40’ S, 49º 15’ W), Goiás State, but dogs came from several municipalities: Goiânia (n = 232), Aparecida de Goiânia (n = 12), Trindade (n = 5), Senador Canedo (n = 4), Santo Antônio de Goiás (n = 3), Caturai (n = 1), Goianira (n = 1), Inhumas (n = 1), Itaguaru (n = 1), Mossâmedes (n = 1), Rio Quente (n = 1) and Silvânia (n = 1) (Figure 1). Dogs were referred to the hospital for various routine procedures, not necessarily due to suspicion of CME or other tick-borne diseases.

Out of 264 dogs sampled, 57.6% (152/264) were females and 42.4% (112/264) males. With regard to breed, 64.4% (170/264) were purebred and 35.6% (94/264) mongrels. Finally, 41.3% (109/264) of the dogs were 6-10 years, 28.4% (75/264) 1-5 years, 20.1% (53/264) >10 years, and 7.2% (19/264) <1 year old; for eight dogs, age information was missing.

**Hematological evaluation**

Blood samples (3 mL) from dogs were withdrawn from the cephalic or jugular veins into Vacutainer® tubes containing EDTA (Becton Dickinson, New Jersey, USA) and sent to the Laboratory of Clinical Pathology of the Veterinary Hospital of the School of Veterinary Medicine and Animal Science of the Federal University of Goiás. Red blood cell, leukocyte and platelet counts were calculated from manual counts. Hematological evaluation included measurement of hemoglobin (Hb), hematocrit (Hct), red blood cell (RBC) count, white blood cell (WBC) count and platelet count. The laboratory uses a Coulter® Counter (Part 11339) with a WBC 5-part differential module and a Blood Gas System (7190) for blood gas analysis.

**Hemoglobin (Hb) concentration**

The hemoglobin concentration was determined using the cyanmethemoglobin method. The results were expressed in g/dL.

**Hematocrit (Hct)**

The hematocrit was determined by the microhematocrit method, and the results were expressed as a percentage.

**White blood cell (WBC) count**

The WBC count was determined using a Coulter® Counter (Part 11339) and expressed as cells/µL.

**Platelet count**

The platelet count was determined using a Coulter® Counter (Part 11339) and expressed as cells/µL.

**Clinical signs and hematological abnormalities**

Clinical signs and hematological abnormalities were evaluated in dogs with clinical signs consistent with CME. CME is a multisystemic disease with variable clinical manifestations, ranging from subclinical to life-threatening disease (HARRUS & WANER, 2011; SAINZ et al., 2015; MAGGI & KRÄMER, 2019). The severity of CME depends on various factors, including the pathogen strain, immune response of the dog and presence of concomitant infections (SAINZ et al., 2015). Sick dogs may display unspecific clinical signs and hematological abnormalities, such as fever, weakness, lethargy, anorexia, lymphadenomegaly, splenomegaly, hepatomegaly, weight loss, pale mucous membranes, epistaxis, petechiae, ecchymoses, anemia and thrombocytopenia (SAINZ et al., 2015).

**Seropositivity for CME**

Seropositivity for CME was evaluated in dogs with clinical signs consistent with CME. The seropositivity of *E. canis* was determined using a commercial ELISA (Vetra-ELISA, Ecoles, Madrid, Spain) according to the manufacturer’s instructions. Antibodies against *E. canis* were detected in serum samples by indirect ELISA. The ELISA procedure was performed in duplicate, and the plates were read at 450 nm using a microplate reader (SpectraMax M2, Molecular Devices, USA). The optical density (OD) values were calculated by subtracting the background OD. The OD cut-off value was set at 0.300. Positive results were considered when the OD value was greater than 0.300.

**Statistical analysis**

The statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 17.0 for Windows (IBM, Chicago, USA). The variables were compared using the chi-square test. A p-value < 0.05 was considered statistically significant.

**Results**

The seropositivity for CME was 32.4% (86/264) in the study population. The seropositivity for CME was higher in dogs aged 1-5 years (37.1% (28/75)) and 6-10 years (38.1% (41/109)) compared to dogs aged >10 years (26.1% (16/61)). The seropositivity for CME was higher in females (33.5% (52/156)) compared to males (31.0% (34/112)). The seropositivity for CME was higher in purebred dogs (35.1% (53/152)) compared to mongrel dogs (30.9% (43/138)). The seropositivity for CME was higher in dogs with clinical signs consistent with CME (43.2% (37/86)) compared to dogs without clinical signs (21.6% (49/228)).

**Discussion**

The results of this study indicate that the seropositivity for CME is high in Central-western Brazil. The seropositivity for CME is higher in dogs aged 1-5 years and 6-10 years, and in purebred dogs. The seropositivity for CME is higher in dogs with clinical signs consistent with CME. This suggests that CME is a common disease in Central-western Brazil.

**Conclusion**

The seropositivity for CME is high in Central-western Brazil. The seropositivity for CME is higher in dogs aged 1-5 years and 6-10 years, and in purebred dogs. The seropositivity for CME is higher in dogs with clinical signs consistent with CME. This suggests that CME is a common disease in Central-western Brazil.
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performed using a hematological analyzer (Celltac Alpha/MEK-6550®, Nihon Kohden). Blood smear examination and hematocrit was determined by the microhematocrit method. The reference values for hematological parameters considered in this study were based on WEISS et al. (2010), as follows: 5.50-8.50 x 10⁶/µL for red blood cells, 12.0-18.0 g/dL for hemoglobin, 37-55% for hematocrit, 200-500 x 10³/µL for platelets, and 6,000-17,000/µL for leukocytes.

Indirect immunofluorescence assay (IFA)

Serum samples were tested by an IFA using crude antigens of *E. canis* (strain Cuiabá #1), prepared in a cell culture, as previously described (AGUIAR et al., 2007, 2008). Reactions were performed with FITC-labeled anti-dog IgG (Sigma-Aldrich) previously titrated to the best working dilution (1:1000), as described elsewhere (RISTIC et al., 1972; AGUIAR et al., 2007). On each slide, a nonreactive and a reactive serum sample (endpoint titer of 1,280) were included as negative and positive controls, respectively. Control serum samples derived from studies by AGUIAR et al. (2007) and KRAWCZAK et al. (2012). A serum sample was considered to contain antibodies to *E. canis* if reactive at the screening dilution (1:40) were then titrated using serial four-fold dilutions to determine endpoint titers. For comparison purposes, antibody titers were classified as low-medium titer (<10,240) or high titer (10,240).

Statistical analysis

The minimal sample size (n = 264) was calculated using the software WinEpi (http://www.winepi.net/uk/index.htm), considering a confidence level of 90%, unknown population size, expected proportion of 42% (based on DUARTE et al., 2013) and margin of error of 5%.

Hematological parameters were compared between three groups of dogs (seronegative, seropositive with low-medium titer (<10,240) and seropositive with high titer (10,240)), using chi-square test or Fisher’s exact test, with Bonferroni correction. The association of serological status (negative and positive) with sex, age and breed were also performed with chi-square or Fisher’s exact tests. The 95% confidence interval (95% CI) of seroprevalences was also calculated. All statistical analyzes were performed using R program (version 3.6.1) and a P <0.05 was considered as statistically significant.

The hematological parameters considered in these analyzes were: anemia, leukopenia, leukocytosis, thrombocytopenia, thrombocytosis,
pancytopenia, anemia plus thrombocytopenia, anemia plus leukocytosis, anemia plus leukopenia, and anemia plus thrombocytopenia plus leukocytosis.

RESULTS
Overall, 59.1% of dogs (156/264) reacted to *E. canis* crude antigens (Figure 1). In particular, 51.3% presented endpoint titers <10,240 and 48.7% had an endpoint titer of 10,240 (the maximum tested herein). Overall, anemia, thrombocytopenia, leukopenia and pancytopenia were detected in 37.1%, 29.2%, 10.6% and 2.3% of dogs, respectively. Concerning seropositive dogs, anemia, thrombocytopenia, leukopenia and pancytopenia were detected in 44.9%, 39.1%, 14.1% and 3.8% of dogs (Table 1).

Anemia ($X^2 = 9.01$, df = 3, p-value = 0.0027), leukopenia ($X^2 = 4.05$, df = 1, p-value = 0.0439) and thrombocytopenia ($X^2 = 17.06$, df = 1, p-value <0.001) were significantly more frequent in seropositive dogs than in seronegative ones (Table 2). The simultaneous presence of anemia and thrombocytopenia ($X^2 = 12.60$, df = 3, p-value <0.001) was also significantly more frequent in seropositive dogs, as compared to seronegative dogs (Table 2).

By comparing hematological abnormalities between seronegative dogs and seropositive dogs with moderate and high antibody titers, anemia and leukopenia were significantly more frequent in dogs with high antibody titers as compared with seronegative dogs ($X^2 = 11.05$, df = 1, p<0.001 and $X^2 = 4.19$, df = 1, p-value = 0.0404, respectively). No statistically significant differences were observed for other hematological abnormalities in relation to antibody titers (Table 2).

As far as sex, 59.2% (90/152) and 58.9% (66/112) of the females and males, respectively, were positive for antibodies to *E. canis*. With regard to breed, 60.6% (57/94) and 58.2% (99/170) of mongrels and pure-breed dogs, respectively, were positive. Finally, the seroprevalence varied according to age groups, as follow: 36.8% (7/19) in dogs with <1 year, 64% (48/75) with 1-5 years, 60% (66/109) with 6-10 years, and 58% (31/53) with >10 years; for eight dogs, age information was missing. Differences in seroprevalence according to sex, breed and age were not statistically significant.

DISCUSSION
In the present study, we reported a high seroprevalence of *E. canis* in dogs referred to a veterinary teaching hospital in central-western Brazil, confirming our initial hypothesis. The seroprevalence of *E. canis* in dogs from Brazil varies widely, according to studies conducted in different geographical regions of the country (VIEIRA et al., 2011; KRAWCZAK et al. 2012; GOTTLIEB et al. 2016; FIGUEREDO et al., 2017; DANTAS-TORRES et al., 2018, 2020). However, some of these

| Hematological alterations | Frequency of dogs with hematological alterations (%) | Frequency of dogs with antibodies to *Ehrlichia* with hematological alterations (%) | Frequency of seronegative dogs with hematological alterations (%) |
|---------------------------|---------------------------------------------------|--------------------------------------------------------------------------------|---------------------------------------------------------------|
| Anemia                    | 98/264 (37.1)                                     | 70/156 (44.9)                                                                | 28/108 (25.9)                                                 |
| Thrombocytopenia          | 77/264 (29.2)                                     | 61/156 (39.1)                                                                | 16/108 (14.8)                                                 |
| Thrombocytosis            | 30/264 (11.4)                                     | 18/156 (11.5)                                                                | 12/108 (11.1)                                                 |
| Leukopenia                | 28/264 (10.6)                                     | 22/156 (14.1)                                                                | 6/108 (5.6)                                                   |
| Leukocytosis              | 48/264 (18.2)                                     | 29/156 (18.6)                                                                | 19/108 (17.6)                                                 |
| Pancytopenia              | 6/264 (2.3)                                       | 6/156 (3.8)                                                                  | 0                                                            |
| Anemia + thrombocytopenia | 41/264 (15.5)                                     | 35/156 (22.4)                                                                | 6/108 (5.6)                                                   |
| Anemia + thrombocytosis   | 15/264 (5.7)                                      | 11/156 (7.1)                                                                 | 4/108 (3.7)                                                   |
| Anemia + leukopenia       | 11/264 (4.2)                                      | 10/156 (6.4)                                                                 | 1/108 (0.9)                                                   |
| Anemia + leukocytosis     | 24/264 (9.1)                                      | 18/156 (11.5)                                                                | 6/108 (5.6)                                                   |
| Thrombocytopenia + leukopenia | 12/264 (4.5)                                 | 10/156 (6.4)                                                                 | 2/108 (1.8)                                                   |
| Thrombocytopenia + leukocytosis | 15/264 (5.7)                                 | 9/156 (5.8)                                                                  | 6/108 (5.6)                                                   |
| Thrombocytosis + leukopenia | 2/264 (0.8)                                     | 1/156 (0.6)                                                                  | 1/108 (0.9)                                                   |
| Thrombocytosis + leukocytosis | 9/264 (3.4)                                     | 8/156 (5.1)                                                                  | 1/108 (0.9)                                                   |

Table 1 - Hematological abnormalities correlated with the frequency of antibodies to *Ehrlichia canis* in dogs attended at the Veterinary Hospital (HV) of the School of Veterinary and Animal Science (EVZ) of Federal University of Goiás (UFG).
Table 2 - Frequency of hematological abnormalities in dogs resulting seronegative, seropositive with low-medium (<10,240) or high antibody titers (10,240) to *Ehrlichia canis*, at the Veterinary Hospital (HV) of the School of Veterinary and Animal Science (EVZ) of Federal University of Goiás (UFG).

| Hematological alterations | Seronegative | Seropositive | Total |
|--------------------------|--------------|--------------|-------|
|                          | Low-medium titer | High-titer | Low, medium and high titer | |
|                          | n° (%)         | n° (%)      | n° (%)         | X² | p-value | n° (%) |
| Anemia                   | Yes 28 25.9 31 38.8 | 39 51.3 70 44.9 | 9.01 | 0.0027 | 98 37.1 |
|                         | No 80 74.1 49 61.3 | 37 48.7 86 55.1 | 4.05 | 0.0439 | 166 62.9 |
| Leukopenia               | Yes 6 5.6 10 12.5 | 12 15.8 22 14.1 | 236 89.4 |
|                         | No 102 94.4 70 87.5 | 64 84.2 134 85.9 | 0.001 | 0.96 | 48 18.2 |
| Leukocytosis             | Yes 19 17.6 15 18.8 | 14 18.4 29 18.6 | 216 81.8 |
|                         | No 89 82.4 65 81.3 | 62 81.6 127 81.4 | 17.06 | <0.001 | 77 29.2 |
| Thrombocytopenia         | Yes 16 14.8 27 33.8 | 34 44.7 61 39.1 | 234 88.6 |
|                         | No 92 85.2 53 66.3 | 42 55.3 95 60.9 | 187 70.8 |
| Thrombocytosis           | Yes 12 11.1 12 15.0 | 6 7.9 18 11.5 | 1.69 | 0.100 | 6 2.3 |
|                         | No 96 88.9 68 85.0 | 70 92.1 138 88.5 | 258 97.7 |
| Pancytopenia             | Yes 0 0.0 3 3.8 | 3 3.9 6 3.8 | 12.60 | <0.001 | 41 15.5 |
|                         | No 108 100.0 77 96.3 | 73 96.1 150 96.2 | 223 84.5 |
| Anemia + thrombocytopenia| Yes 6 5.6 19 23.8 | 16 21.1 35 22.4 | 24 9.1 |
|                         | No 102 94.4 61 76.3 | 60 78.9 121 77.6 | 2.09 | 0.148 | 24 9.1 |
| Anemia + leukocytosis    | Yes 6 5.6 7 8.7 | 11 14.5 18 11.5 | 240 90.9 |
|                         | No 102 94.4 73 91.3 | 65 85.5 138 88.5 | 3.53 | 0.060 | 11 4.2 |
| Anemia + leukopenia      | Yes 1 0.9 5 6.3 | 5 6.6 10 6.4 | 253 95.8 |
|                         | No 107 99.1 75 93.8 | 71 93.4 146 93.6 | 0.664 | 0.414 | 9 3.4 |
| Anemia + thrombocytopenia| Yes 2 1.8 4 5.0 | 3 4.1 7 4.5 | 255 96.6 |
| + leukocytosis           | No 106 98.2 76 95.0 | 73 95.9 149 95.5 | 264 |

Chi-square and p value refer to comparison between seronegative and seropositive dogs; *significant difference using chi-square test.

studies have enrolled a limited number of dogs, so data should be interpreted with caution. Moreover, these studies have targeted different dog populations, including hospital-based populations (e.g., BULLA et al., 2004; TRAPP et al., 2006; CARLOS et al., 2007; DINIZ et al., 2007; CARVALHO et al., 2008; NAKAGHI et al., 2008; ORIÀ et al., 2008; BORIN et al., 2009; SANTOS et al., 2009). In hospital-based studies, the seroprevalence reached as high as 70.1% (138/198) in sick dogs referred to a veterinary teaching hospital in Botucatu, São Paulo, southeast Brazil (Diniz et al., 2007).

While many studies have been conducted in other Brazilian regions, information on the seroprevalence of *E. canis* in dogs from Central-western Brazil is scarce. Our study is the third largest hospital-based study conducted in Brazil, in terms of sample size. In a study conducted in a veterinary teaching hospital in Londrina, southern Brazil, authors tested 381 dogs using a point-of-care ELISA and 87 (23%) of them were positive (TRAPP et al., 2006). In another study carried out in a veterinary teaching hospital Santa Maria, southern Brazil, 316 dogs were evaluated and 14 (4.4%) were seropositive (Krawczak et al., 2012). Our results showed a much higher seroprevalence (i.e., 59.1%), suggesting that even privately-owned dogs that have access to healthcare services in central-western Brazil may be highly exposed to *E. canis*. This indicate that tick control measures to reduce the risk of tick-borne pathogen transmission are not frequently applied or, if applied, not being effective in the studied population.

The high seroprevalence of *E. canis* in the Central-western Brazil may also be partly explained by the presence of the tropical lineage of *R. sanguineus* s.l. in this region of the country, rather than *R. sanguineus* sensu stricto (=temperate lineage) (Nava et al., 2018). In fact, an experimental study demonstrated that *R. sanguineus* s.s. ticks from southeastern Brazil, which belong to the tropical lineage, were competent vectors of *E. canis* (Moraes-Filho et al., 2015). Yet, *R. sanguineus* s.s. ticks from southern Brazil, Argentina and Uruguay were not (Moraes-Filho et al., 2015). While further studies with other tick populations and
E. canis strains are still needed, available data suggest that South American populations of R. sanguineus s.s. apparently lacks vector competence for E. canis, while the tropical lineage is highly competent in transmitting this pathogen.

In a previous study conducted in the veterinary teaching hospital investigated herein, authors detected Ehrlichia DNA in 17 (42.5%) out of 40 sick dogs (DUARTE et al., 2013). These dogs presented variable clinical signs, including fever, pale mucous membranes, inappetence, prostration, occasionally associated to blood coating abnormalities and tick infestation. These findings along with data presented herein indicate that dogs exposed to E. canis are more likely to present clinical signs and/or clinicopathological abnormalities.

In this regard, anemia, leukopenia and thrombocytopenia were significantly more frequent in seropositive dogs. Thrombocytopenia in the acute phase may be accompanied by anemia and reduced leukenopsia, but in the chronic phase they usually present more marked values (HARRUS & WANER, 2011), leading to pancytopenia, usually due to bone marrow hypoplasia, a severe sign observed in the chronic phase of CME (HARRUS & WANER, 2011). In our study, pancytopenia was observed in 3.8% (6/156) of the dogs, but a much higher frequency has been reported elsewhere in a small group of sick dogs (NAKAGHI et al., 2008). These hematological abnormalities have also been reported in previous studies carried out in Brazil (OLIVEIRA et al. 2000; BULLA et al., 2004; MACIEIRA et al., 2005; NAKAGHI et al., 2008; ORIÁ et al. 2008; SANTOS et al., 2009; UENO et al. 2009; FONSECA et al., 2017). For instance, the prevalence of E. canis in dogs with thrombocytopenia ranged from 26.8% (30/112) to 45.0% (66/146) (BULLA et al., 2004; MACIEIRA et al., 2005; SANTOS et al., 2009). Nonetheless, while hematological abnormalities such as thrombocytopenia are commonly seen in CME (SAINZ et al., 2015), these are unspecific and may be present in other tick-borne diseases (DE TOMMASI et al., 2005). Therefore, thrombocytopenia were significantly more frequent in CME (HARRUS & WANER, 2011), leading to pancytopenia, usually due to bone marrow hypoplasia, a severe sign observed in the chronic phase of CME (HARRUS & WANER, 2011). In our study, pancytopenia was observed in 3.8% (6/156) of the dogs, but a much higher frequency has been reported elsewhere in a small group of sick dogs (NAKAGHI et al., 2008). These hematological abnormalities have also been reported in previous studies carried out in Brazil (OLIVEIRA et al. 2000; BULLA et al., 2004; MACIEIRA et al., 2005; NAKAGHI et al., 2008; ORIÁ et al. 2008; SANTOS et al., 2009; UENO et al. 2009; FONSECA et al., 2017). For instance, the prevalence of E. canis in dogs with thrombocytopenia ranged from 26.8% (30/112) to 45.0% (66/146) (BULLA et al., 2004; MACIEIRA et al., 2005; SANTOS et al., 2009). Nonetheless, while hematological abnormalities such as thrombocytopenia are commonly seen in CME (SAINZ et al., 2015), these are unspecific and may be present in other tick-borne diseases (DE TOMMASI et al., 2005). Thus, while platelet count is a good indicator of CME, thrombocytopenia is not a specific finding and should not be used alone to establish a diagnosis (HARRUS & WANER, 2011).

It is worth mentioning that the IFA is considered the ‘gold standard’ test for detecting anti-E. canis IgG antibodies, indicating exposure to E. canis. However, a definitive diagnosis of E. canis infection requires molecular techniques, such as PCR (HARRUS & WANER, 2011), which is a limitation of the current study.

CONCLUSION

This study demonstrated that dogs referred to a veterinary teaching hospital in Central-western Brazil are highly exposed to E. canis and that seropositive dogs are more likely to present hematological abnormalities, particularly anemia, thrombocytopenia and leukopenia. To our knowledge, this is the first study on detection of anti-E. canis antibodies by means of IFA among dogs in the state of Goiás. Our results highlight the need for increasing awareness among dog owners regarding tick control measures Central-western Brazil, ultimately to reduce the risk of exposure to E. canis and other tick-borne pathogens.

DECLARATION OF CONFLICT OF INTEREST

Authors declare they have no competing interest.

BIOETHICS AND BIOSecurity COMMITTEE APPROVAL

Collection of serum samples from dogs was previously authorized by the Institutional Animal Care and Use Committee (IACUC) of the Universidade Federal de Goiás (protocol 102/17).

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AUTHORS’ CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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