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Prednisolone associated with doxycycline on the hematological parameters and serum proteinogram of dogs with ehrlichiosis

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ABSTRACT: *The objective of the present study was to assess the treatment of dogs with ehrlichiosis (tropical canine pancytopenia (TCP)) with doxycycline hydrochloride associated or not with prednisolone on the hematological profile and serum proteins. Ten dogs with TCP were selected in the Small Animal Medical Clinic Veterinary Hospital, Federal University of Campina Grande-UFCG-PB, Brazil. The diagnosis was obtained by clinical examination, hemogram and immunochromatographic test (with anti Ehrlichia canis antibodies). Samples were distributed randomly in two experimental groups of five animals each (n = 5), named GD and GDP. The GD group was treated with doxycycline (5 mg/kg, VO, BID for 28 days) and the GDP group was treated with doxycycline at the same dose and duration and prednisolone (2 mg/kg, VO, BID, for five days). Four blood collections were made during the treatment period: a base collection (M0), one at 10 days (M10), a second at 21 days (M21) and another at the end of the treatment (M28). These samples were used for the tests erythrogram, leucogram, plateletgram and proteinogram (dose of total proteins, pre-albumin, albumin, albumin, α-1 globulin, α-2 globulin, ß-globulin, γ-globulin and C- reactive protein) in the Clinical Pathology Laboratory, Veterinary Hospital-UFCG-PB. Results, that presented normal distribution, was submitted to the Tukey test (P<0.05). Comparison of treatments GD and GDP showed that both promoted discreet and similar response in the hematological parameters at different times. Results obtained allowed the conclusion that both therapeutic protocols resulted in clinical, hematological parameter and proteinogram improvement, but the use of prednisolone at the dose administered during the first five days of treatment did not show more beneficial effects than isolated administration of doxycycline.*
Key words: *tick, hemoparasitoses, immunoglobulins, protein.*

Efeitos da influência da prednisolona associada à doxiciclina nos parâmetros hematológicos e proteinograma sérico de cães com erliquiose

RESUMO: *Objetivou-se com este estudo avaliar os efeitos do tratamento de cães com erliquiose monocítica canina (EMC) com cloridrato de doxiciclina associada ou não à prednisolona sobre o perfil hematológico e de proteínas séricas. Foram selecionados, na Clínica Médica de Pequenos Animais do Hospital Veterinário da Universidade Federal de Campina Grande (UFCG), 10 cães com EMC. O diagnóstico foi obtido através de exame clínico, hemograma e teste de imunoensaio imunocromatográfico (com anticorpos anti Ehrlichia canis). As amostras foram distribuídas aleatoriamente em dois grupos experimentais, com cinco animais cada (n=5), denominados GD e GDP. O grupo GD foi tratado com doxiciclina (5 mg/kg, VO, BID durante 28 dias) e o grupo GDP, tratado com doxiciclina na mesma dose e duração e prednisolona (2 mg/kg, VO, BID, durante 5 dias). Durante o período de tratamento, foram realizadas quatro coletas de sangue; uma coleta basal (M0), uma com 10 dias (M10), uma aos 21 dias (M21) e outra ao final do tratamento (M28). Dessas amostras foram realizados eritrograma, leucograma, plaquetograma e proteinograma (dosagem de proteínas totais, pré-albumina, albumina, α-1 globulina, α-2 globulina, ß-globulina, γ-globulina e proteína C-reativa) no Laboratório de Patologia Clínica do Hospital Veterinário da UFCG. Os resultados que apresentaram distribuição normal foram submetidos ao teste de Tukey (P<0,05). Ao comparar os tratamentos GD e GDP, observou-se que os dois promoveram resposta discreta e semelhante dos parâmetros hematológicos nos diferentes momentos. Os resultados obtidos permitem concluir que ambos os protocolos terapêuticos resultaram em melhora clínica e dos parâmetros hematológicos e do proteinograma. Porém, o uso da prednisolona na dose empregada durante os primeiros cinco dias de tratamento não demonstrou efeitos mais benéficos do que a administração isolada da doxiciclina.*
Palavras-chave: *carrapato, hemoparasitose,imunoglobulinas, Proteína.*

INTRODUCTION

Canine monocytic ehrlichiosis (CME) is a severe infectious disease caused by bacteria of the genus *Ehrlichia*. In Brazil, the disease is distributed in all regions of the country. This is mainly due to its vector, the tick *Rhipicephalus sanguineus*. It is one of the most important diseases in dogs, represented by

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approximately 20% to 30% of the cases received in veterinary hospitals and clinics in Brazil (TRAPP et al., 2006; AGUIAR et al., 2007). In the city of Patos/PB, an occurrence ranging from 69.4% (TANIKAWA et al., 2013) to 72.5% (AZEVEDO et al., 2011) was observed. The relevance of this disease in the routine of the medical clinic is due to the high casuistry, the high morbidity and mortality caused by the infection and the lack of a vaccine.

The disease is multisystemic, being classified as acute, subclinical or chronic, according to clinical changes (WOODY & HOSKINS, 1991). Clinical manifestations are nonspecific, which makes diagnosis difficult. In general, dogs have depression, weight loss, anorexia, fever, lymphadenopathy and hemorrhage (HARRUS et al., 1996).

Doxycycline hydrochloride is the drug of choice for the treatment of CME, and its effectiveness in treating infected dogs has been well documented (NEER et al., 2002; LAPPIN, 2015; MYLONAKIS et al., 2019). However, the use of glucocorticoids in immunosuppressive doses as an adjunct to treatment is common (MYLONAKIS et al., 2019) Serum proteins participate in inflammatory processes, in immune-mediated reactions and in the formation of immune complexes (LAPPIN, 2015; NEER & HARRUS, 2015). Considering the lack of studies evaluating the profile of these proteins in the pathological process of CME treated with doxycycline and glucocorticoids in immunosuppressive doses, the objective was to evaluate the hematological and serum protein profile of dogs diagnosed with *Ehrlichia canis.*

MATERIALS AND METHODS

Ten dogs were selected for the study, between males and females, of different ages and weights, clinically diagnosed with CME from the outpatient care of the Small Animal Medical Clinic (CMPA), at the Professor Ivon Macêdo Tabosa University Veterinary Hospital (HVUIMT), from the Federal University of Campina Grande (UFCG), Patos, attended from October 2014 to January 2016.

For inclusion in the experiment, an initial evaluation of the animals was carried out, consisting of general clinical examination and determination of clinical parameters. After the clinical examination, whole blood was collected and sent to the Clinical Pathology Laboratory at HVUIMT/UFCG for a complete blood count. The animals with clinical signs and blood count suggestive of the disease (thrombocytopenia) were submitted to an immunochromatographic test (Alere Ehrlichiosis Ac Test Kit) that qualitatively detects (positive or negative) antibodies (IgG and IgM) anti *Ehrlichia canis* using whole blood samples.

Selected animals were divided into two groups with five animals each (n = 5) named doxycycline treated (GD) and doxycycline and prednisolone treated (GDP). Animals in the GDP group were chosen according to the intensity of their hematological changes. The dose used for doxycycline was 5 mg/kg, orally, every 12 hours, for 28 days. The dose adopted for prednisolone was 2 mg/kg, orally, every 12 hours, for 5 days (LAPPIN, 2015).

Hematological parameters were assessed at the following times: initial (M0), after 10 days (M10), after 21 days (M21) and at the end of treatment (M28). At each moment, 6 ml of blood were collected, which were distributed in equal parts in tubes with 10% Ethylenediamine Tetraacetate (EDTA) for blood count and tubes without EDTA for serum separation and determination of serum proteins.

Laboratory analyzes were performed at the Clinical Pathology Laboratory at HVUIMT/UFCG. The EDTA samples were counted on the Sysmex pocH-100iV™ automatic veterinary analyzer. The erythrocyte, leukocyte and platelet series were analyzed simultaneously, blood smears stained by the rapid panotic method (Laborclin) were performed for the differential count of leukocytes (monocytes, lymphocytes, neutrophils, eosinophils and basophils) and search *for E. canis* morulas.

Measurements of total plasma proteins and albumin were performed using colorimetric methods in a semi-automatic biochemical analyzer (BIO-START 200) according to the manufacturer's recommendations (Total Proteins Labtest and Albumin Vet Labtest). The measurement of C-reactive protein (PCR) was performed using the immunoturbidimetry method in an automatic analyzer (Cobas C 111). Serum protein concentrations of the pre-albumin, albumin, alpha 1, alpha 2, beta and gamma globulin fractions were determined by electrophoresis with the method adapted from LISBÔA et al. (2016). The proteinogram was obtained by horizontal electrophoretic fractionation on a 1% (w/v) agarose gel.

Results were subjected to normality tests. Those with normal distribution were submitted to Tukey's test ($P<0.05$). The deterministic trend of variables in the GD and GDP groups was analyzed using the logarithmic regression method. Changes in the mean of the data series were adjusted (measured variable) according to the treatment time. The model used was the logarithmic trend, described in the

equation: y = c ln x + b, where c and b are constant, and ln is the function of the natural logarithm.

RESULTS AND DISCUSSION

When analyzing the groups treated with doxycycline hydrochloride (GD) and treated with doxycycline hydrochloride associated with prednisolone (GDP) regarding the erythrocyte values, a statistical difference (P<0.05) was observed at the moments M0, M10 and M28 (Table 1), where only the GD remained within the normal range. The difference observed between the groups regarding the erythrocyte values is due to the possibility that the animals of the GD are at a stage of the disease in which the organism has already recovered from hemolysis or suppression of hematopoietic activity and; therefore, regardless of treatment, there would be no great variation in the values.

In GDP, it was possible to observe the presence of moderate to severe anemia in two animals, in which an increase in red blood cell count was observed from the 10th day of evaluation until M28. The anemic condition may be associated with a type II hypersensitivity immune response, which in immune-mediated or infectious diseases, such as ehrlichiosis, can stimulate an excess of phagocytic activity of macrophages and the recognition of erythrocytes as immunologically foreign by antibodies and complement, causing the lysis of these cells (TIZARD, 2008). The suppression of erythropoiesis in the bone marrow is another mechanism suggested in the etiopathogenesis of anemia in ehrlichiosis (MOREIRA et al., 2003). Spinal aplasia is in many cases associated with *E. canis* infection and is characterized by a decrease in all cell precursors, being able to be responsive within two weeks after removal of the underlying cause, in the case of the acute form, and when in the chronic form, recover after months, or fail to respond (FELDMAN et al., 2000).

Regarding hemoglobin content (Table 1), the treatments GD and GDP differed from each other (P<0.05) at all times. As observed regarding red blood cells, the averages of Hb and hematocrit content remained below the reference values in GDP throughout the evaluation period. However, there was an increase in these parameters from M10 on.

Considering the hematological changes observed in the GDP, which varied from moderate to severe, in addition to the presence of hypergammaglobulinemia and more severe or absent clinical changes, it is suggested that these animals were in the subclinical or chronic phase of the disease, since such changes are detected mainly in these phases (WANER et al., 1997; SYKES, 2013).

Table 1 - Means and variation coefficients of the erythrogram and platelet values of the group of animals treated with doxycycline hydrochloride (GD) and the group treated with doxycycline associated with prednisolone (GDP).

	Group	M0	M10	M21	M28	Reference values
Erythrocytes (x10⁶ /mm³)	GD	6.17 Aa	6.83 Aa	6.72 Aa	6.82 Aa	5.5 – 8.5 x 106/µL*
	GDP	4.24 Ba	5.22 Ba	5.32 Aa	5.14 Ba	
	CV(%)	24.91	17.89	16.14	15.26	
Hemoglobin content (g/dL)	GD	13.62 Aa	15.30 Aa	15.18 Aa	15.12 Aa	12 – 18 g/dL*
	GDP	9.28 Ba	11.50 Ba	11.60 Ba	11.22 Ba	
	CV (%)	23.64	17.93	17.20	14.99	
Hematocrit (%)	GD	38.78 Aa	43.32Aa	42.54 Aa	43.4 Aa	37 – 55 %*
	GDP	27.62 Ba	34.44 Aa	34.4 Aa	33.2 Ba	
	CV (%)	22.18	16.18	15.79	13.75	
Platelets (µL)	GD	123000 Aa	235300 Aa	147400 Ba	195600 Aa	200.000 – 500.000 µL*
	GDP	86000 Ab	166200 Aab	231600 Aa	178000 Aab	
	CV (%)	74.30	32.61	29.49	33.32	
TPP (g/dL)	GD	8.72 Aab	9.16 Aa	7.86 Ab	7.68 Ab	6.0–8.0 g/dL*
	GDP	9.48 Aa	8.96 Aa	9.04 Aa	8.36 Aa	
	CV (%)	11.08	13.82	13.46	9.50	

Means followed by different capital letters in the columns indicate a significant difference between groups (P<0.05). Means followed by different lowercase letters on the lines indicate a significant difference between moments (P<0.05), using Tukey's test. *Kaneko et al. (2008).

As shown in table 1, TPP did not differ statistically (P> 0.05) between the two treated groups. There were averages above the normal values in the GDP during all the evaluated moments, whereas in the DG, the values were above the reference only in M0 and M10, observing decrease and normality from M21 until the end of the treatment, evidenced statistically.

It was reported that in both treated groups, thrombocytopenia was present until the last evaluation (M28), with statistical difference (P<0.05) between GD and GDP only in M21, with an increase in the platelet values of GDP. The maintenance of hemoglobin and hematocrit values below the reference range at the end of treatment associated with corticosteroids suggested that the pathogen still persists in these animals.

Only in GDP means above normal values were detected for total plasma proteins (TPP), as it can be attributed to the hyperglobulinemia observed in this group. As for the evaluation of platelets, a better therapeutic response was observed in GDP, evidenced by an increasing trend curve (Figure 1).

In this study, the variation in platelet count can be justified by the immune reactions triggered by ehrlichiosis, such as increased consumption associated with vasculitis, immune-mediated destruction by antiplatelet antibodies, usually of the IgG subtype and destruction by complement fixation or phagocytosis (WANER et al., 1995; GRINDEM et al., 1999; TIZARD, 2008).

Animals with a more severe disease condition may induce a greater persistence of antiplatelet IgG titers, which may have influenced the decrease in platelet count at the last moment of collection, also suggested by the hypergammaglobulinemia observed during treatment in both groups (GRINDEM et al., 1999). The return of values to normal levels showed the spinal response capacity (XAVIER et al., 2009).

The studied groups showed statistical difference (P<0.05) regarding the total leukocyte count in M10, M21 and M28 (Table 2). GDP averages were significantly lower (P<0.05). As for the differential count, the groups did not present significant statistical variation (P>0.05) in the values of monocytes, segmented and banded neutrophils. Significantly lower values for leukocyte counts in GDP were attributed to the marked leukopenia observed in three animals, indicating that they were chronically infected. Such pathological clinical alteration is often seen at this stage of the disease and it is associated with bone marrow hypocellularity (BIRCHARD & SHERDING, 2005; LAPPIN, 2015).

As for the lymphocyte count, the treatments differed from each other (P<0.05) at moments M10, M21 and M28, where the values were

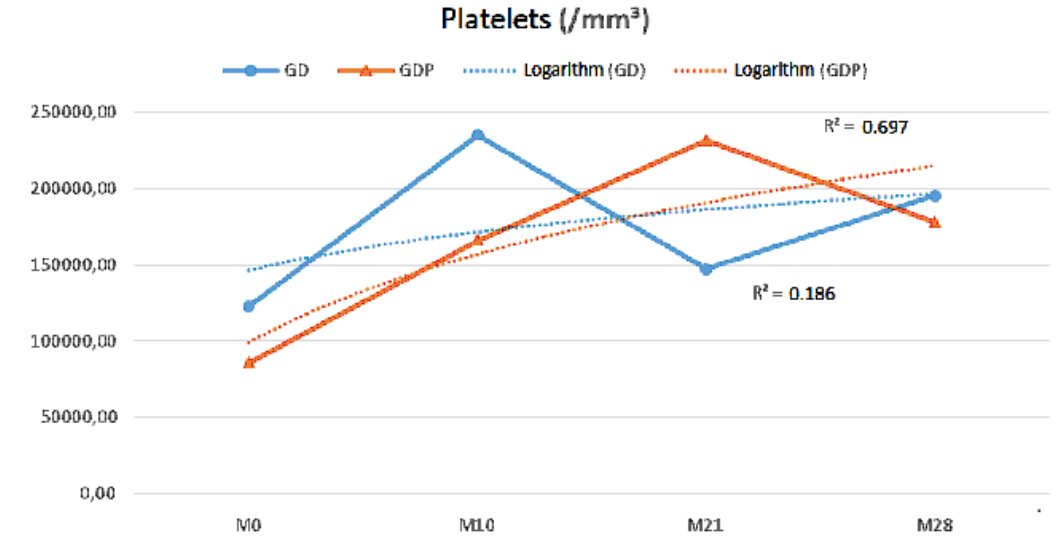

Figure 1 - Graphical representation of logarithmic trend of the group of animals treated with doxycycline hydrochloride (GD) and the group treated with doxycycline associated with prednisolone (GDP) averages, at each evaluation moment (M0, M10, M21 and M28), in dogs diagnosed with ehrlichiosis, at the HVUIMT/UFCG campus in Patos.

Table 2 - Means and variation coefficients of the leukogram values of the group of animals treated with doxycycline hydrochloride (GD) and the group treated with doxycycline associated with prednisolone (GDP).

	Group	M0	M10	M21	M28	Reference Values
Leukocytes (µL)	GD	11320 Aa	12380 Aa	14080 Aa	12480 Aa	6.000 – 17.000 µL*
	GDP	7390 Aa	6306 Ba	7944 Ba	6954 Ba	
	CV (%)	54.50	21.18	29.32	34.23	
Monocytes (µL)	GD	361.40 Aa	352.80 Aa	518.40 Aa	374.80 Aa	0 – 1.200 µL*
	GDP	674.50 Aa	234.40 Aa	153.40 Aa	103.80 Aa	
	CV (%)	73.10	64.18	94.71	120.15	
Lymphocytes (µL)	GD	3680.2 Aa	3976.6 Aa	4429.4 Aa	2886 Aa	1.000 – 4.800 µL*
	GDP	2195 Aa	863.4 Ba	1186.2 Ba	966.8 Ba	
	CV (%)	80.01	75.15	70.59	55.40	
Segmented neutrophils (µL)	GD	6570.80 Aa	6698.40 Aa	7789.80 Aa	7458.80 Aa	3.000 – 11.500 µL*
	GDP	6127 Aa	4753.60 Aa	6151.60 Aa	5500 Aa	
	CV (%)	66.76	24.28	44.96	44.93	
Banded neutrophils (µL)	GD	0.00 Aa	89.60 Aa	28.60 Aa	0.00 Aa	0 – 300 µL*
	GDP	0.00 Aa	14.20 Aa	0.00 Aa	4.80 Aa	
	CV (%)	0	173.74	316.23	316.23	
Eosinophils (µL)	GD	707.60 Aa	1241.40 Aa	1513.80 Aa	1760.40 Aa	100 – 1.250 µL*
	GDP	704.67 Aa	440 Aa	453.20 Ba	379.20 Ba	
	CV (%)	109.64	84.73	40.39	71.99	

Means followed by different capital letters in the columns indicate a significant difference between groups (P<0.05). Means followed by different lowercase letters on the lines indicate a significant difference between moments (P<0.05), using Tukey's test. *THRALL et al. (2012).

lower in GDP. From the five animals evaluated in this group, four presented lymphopenia in one or more evaluations. The lymphopenia observed in the GDP animals may be related to the pathogenicity of the agent, as well as the immunological capacity of the subjects evaluated, as they had lymphopenia since the first evaluation and even with the administration of exogenous glucocorticoid, the animals showed a slight to moderate increase in lymphocyte count.

The decrease in lymphocyte count is generally attributable to the response to endogenous or exogenous steroids. These contributed to the sequestration of lymphocytes in lymphoid organs, including bone marrow, in addition to potentiating the apoptosis of sensitive lymphocytes. In severe systemic diseases, the release of endogenous glucocorticoids plays an important role in the lymphopenia observed under these conditions (HARVEY, 2012; THRALL et al., 2012). In CME, lymphocytosis is the most reported change in the acute, subclinical and chronic phases (LITTLE, 2010; MCCLURE et al., 2010; LAPPIN, 2015); although, lymphopenia can be seen in several affected animals (SYKES, 2013). In the eosinophil count, the evaluations in M21 and M28 were statistically different (P<0.05), with lower averages in GDP.

There were no significant variations (P>0.05) in the evaluation of total proteins between the two treated groups (Table 3). There was a statistical difference (P<0.05) regarding the values of albumin and globulins.

Regarding the evaluation of total proteins, it is relevant that this parameter is not evaluated in isolation. As observed in the GDP, the total protein values slightly increased or within the normal range do not indicate the severity of the clinical condition, which in animals in this group was associated with hypoalbuminemia and hyperglobulinemia.

Albumin differed statistically between treatments at moments M10 and M21. GDP showed lower averages (P<0.05) than GD in all times evaluated for this parameter. The main factors associated with hypoalbuminemia when we do not observe concomitant decreases in globulins are the decrease in the production of albumin or its greater loss. As the liver has a reserve capacity, it would be necessary to lose 60% to 80% of its functional capacity to lead to a serum decrease in albumin. (THRALL et al., 2012).

The changes caused by *E. canis* in liver cells would have only a limited effect in altering the concentrations of this protein and for a short

Table 3 - Means and coefficients of variation of the serum proteinogram values of the group of animals treated with doxycycline hydrochloride (GD) and the group treated with doxycycline associated with prednisolone (GDP).

		M0	M10	M21	M28	Reference Values
Total proteins (g/dL)	GD	6.54 ± 0.53Aa	6.6 ± 0.81 Aa	6.7 ± 0.3 Aa	6.56 ± 0.66 Aa	5.4 – 7.1 g/dL*
	GDP	7.6 ± 1.02 Aa	7.5 ± 1.03 Aa	6.7 ± 0.65 Aa	6.8 ± 1.40 Aa	
	CV (%)	11.59	13.19	7.54	16.49	
Albumin (g/dL)	GD	3.71 ±0.85 Aa	3.774 ±1.04 Aa	4.174 ±1.39 Aa	3.696 ±1.43 Aa	2.6 – 3.3 g/dL*
	GDP	2.504 ±0.80 Aa	2.432 ±0.52 Ba	2.58 ±0.53 Ba	2.352 ±0.62 Aa	
	CV (%)	26.67	26.76	31.29	36.60	
Globulins (g/dL)	GD	2.83 ± 1.03 Ba	2.82 ± 1.28 Ba	2.52 ± 1.27 Ba	2.86 ± 1.20 Aa	2.7 – 4.4 g/dL*
	GDP	5.09 ± 1.00 Aa	5.07 ± 1.12 Aa	4.2 ± 0.81 Aa	4.45 ± 1.71 Aa	
	CV (%)	25.76	30.67	31.80	40.55	
C-reactive protein (mg/dL)	GD	0.01 ± 0.00 Ba	0.01 ± 0.01 Aa	0.02 ± 0.01 Aa	0.01 ± 0.00 Aa	Até 5.0 mg/L**
	GDP	0.022 ± 0.0 Aa	0.028 ± 0.01 Aa	0.04 ± 0.02 Aa	0.035 ± 0.02 Aa	
	CV (%)	0	53.74	60.18	48.30	
Pre-albumin	GD	0.536 ±0.22 Aa	0.5 ±0.21 Aa	0.41 ±0.12 Ba	0.538 ±0.22 Aa	-
	GDP	0.752 ±0.39 Aa	0.826 ±0.38 Aa	0.84 ±0.23 Aa	0.902 ±0.29 Aa	
	CV (%)	49.70	46.75	30.25	37.05	
Alfa-1	GD	0.172 ±0.06 Aa	0.174 ±0.07 Aa	0.21 ±0.14 Aa	0.206 ±0.08 Aa	0.62 – 0.92 g/dL*
	GDP	0.33 ±0.25 Aa	0.294 ±0.26 Aa	0.27 ±0.09 Aa	0.31 ±0.12 Aa	
	CV (%)	75.47	82.35	51.96	41.50	
Alfa-2	GD	0.34 ±0.15 Aa	0.278 ±0.10 Aa	0.332 ±0.25 Aa	0.344 ±0.23 Aa	0.37 – 0.71 g/dL*
	GDP	0.648 ±0.39 Aa	0.724 ±0.54 Aa	0.588 ±0.20 Aa	0.694 ±0.34 Aa	
	CV (%)	60,77	77,83	51,02	57,65	
Beta	GD	0.822 ±0.36 Aa	0.942 ±0.54 Aa	0.812 ±0.46 Aa	0.874 ±0.48 Aa	1.14 – 2.14 g/dL*
	GDP	1.316 ±0.49 Aa	1.1 ±0.55 Aa	1.07 ±0.16 Aa	1.142 ±0.24 Aa	
	CV (%)	40.73	53.93	36.99	37.91	
Gama	GD	0.956 ±0,38 Aa	0.932 ±0.48 Aa	0.764 ±0.36 Aa	0.898 ±0.36 Aa	0.43 – 0.81 g/dL*
	GDP	1.698 ±0.73 Aa	1.436 ±0.90 Aa	1.384 ±0.60 Aa	1.534 ±0.82 Aa	
	CV (%)	44.34	61.53	46.56	52.38	

Means followed by different capital letters in the columns indicate a significant difference between groups (P<0.05). Means followed by different lowercase letters on the lines indicate a significant difference between moments (P<0.05), using Tukey's test. *ABATE et al. (2000) **Cerón et al. (2005).

period of time, especially in the acute phase, when hypoalbuminemia was observed associated with the development of the expansion of foci of monocytic phagocyte cells around hepatic sinusoids, causing compression and necrosis of adjacent hepatocytes (REARDON & PIERCE, 1981).

The GDP albumin values observed in this study were below the normal range. Such alteration may be related to a chronic condition, with glomerular loss and reduction in the synthesis of albumin due to the increase in the production of globulins (HARRUS et al., 1999), as it was observed regarding the latter parameter. This variation between groups is due to the tendency to choose the treatment protocol associated with prednisolone in those animals that demonstrated a more severe clinical and hematological condition, justified by the supposed protective action of glucocorticoids against immunomediated events and the consequent development of more serious changes. (BIRCHARD & SHERDING, 2005; SYKES, 2013).

Glucocorticoids have several anti-inflammatory effects and endogenous release is one of the means of suppressing the inflammatory response when it is no longer needed, so that there is less tissue damage. Thus, one of the antagonistic actions of corticosteroids is related to capillary blood flow and vasodilation, preventing the formation of edema and loss of proteins to the damaged tissue. There is a moderate increase in the serum values of albumin induced by the use of glucocorticoids, even at anti-inflammatory doses (MARTINEZ-SUBIELA et al., 2004). In this study, the use of prednisolone

at immunosuppressive doses resulted in a slight increase in albumin values in three of the five treated animals; although, all remained below the reference range (THRALL et al., 2012).

As for globulins, in three moments (M0, M10 and M21) there was statistical variation (P<0.05) between the groups, with higher values being observed in all assessments of GDP. As for the fractions of α-1 globulin, α-2 globulin, ß-globulin and ɣ-globulin, there was no statistical difference (P>0.05) between the groups studied;although, the means of these parameters of GDP were higher in all the moments of evaluation, evidenced by the high coefficient of variation (CV).

Haptoglobin is one of the main proteins of the acute phase in dogs and it is located in the α-2 region of the electrophoretic tracing, presenting itself moderately elevated in acute inflammatory processes (TIZARD, 2008; MUNHOZ et al., 2012). Its increase has also been reported in hemolytic diseases, in diabetic dogs or after administration of glucocorticoids (HARVEY & WEST, 1987; ABATE et al., 2000; LOWRIE, 2009). It is suggested that the last factor is associated with the higher values of α-2 globulins reported in GDP compared to GD, since the other parameters evaluated in this group indicated that the animals were not acutely affected. However, results for α-2 globulins reported in this study do not corroborate those reported by other authors (HARVEY & WEST, 1987; LOWRIE, 2009). According to the authors cited above, α-2 globulin values remained significantly above the reference values, even after 9 days of glucocorticoid administration. Values reported in GDP remained within normal values in all assessments. As for the values of C-reactive protein, no significant variations were observed (P>0.05) among the moments of each treatment or among the means of each group.

Electrophoretic tracing revealed five protein fractions (pre-albumin, albumin, α-1 globulin, α-2 globulin, ß-globulin and ɣ-globulin). In the pre-albumin evaluation, values differed statistically (P<0.05) only at 21 days of treatment (M21). The GDP showed higher values of this protein throughout the treatment when compared to GD. In both groups, an increase in ɣ-globulin was observed, with markedly higher averages in GDP. The decrease observed in eosinophil values in animals treated with prednisolone was associated with the effects of glucocorticoid (THRALL et al., 2012; SYKES, 2013).

In both groups, hypergammaglobulinemia was observed, with markedly higher averages in GDP. The increase in gamma globulin values is a consistent finding in *Ehrlichia canis* infection and it can be observed from the first three weeks after infection and it persists indefinitely. (REARDON & PIERCE, 1981; KATAOKA et al., 2008; NEER & HARRUS, 2015). Its increase is represented by a non-specific humoral response and; although, two to three weeks after infection the anti-*Ehrlichia canis* antibodies can be identified, these are not the main source of gamma globulins (BURGHEN et al., 1971; BUHLES et al., 1974; NEER & HARRUS, 2015).

CONCLUSION

Results obtained in this research allowed us to conclude that both use of doxycycline and doxycycline associated with prednisolone resulted in clinical improvement, in hematological parameters and in serum proteinogram. However, the use of prednisolone during the first five days of treatment did not demonstrate any more beneficial effects than the administration of doxycycline alone.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The project was approved by the Research Ethics Committee (CEP) of the Rural Health and Technology Center (CSTR) of the Federal University of Campina Grande (UFCG), under protocol No. 202-2014.

DECLARATION OF CONFLICTS OF INTERESTS

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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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