DOI: 10.5433/1679-0359.2019v40n2p981

Kidney disease in natural infection by *Ehrlichia canis* in dogs

Doença renal na infecção natural por *Ehrlichia canis* em cães

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Abstract

Canine monocytic ehrlichiosis, caused by the intracellular bacterium *Ehrlichia canis*, can affect different organs, including the kidneys, in different stages of infection, and kidney involvement is considered one of the main causes of death related to the disease. This study aimed to investigate the occurrence of kidney disease in dogs naturally infected with *E. canis* and to correlate antibody levels with the severity of renal disease. Serum concentrations of urea, creatinine, and proteins (albumin and globulin), along with urine concentration, urine gamma-glutamyl transferase, and urine protein levels, were evaluated in 60 dogs with *E. canis* infection diagnosed by polymerase chain reaction. The detection of anti-*E. canis* antibodies was also performed for each dog. Of the 60 dogs with *E. canis* infection, 73.33% presented anti-*E. canis* antibodies. Laboratory abnormalities consistent with renal disease were observed in 33 (55%) infected dogs, and of these, 43.3% were in stage I chronic kidney disease. A positive correlation was observed between antibody levels and total plasma protein ($p = 0.0332$) and serum globulin ($p = 0.0057$) levels. In this study, renal disease was observed on routine laboratory testing in 55% of dogs with monocytic ehrlichiosis; however, there was no correlation between the stage of renal disease and the antibody titer against *E. canis*.

Key words: Monocytic ehrlichiosis. Dog. Glomerulonephritis. Biomarker.

Resumo

A Erliquiose monocítica canina, causada pela bactéria intracelular *Ehrlichia canis,* pode acometer diferentes órgãos inclusive os rins, nas distintas fases da infecção, sendo considerada uma das principais causas de óbito relacionadas a essa doença. Este trabalho teve por objetivo investigar a ocorrência de doença renal em cães naturalmente infectados por *E. canis* correlacionando à gravidade da doença renal. Sessenta cães com infecção por *E. canis* diagnosticados pela reação em cadeia pela polimerase (PCR) foram avaliados a concentração sérica de ureia e creatinina, proteínas (albumina e globulinas), urinálise, gamaglutamil transferase urinária e proteinúria. Paralelamente foi pesquisado a presença de anticorpos anti-*E. canis* pelo ensaio imunoenzimático (dot ELISA). Dos 60 cães com infecção por *E. canis*, 73,33% apresentaram anticorpos anti- *E. canis*, enquanto, 33 (55%) cães apresentaram achados laboratoriais condizentes com doença renal, e destes 43,3% dos cães encontravam-se no estágio I da doença renal. Correlação positiva foi observada entre os níveis de anticorpos, globulina sérica (p=0,0057) e proteínas plasmáticas totais (p=0,0332). Neste estudo, a doença renal foi observada em

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Received: July 19, 2018 - Approved: Dec. 18, 2018

55% dos cães com erliquiose monocítica, utilizando exames laboratoriais empregados na rotina clínica, sem correlação com o estadiamento da doença renal, apesar dos altos títulos de anticorpos contra *E. canis*.
Palavras-chave: Erliquiose monocítica. Cão. Glomerulonefrite. Biomarcador.

Canine monocytic ehrlichiosis is caused by the intracellular bacterium *Ehrlichia canis* of the family Anaplasmataceae and has a worldwide distribution. Infected dogs may present in the acute or subclinical phases, while those who do not clear the infection advance to the chronic phase (WANER; HARRUS, 2013).

Clinical signs induced by *E. canis* include fever, anorexia, weakness, lethargy, lymphadenomegaly, hemorrhagic disorders, neurologic abnormalities and chronic kidney disease. In the acute phase of renal injury induction, mild glomerulopathy may occur, evidenced by fusion of the podocyte processes, leading to proteinuria (CODNER et al., 1992; CASTRO et al., 2004). In the subclinical phase, basement membrane thickening, mesangial proliferation, focal segmental glomerulosclerosis, and synechia, associated with strong IgM and C3 positivity, suggest that the deposition of immune complexes may cause renal damage (CRIVELLENTI et al., 2015). Similarly, chronic glomerulonephritis may be induced by the deposition of immune complexes (IQBAL; RIKIHISA, 1994).

Furthermore, chronic stimulation caused by *E. canis* may induce silent glomerulonephrotic changes that contribute significantly to the development of severe renal disease in dogs (HEIENE et al., 2007; GOLDSTEIN et al., 2013). Therefore, this work aimed to investigate the occurrence of renal disease in dogs naturally infected by *E. canis* and to evaluate the correlation between antibody titer and the stage of renal disease.

Dogs of different breeds, ages, and sexes, examined at a university veterinary hospital in the city of Cuiabá, Mato Grosso, with clinical signs and amplification of *E. canis* DNA by nested polymerase chain reaction (PCR) (MURPHY et al., 1998) from blood and/or bone marrow samples, were included in the study, after obtaining written free and informed consent from the owners.

During the clinical evaluation of the dogs, blood samples were collected by cephalic or external jugular puncture into tubes with and without the anticoagulant ethylenediaminetetraacetic acid (EDTA). In addition, samples of the first urine of the day were obtained by urethral catheterization or cystocentesis.

A complete blood count was performed using the PocH-100iv Diff Automated Hematology Analyzer (Roche®), considering reference values determined by Jain (1993). Measurements of serum urea, creatinine, total protein, and albumin levels were performed using commercial kinetic and/or colorimetric (Gold Analisa®) kits on a semiautomatic biochemical analyzer (SB 190-CELM®). The globulin level was calculated as the difference between total protein and albumin, and the albumin: globulin ratio was computed.

The urine samples were submitted to physical-chemical analysis and sedimentation, according to Kantek and Navarro (1996). In addition, urine creatinine and protein levels were measured using commercial kits (Gold Analisa®) to establish the urine protein: creatinine (UPC) ratio and the urine gamma-glutamyl transferase (GGT): creatinine ratio.

Serum samples were processed for the detection of IgG antibodies using the ImmunoComb® Canine *Ehrlichia* semiquantitative method, according to the manufacturer's instructions.

For data analysis, the age groups were defined as follows: puppies (0 to 12 months), adults (13 to 60 months), and elderly dogs (over 60 months). Breeds were categorized as those with a defined breed (CRD) and those without a defined breed (SRD).

For staging of the chronic renal disease (CKD), dogs were divided by serum creatinine level, urine concentration ability, and UPC, according to the International Renal Interest Society (IRIS, 2013) guidelines, as follows: stage 1 (creatinine < 1.4 mg/dl, non-azotemic animal, with isosthenuria and/or proteinuria of ≥ 0.5), stage 2 (creatinine between 1.4 and 2 mg/dl, mild azotemia), stage 3 (creatinine between 2.1 and 5 mg/dl, moderate azotemia), and stage 4 (serum creatinine > 5 mg/dL, severe azotemia). For analysis by Pearson's correlation between the antibody titer against *E. canis* and the stage of renal disease, the groups were subdivided as follows: Group 0 (dogs without renal disease), Group 1 (IRIS stage I renal disease dogs), and Group 2 (IRIS stage II, III, and IV renal disease dogs). The hematological, serum, and urine results were analyzed and were correlated with the antibody titer to *E. canis* by the Spearman or Pearson method, in the presence of normality, using Graph Pad Prism v.4.0 software.

During the study period, 60 dogs were diagnosed with monocytic ehrlichiosis. Of these, 39 (65%) were males and 21 (35%) females; 22 (36.7%) were puppies, 27 (45%) adults, and 11 (18.3%) elderly; and 30 (50%) were CRD and 30 (50%) SRD. On the serological analysis, 16 (26.7%) dogs had no antibodies to *E. canis*, 5 (8.3%) had antibody titers of 1:80, 19 (31.7%) had titers of 1: 320, and 20 (33.3%) had titers of 1: 640.

The mean, standard deviation, and minimum and maximum values of the hematological findings and serum and urine biochemistry results are shown in Table 1. No statistically significant correlation was found between the antibody titers against *E. canis* and routinely used tests for the detection of renal disease ($p \geq 0.05$) (Table 2). However, a positive correlation with total plasma protein ($r = 0.41$) and serum globulin ($r = 0.35$) and a negative correlation with serum albumin ($r = -0.27$) and albumin: globulin ratio ($r = -0.45$) were observed.

Table 1. Mean, standard deviation, and minimum and maximum values of the hematological findings and serum and urine biochemistry results of 60 dogs with monocytic ehrlichiosis.

Parâmetros	Cães infectados por *Ehrlichia canis* (n=60)				
	Média	Desvio	Mediana	Mínima	Máxima
Hematocrit (%)	30,35	11,16	30,5	8,2	49
Platelets (10^3/ul)	132,2	122,5	82,0	01	459
Total Plasma Protein (g/dl)	7,25	1,46	7,0	3,6	12
Urea (mg/dl)	61,11	74,53	42	14	500
Creatinine (mg/dl)	1,35	1,73	0,9	0,4	9,3
GGTU (UI/L)	93,53	73,19	76	15	391
GGT:CRU	1,132	1,88	0,48	0,01	8,88
UPC	0,83	1,14	0,29	0,00	5,04
Urinary Density	1037	16,82	1042	1006	1096
Total Serum Proteins	6,68	1,87	6,70	2,60	13,70
Albumin	2,43	0,83	2,50	1,00	4,20
Globulin	4,24	1,77	3,95	1,60	12,10
Albumin:Globulin	0,65	0,34	0,57	0,13	1,90
Sorology	316,8	252,7	320	00	640

GGTU: urine gamma-glutamyl transferase; GGT:CRU: urine gamma-glutamyl transferase (GGT): creatinine ratio; UPC: urine protein: creatinine (UPC) ratio; Albumin:Globulin: albumin: globulin ratio.

Considering the IRIS classification, 33 (55%) dogs had renal impairment in various stages as follows: 26 (43.4%) in stage I, 1 (1.6%) in stage II, 3 (5%) in stage III, and 3 (5%) in stage IV. The remaining 27 (45%) had no kidney disease. A statistically significant correlation was observed between antibody titer and total plasma protein ($p = 0.03$, $r = 0.54$), total serum protein ($p = 0.01$, $r = 0.45$), and globulin concentration ($p = 0.00$, $r = 0.56$) in Group 0 and the albumin: globulin ratio ($p = 0.01$; $r = -0.48$) in Group 1.

Renal involvement in canine monocytic ehrlichiosis has been documented in natural and experimental infections (CODNER et al., 1992; LUCKSCHANDER et al., 2003). Renal damage has been associated with the elevated production of antibodies to *E. canis*, which induces the deposition of glomerular immune complexes (IQBAL; RIKIHISA, 1994; HEIENE et al., 2007; GOLDSTEIN et al., 2013). In this study, 73.3% of the dogs with *E. canis* infection had antibody titers higher than 1:80; however, a positive and statistically significant correlation was observed only with total plasma protein concentration and serum concentration of globulin, and a negative correlation was observed with serum albumin concentration and albumin: globulin ratio. This significance was also present when analyzed by IRIS group. Such results may reflect the increase of gamma globulins present in the blood due to the antigenic stimulation provided by the bacterium, leading to an alteration in the albumin: globulin ratio and an increase in total plasma and serum proteins (Table 2).

Table 2. Correlation between the anti-*Ehrlichia canis* antibody titers and the hematological findings and serum and urine biochemistry results of 60 dogs with monocytic ehrlichiosis.

Parâmetros	R	p-value
Hematocrit (%)	-0,2511	0,0529
Platelets (10^3/ul)	0,04104	0,7555
Total Plasma Protein (g/dl)	0,4117	0,0011*
Urea (mg/dl)	0,1058	0,4213
Creatinine (mg/dl)	0,1831	0,1615
GGTU (UI/L)	-0,1219	0,3576
UPC	0,2364	0,0690
GGT:CRU	-0,05357	0,6844
Total Serum Proteins	0,2103	0,1068
Albumin	- 0,2754	0,0332*
Globulin	0,3525	0,0057*
Albumin:Globulin	-0,4549	0,0003*
Urinary Density	-0,2100	0,1104

* statistically significant ($p< 0,05$); GGTU: urine gamma-glutamyl transferase; GGT:CRU: urine gamma-glutamyl transferase (GGT): creatinine ratio; UPC: urine protein: creatinine (UPC) ratio; Albumin:Globulin: albumin: globulin ratio.

Approximately 20% of the infected dogs displayed hyposthenuria, uremia, increased urine GGT, and proteinuria, which indicate renal injury, although the values were not statistically significant. According to Pressler (2013), urine GGT in dogs is considered not only an early marker of renal damage but also a persistent marker, which, together with hyposthenuria, may indicate a severe impairment of renal function (BARTGES, 2012). The observation of proteinuria in 43.3% of infected

dogs in this study may be associated with constant stimulation of the immune system leading to glomerulonephritis (CRIVELLENTI et al., 2015). However, the increase in urine protein may also be due to increased inflammatory proteins associated with the infectious disease (BONFANTI et al., 2004). These proteins have a low molecular weight and can cross the glomerular membrane freely; however, the determination of the specific proteins is an aspect not pursued in this study.

Serum creatinine is the endogenous marker most commonly used to evaluate renal function (FREITAS et al., 2014). Although only 11.7% of the infected dogs had an elevated serum creatinine, 43.3% of the dogs were diagnosed with stage I CKD. These dogs were non-azotemic, but had some renal changes, such as loss of urine concentration and/or renal proteinuria; however, no significant correlation was observed between serum creatinine level and IRIS group. In 10% of dogs with *E. canis* infection, the azotemia was considered moderate to severe, indicating severe renal impairment (IRIS, 2013).

In this study, renal disease was observed on routine laboratory tests in 55% of dogs with monocytic ehrlichiosis, without a correlation between the stage of renal disease and the antibody titer against *E. canis*.

Ethics Committee

This research was approved by the Committee on Ethics in the Use of Animals (CEUA) of UFMT under nº 23108.075691/2015-97.

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