

Hyperadrenocorticism in dogs attended at the Animal Health Service in the City of Rio de Janeiro, Brazil*

Maria Isabel Menezes Ramos¹, Paulo Daniel Sant'Anna Leal²⁺, Larissa Licurci de Oliveira Barbosa¹ and Carlos Wilson Gomes Lopes³

ABSTRACT. Ramos M.I.M., Leal P.D.S., Barbosa L.L.deO. & Lopes C.W.G. **Hyperadrenocorticism in dogs attended at the Animal Health Service in the City of Rio de Janeiro, Brazil.** [Hiperadrenocorticismo em cães atendidos em serviço de saúde animal na cidade do Rio de Janeiro, Brasil.] *Revista Brasileira de Medicina Veterinária*, 38(Supl. 3):42-48, 2016. Programa de Pós-Graduação em Ciências Veterinárias, Anexo 1, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR 465 Km 7, Campus Seropédica, RJ, 23.890-000, Brasil. E-mail: mariaisabelmrm@gmail.com

The hyperadrenocorticism (HAC), or Cushing's syndrome (CS) is an endocrine disease diagnosed in dogs associated with excessive endogenous glucocorticoid by pituitary or adrenal neoplasm, by iatrogenic (IHAC) induced by excessive administration of oral glucocorticoids, parenteral or topical. These changes are identified by physical examination, no specific laboratory tests (blood counts, urinalysis, lipid profile, alkaline phosphatase dosage and liver function profiles) and confirmed by a specific test. Clinical and laboratory manifestations vary among animals due to individual differences in cortisol sensitivity, with the absence or presence of clinical and laboratory signs. Due to the importance of this disease in the dog clinic for producing systemic effects, this work had the objective of studying in 21 dogs where they were tested by stimulation of adrenocorticotrophic hormone (ACTH) for HAC. Animals, which had some consistent factor for HAC as recurrent urinary tract infections, cholesterol or triglycerides and after fasting for more than 12 hours or alkaline phosphatase levels above the normal range without presenting hepatic and bone disease, they underwent adrenocorticotrophic hormone stimulation test (ACTH-adrenocorticotrophic hormone) synthetic dose 0.25 mL/ dog. Dogs with cortisol results after stimulation by ACTH, above 20 mcg/dL, had a confirmed diagnosis of hyperadrenocorticism. Of 21 dogs studied, 10 were diagnosed for HAC. Of these, eight dogs were positive for some species of blood parasites where six with monoinfection by *Anaplasma platys* and only one dog had multiple infection by *A. platys* and *Mycoplasma canis*. The hematological findings showed three dogs with anemia, four with thrombocytopenia, two with thrombocytosis, two with leukocytosis, six with eosinopenia, seven with neutrophilic, six with lymphopenia, five with monocytopenia and seven dogs with neutrophils on rods and left shunt, and six with hyperproteinaemia. Just a positive HAC dog

* Received on July 21, 2016.

Accepted for publication on November 17, 2016.

¹ Médica-veterinária. PPGCV. Anexo 1, IV, UFRRJ. BR 465 km 7. Campus Seropédica, 23.890-000, RJ. E-mails: mariaisabelmrm@gmail.com; larissalicurci@gmail.com - CNPq scholarship.

² Médico-veterinário, DSc. Programa de Pós-Graduação de Ciências Veterinárias (PPGCV). Anexo 1, Instituto de Veterinária (IV), Universidade Federal Rural do Rio de Janeiro (UFRRJ). BR 465 km 7. Campus Seropédica, 23.890-000, RJ. *Author for correspondence. E-mail: pauloleal@ctiveterinario.com.br - post doctoral training program.

³ Médico-veterinário, PhD, LD. Departamento em Parasitologia Animal, Anexo 1, IV, UFRRJ. BR 465 km 7. Campus Seropédica, 23.890-000, RJ. E-mail: lopescwg@ufrj.br - CNPq fellowship.

did not have any concomitant infection. The results of biochemical evaluation showed eight animals with elevated alkaline phosphatase. Finally, when diagnosing hyperdrenocorticism in dogs, no correlation was observed with laboratory findings, other than alkaline phosphatase above 984 U/L and positive dogs for HAC.

KEY WORDS. Cushing's syndrome, dogs, cortisol, adrenal, phosphatase alkaline.

RESUMO. O hiperadrenocorticismo (HAC), ou síndrome de Cushing (CS), é uma doença endócrina diagnosticada em cães associados à glicocorticoides endógenos excessivos por neoplasia pituitária ou adrenal, por iatrogenia (IHAC) induzida pela administração excessiva de glicocorticoides por via oral, parenteral ou tópica. Estas alterações são identificadas por exame físico, sem testes laboratoriais específicos como hemograma, análise de urina, perfil lipídico, dosagem de fosfatase alcalina e perfis de função hepática e confirmados por um teste específico. As manifestações clínicas e laboratoriais variam entre os animais devido às diferenças individuais na sensibilidade ao cortisol, com ausência ou presença de sinais clínicos e laboratoriais. Dada à importância desta doença na clínica de cães por produzir efeitos sistêmicos, este trabalho teve como objetivo estudar em 21 cães que foram testados com estimulação por hormônio adrenocorticotrópico (ACTH) para HAC. Cães que tinham algum fator consistente para HAC, como infecções urinárias recorrentes, colesterol ou triglicérides. Após jejum por mais de 12 horas ou níveis de fosfatase alcalina acima do intervalo normal sem apresentar doença hepática e óssea, foram submetidos a uma dose sintética de hormônio adrenocorticotrópico (ACTH-adrenocorticotropic hormone) 0,25 mL/cão. Cães com resultados de cortisol após estimulação com ACTH, acima de 20 mcg/dL, tiveram diagnóstico confirmado de hiperadrenocorticismo. Dos 21 cães estudados, 10 foram diagnosticados para HAC, destes, oito cães foram positivos para algumas espécies de parasitas sanguíneos onde seis com monoinfecção por *Anaplasma platys* e apenas um cão teve infecção múltipla por *A. platys* e *Mycoplasma canis*. Os achados hematológicos mostraram três cães com anemia, quatro com trombocitopenia, dois com trombocitose, dois com leucocitose, seis com eosinopenia, sete com neutrofílica, seis com linfopenia, cinco com monocitopenia e sete cães com neutrófilos em hastes e shunt esquerdo e seis com Hiperproteinemia. Apenas um cão HAC positivo não teve qualquer infecção concomitante. Os resultados da avaliação bioquímica mostraram oito com fosfatase alcalina elevada. Nenhuma das alterações hematológicas

observadas tem correlação positiva, embora somente os cães com fosfatase alcalina acima de 984 U/L tenham sido positivos para HAC. Finalmente, ao diagnosticar o hiperdrenocorticismo em cães, não foi observada correlação com achados laboratoriais, exceto a fosfatase alcalina acima de 984 U/L e cães positivos para HAC.

PALAVRAS-CHAVE. Síndrome de Cushing, cães, cortisol, adrenal, fosfatase alcalina.

INTRODUCTION

Pets are integrated to the families of intimate and very emotional way, which allows a higher quality of life for benefit, through the zeal of his tutors, the advances available in veterinary medicine. Consequently, it has been promoting longevity that is intimately associated with an increased incidence of diseases in which age may play a risk factor, such as oncology and endocrine diseases. Regarding these groups, hyperadrenocorticism is the main endocrine disorder observed in dogs (Ramsey & Ristic 2007, Parry 2012, O'Neill et al. 2016, Pöppel et al. 2016).

The hyperadrenocorticism (HAC), or Cushing's syndrome (CS) is an endocrine disease diagnosed in dogs associated with excessive endogenous glucocorticoid by pituitary or adrenal neoplasm, by iatrogenic (IHAC) induced by oral, parenteral or topical by excessive glucocorticoids administrations (Parry 2012, Bhavani et al. 2015, Scudder & Niessen 2015). The effects of clinical and laboratory manifestations vary among animals, due to the individual differences in cortisol sensitivity (Peterson 2007, Ramsey & Ristic 2007, Behrend et al. 2013), where the majority of dogs with HAC presenting hypercoagulability (Pace et al. 2013, Rose et al. 2013) and hyperalbuminemia (Chen et al. 2016). The clinical signs are consisted by polyuria, polydipsia, abdominal distension, polyphagia, muscle weakness, respiratory, neurological and skin changes (Ling et al. 1979, Huang et al. 1999, Feldman 2008, Nelson & Couto 2010, Nagata & Yuki 2015) associated with laboratory abnormalities as lymphopenia, eosinopenia, serum cholesterol levels, urine low density, elevation of serum alkaline phosphatase values, cholesterol and tri-

glycerides that even common in dogs with HAC, but they were not specific for all suspect animals (Ling et al. 1979, Teske et al. 1989, Bhavani et al. 2015), or do not have clinical repercussions (Ramsey & Ristic 2007). The suspicion of hyperadrenocorticism in dogs can be made from clinical signs, physical examination, routine laboratory tests and imaging diagnostic, but the diagnosis must be confirmed by the use of pituitary-adrenal function tests, based on corticosteroids values abnormally high in response to an intramuscular injection of adrenocorticotrophic hormone (Ling et al. 1979, Behrend et al. 2013, Mawby et al. 2014, Midence et al. 2015, Frank et al. 2015). Other screening tests include dexamethasone suppression test in a low dose, and urine cortisol/creatinine ratio. All tests can have false-negative/positive results (Peterson 2007), therefore, a combination of historical findings, clinical signs and laboratory evaluations are important in the diagnosis of CS in dogs (Parry 2012, Behrend et al. 2013).

CAH patients are immunosuppressed and inflamed patients in need, be assisted by the diagnosis of systemic concomitant diseases, including pancreatitis (Mawby et al. 2014), recurrent urinary tract infections (Ling et al. 1979) and high risk of sepsis, where serial blood tests may be useful in monitoring inflammation and systemic infections (Bastos et al. 2016), besides receiving cardiocirculatory monitoring (Soares et al. 2016). They require specialized monitoring for presenting risk of thrombosis due to hyper coagulation and acquired abnormalities, that are associated with thromboembolism and platelet changes (Kittrell & Berkwitz 2012, Rose et al. 2013, Pace et al. 2013, Park et al. 2013, Kang et al. 2016), plus cardiocirculatory monitoring at diagnosis and during treatment (Soares et al. 2016).

According to O'Neill et al. (2016) and Pöppel et al. (2016) to diagnose the disease, some risk factors should be considered. In addition to the clinical signs, blood count examinations, biochemical analysis, urinalysis, suppression test by dexamethasone in low or high dose and stimulation test by ACTH may not lead to definitive diagnosis because no biochemical laboratory tests or specific tests insulated hyperadrenocorticism are perfect (Parry 2012, Behrend et al. 2013). The most common signs observed are thinning of the skin, bilaterally symmetric alopecia, muscle atrophy, elevated alkaline phosphatase, alanine aminotransferase, triglycerides and cholesterol (Ling et al. 1979, Huang et al. 1999, Nagata & Yuki 2015). In addition to them, the increase

in renal resistance index, which also is a predictor of mortality in dogs with HAC (Chen et al. 2016).

Given the importance of this disease in the dog clinic, and because of the systemic effects produced, this study aims to study 21 suspect dogs that were tested by ACTH stimulation for the HAC and to correlate them with laboratory findings.

MATERIAL AND METHODS

Study conduction

The study was conducted from January 2014 to September 2016, where a total of 21 dogs suspected of having hyperadrenocorticism was attended by the veterinary service center *Intensive Care and Emergency Veterinary*, at Barra da Tijuca in the City of Rio de Janeiro, RJ. As the need for laboratory tests for diagnosis, blood samples were collected from dogs in a disposable syringe with needle 25x7mm, 4 to 5 mL. The total volume of blood collected from each patient, 2 ml was placed in a pediatric test tube without anticoagulant, while the remaining volume was placed in a pediatric test tube with ethylenediaminetetraacetic anticoagulant acid (EDTA). With the own syringe blood material was prepared two blood smears, as well as the preparation of two concentrate spurts of platelets and leukocytes on glass slides after separation of formed elements and plasma by the sample microhematocrit preparation without storage occur in cooling. Then it was arranged in a microcentrifuge (Micro-Hematócrito E3500108 **Microspin** CDR, Jaboticabal, SP). Blood samples were processed using an automatic machine (Ms4-Vet-Melet Schloesing Laboratories coulter) for white blood cell count, platelet parameters and erythrocyte, and measurement of total plasma protein was used a manual clinical refractometer model Q667 (**Quimis Aparelhos Científicos**). The samples without anticoagulant were centrifuged in centrifuge (Mod. 208N, Excelsa Baby, Fanem) at 350 x G for 10 minutes for serum separation. For alkaline phosphatase dosage (LAF) was used an automatic pipette 32µL serum and placed in reflectance photometry device *in vitro* Reflotron® Plus (Roche Diagnostics GmbH, RFA). The value ranges were divided into formal (<164 U/L), and the results above the reference values were stratified into: 164 to 328, >328 to 656, >656 to 984, and >984 U/L (Solter et al. 1993).

Staining and smears observation

blood smears and concentrates of leukocytes and platelets were stained with Quick Panotic (LB-Laborclin produtos para laboratórios Pinhais, PR) and observed with the aid of a binocular microscope (**Eclipse E200**, Nikon Instruments Inc. Japão) to evaluate the morphology of the blood cells and the presence of hematozoa.

Identification and diagnosis of hemoparasites

Parasites were classified according to the cells and formed elements of parasitized blood, staining and morphology.

Babesia canis vogeli and *Mycoplasma canis* (= *Mycoplasma haemocanis*) When observed in erythrocytes, in the forms of merozoites and trophozoites and in the form of cocci, respectively, or even assuming amórficas characteristics or amoeboid which correspond to different stages of the binary division process, when stained by quick Panotic present cytoplasm blue and pink core for the merozoites of the genus *Babesia* (Duh et al. 2004, Kemming et al. 2004).

Anaplasma platys and species of the genus *Ehrlichia* when observed in platelets and mononuclear cells, respectively, following morphology and staining characteristics of each species when stained by Quick Panotic were considered positive (Sainz et al. 2015).

Diagnosis Hyperadrenocorticism

Dogs that had some consistent factor for HAC, as recurrent urinary tract infections, cholesterol or triglycerides after fasting for 12 hours or elevated alkaline phosphatase levels above the normal range (Roche Diagnostics GmbH, RFA), without presentation of liver and bone disease (Ling et al. 1979, Teske et al. 1989, Ramsey & Ristic 2007, Bhavani et al. 2015, Çolakoğlu et al. 2015), they were subjected to stimulation test with adrenocorticotrophic hormone (ACTH-Adrenocorticotrophic hormone) sintético (Synachten®, Novartis Pharma S.A., France), dose 0.25mg/dog, applied intramuscularly. Blood samples collected after one hour of stimulation with ACTH, were intended for determination of serum cortisol by radioimmunoassay (Jericó et al. 2002). Dogs with cortisol results after stimulation with ACTH, above 17 mcg/dL, had a confirmed diagnosis of hyperadrenocorticism (Ramsey & Ristic 2007).

Ethics Statement, Competing Interests and Statistical analysis

This research was conducted with consent approved by CEUA/IV/UFRRJ #133/2014. The authors have declared that no competing interests exist. Statistic analysis were based on Ayres et al. (2007) and Sampaio (2002).

RESULTS AND DISCUSSION

A total of 21 dogs (Table 1), seven (33.3%) were diagnosed with HAC hyperadrenocorticism, a result near to that observed in a study done in the State of Rio Grande do Sul, Brazil, where 37% of the animals studied were HAC positive with diagnosis based on laboratory tests, Hormonal tests and ultrasound imaging (Pöppel et al. 2016). These results were much higher than observed in an epidemiological report of a hospital population of dogs with a diagnosis of hyperadrenocorticism (O'Neill et al. 2016), a result explained by the fact that in the present study, the dogs tested had some clinical signs or laboratory results compatible with the HAC, agreeing that hyperadrenocorticism is one of the endocrinopathy among canine disorders

(Ramsey & Ristic 2007) and that the possibility of a patient having hyperadrenocorticism is based on the history and physical examination and endocrine tests, which should be performed only when Clinical signs or laboratory results are consistent with HAC, according to Behrend et al. (2013). The diagnosis was obtained through the adrenocorticotrophic hormone stimulation test using cutoff point above 20 mcg/dL, according to the reference protocols (Ramsey & Ristic 2007, Peterson 2007). Although the ACTH stimulation test does not identify 100% of dogs with hyperadrenocorticism (Peterson 2007), it is one of the most used and reliable, with more than 95% use for diagnosis, being the diagnostic test used in the UK, agreeing with the choice of the present study, confirmed by the statistical test, with $p = 0.003b$ (Table 1) (O'Neill et al. 2016). The dogs were divided according to sex: four males (three orchietomized) and three females (all ovarian aldosteronectomized). This result is opposite to that observed in a study with 1,400 dogs, compiled from a specialized center of endocrinology between 2004 and 2014, explained by our number of samples being much lower than the study cited (Pöppel et al. 2016).

Five dogs (71.4%) were positive for some haemoparasites: four with *Anaplasma platys* and one with concomitant infection with *Mycoplasma canis* and *A. Platys*. A similar result was observed in dogs, in routine veterinary care, where a frequency of 64.7% was positive for some haemoparasites infection and showed no clinical signs of parasitic infection (Leal et al. 2015).

The hematological results of dogs with HAC showed three dogs with anemia, which is not indicative or suggestive of HAC, although the statistical test showed a greater chance of anemia for the HAC patient ($p = 0.05$) (Table 1), Presented 38% of median, with the globular volumes (VG) of HAC patients, presented values below the negative dogs, since the clinical findings are varied and very common to other conditions, as observed by Parry (2012), being confirmed due to the lack of significance for hemoglobin values.

Three of the dogs with anemia had haemoparasites infections, which are associated with anemia, with variable intensity, but with absence of clinical signs (Chalker 2005, Leal et al. 2015). The worsening of clinical signs are related to associations with other haemoparasites and other concomitant diseases, in addition to the high age group, as observed in other studies (Goldston & Hoskins 1999, Sasaki et al. 2008), confirming our results, where

Table 1. The comparison between dogs with or without hyperadrenocorticism attended at a veterinary health service in Barra da Tijuca, Rio de Janeiro, RJ.

Variables	Median Range		Values		Reference values a
	Negative	Positive	U	p	
Clinical findings:					
Age (years)	10.0 (1.8 -14.3)	11.4 (1.1 -17.0)	39.5	0.36 ^b	-
Body Weight (Kg)	10.0 (2.1-32.0)	8.30(4.5-19.2)	51.5	0.97 ^b	-
Plasma protein (g/dL)	7.20 (5.0-9.0)	7.40(6.0- 8.4)	48.0	0.77 ^b	5.5 - 7.7
Alcaline phosphatase	144.0(38.9-1,115.4)	769.0(23.2-5,496)	31.0	0.13 ^b	< 164 ^d
Cortisol after ACTH (mcg/dL)	10.0 (2.5-17.2)	22.5(20.1 -35.0)	0.0	0.003 ^c	6 a 17
Globular volume (%)	44.2(27-53)	38 (24-44)	21.0	0.05 ^c	37 - 55
Hemoglobin (g/dl)	15.3(8.3 - 20.2)	12.2(8.7 -15.9)	28.5	0.13 ^b	12 - 18
Blood figures:					
Platelets (mm ³)	424.0(129.0-545.0)	267.0(150.0-809.00)	44.0	0.71 ^b	200 - 500
Volume plaquetário médio (fL)	9.30(6.4-10.2)	9.10(8.2-10.3)	43.5	0.68 ^b	6.7 - 11.1
Leucócitos/mm ³	11,000(8,400-17,700)	14,800(8,400-17,700)	26.5	0.09 ^b	6,000 - 17,000
Eosinófilos/mm ³	301(0-1,032)	0(0-1,032)	34.0	0.26 ^b	100 - 1,250
Linfócitos/mm ³	1,174.5(345-4,472)	1,416.0(336- 3.612)	47.0	0.88 ^b	1,000 - 4,800
Monócitos/ mm ³	295.5(0-1.260)	344.0(0-504)	43.5	0.68 ^b	150 - 1,350
Neutrófilos/mm ³	7,7595(1,6552-27,520.0)	11,524.0(7,055.0-15,576.0)	26.0	0.09	3,000 - 11,500
Bastonetes/mm ³	140 (0-504)	425 (336-688)	19.5	0.03 ^c	0 - 300
Eritrócitos/mm ³	6.1(3.8 - 7.4)	5.9(3.0 - 6.9)	36.5	0.35 ^b	5.5 - 8.5

^a reference values according to (Thrall 2007); Mann-Whitney U test, ^b NS - not significance; ^c considered significant between hyperadrenocorticism (n = 7) and negative (n = 14); ^d Roche Diagnostics GmbH, Mannheim-Baden-Württemberg, RFA.

there is no significance In relation to age and HAC (Table 1). Most of the positive dogs were aged between one and twelve years (19%), three (14.4%) were over the age of twelve, agreeing that hyperadrenocorticism is a common condition in older dogs (Parry 2012), especially above Of the six years (Ling et al 1979, Pöppl et al. 2016), but this deduction is unrelated to the statistical test, where no significance was observed, due to the ages presented by the study dogs, not to be discordant between positive and negative for the HAC (Table 1).

Three dogs with thrombocytopenia, showing that patients with HAC, tend to present a low platelet variation, but not significant (Table 1), one with thrombocytosis, one with platelet aggregates, one with chips and one with reactive platelets. We did not observe clinical coagulation alterations, although the number and morphology of platelets were altered, common in the endocrine changes, but without indicating clinical presence of platelet hypofunction (Park et al. 2013, Kang et al. 2016). Coagulation changes call attention to research suggesting that dogs with CAH are at risk of developing thromboembolic complications, including respiratory distress, due to pulmonary artery thrombosis and should be suspected in cases of intractable dyspnea, unexplained right heart failure, and unexplained acute death (Burns et al., 1981). Such signs were not observed in the present study. Changes due to hypercoagulability with alteration in hemostasis, which may progress to thrombosis, due to HAC promoting a decrease in anticoagulant

factors (antithrombin levels) and decreased fibrinolysis time, and many are hyperfibrinogenemic, which makes it increasingly important to adopt Of preventive measures and appropriate therapy to avoid acquired abnormalities associated with thromboembolism (Burns et al., 1981, Kittrell & Berkwitz 2012, Kol et al. 2013, Paco et al. There is a significant decrease in antithrombin levels in dogs with HAC and there is an increase in platelet aggregation in dogs with high glucocorticoids, a result observed in only one HAC dog (Romão et al. 2013) associated with hypercholesterolemia, common in HAC dogs , Which leads to the accumulation of cholesterol in macrophages and other defense cells, promising inflammatory responses and the production of monocytes and neutrophils in the bone marrow and spleen, affecting leucocytosis, agreeing with the results obtained (Table 1), where a dog was carrying HAC, pathophysiology that aggravates diseases associated with chronic metabolic inflammation (Tall & Yvan-Charvet 2015). Studies comparing variables such as age, body weight, platelet count and mean platelet volume found no association of coagulation changes with HAC and the same was observed in the present study (Table 1), where no HAC positive dogs presented changes for mean volume (Klose et al. 2011).

Confirmation of changes in coagulability and deficiency in clot stabilization due to decreased fibrinolysis time are often impractical in the medical clinic, without even having reliable results, such as thrombolysis (TEG), which is a diagnostic tool

that allows The detection of hypercoagulability in a clinical setting (Klose et al. 2011). Therefore, the hematological evaluation through the hemogram, cytospin, following its own methodology, can be useful as performed in the present study and agreeing with bedside monitoring through the cytospin of the blood figure elements (Bastos et al. 2016), allowing the use of Specific platelet antiplatelet therapy, agreeing that platelets are of crucial importance in hemostasis and play an important role in inflammation (Stoppelaar et al. 2014). Plasma concentrations of proinflammatory cytokines TNF- α and IL-6 are elevated by thrombocytopenia, recognizing the risk of this alteration that was observed in three of the studied dogs with HAC, being a risk factor for sepsis, in addition, platelets protect Of septic shock inhibiting macrophage-dependent inflammation (Xiang et al. 2013).

Two of dogs had leukocytosis, three with eosinopenia, five with neutrophilia, four with lymphopenia, four with monocytopenia and five with rod neutrophils (left shunt). Results similar to those observed in a study with 117 dogs with HAC (Ling et al. 1979), remembering that concomitant diseases and opportunistic infections interfere in the results, making them of varied presentation and many are common to other conditions (Parry 2012), Mainly due to stress, hormonal diseases and excess glyco-corticoides possess immunosuppressive action, favoring infections, mainly infections of the urinary tract and skin (Ling et al. 1979, Pruett 2001, Bryden et al. 2004, Leal et al. 2016). In addition, they may present an urgent character with nonspecific signs and symptoms, often precipitated by concomitant diseases, making early identification; management and treatment even more difficult (Koenig 2013). Only a HAC positive mongrel dog, which agrees with Ling et al. (1979) and O'Neill et al. (2016) that say small breed dogs are five times more likely to develop HACs.

The alkaline phosphatase evaluation results showed two dogs with normal values and five with alkaline phosphatase elevation (Table 1). Four of these had alkaline phosphatase levels above 984 U / L and all were positive (100%) for HAC. These results confirm that elevation of alkaline phosphatase is a common finding in HAC, according to statistical comparison between positive and negative for HAC, compared to alkaline phosphatase values (Ling et al. 1979, Teske et al. 1989). Its lack of specificity renders it unsuitable as a diagnostic test and does not present any significance when studied in an unfractionated way (isoenzymes). The main ap-

plication is in the selection for suspected hyperadrenocorticism, with a positive predictive value for the presence of hyperadrenocorticism, agreeing with the current study, through clinical deduction of the higher the alkaline phosphatase values, the greater the chance of HAC present (Teske et al. 1989).

The other laboratory abnormalities and physical characteristics, such as weight and plasma protein, were not correlated positively with HAC, results observed in previous studies (Ling et al. 1979, Teske et al. 1989, Peterson 2007, Ramsey & Ristic 2007, Parry, 2012, Behrend et al. 2013, O'Neill et al. 2016) confirming the need for specific tests, such as the ACTH stimulation test (Table 1).

Based on the data observed in this study, which was performed from a clinical point of view, since the reduced number of samples used was linked to the fact that the diagnostic test for this endocrinopathy was economically unfeasible for its owners, statements previously commented by Ramsey & Ristic (2007) and O'Neill et al. (2016).

CONCLUSIONS

The diagnosis of CAH has been a challenge for the Veterinarian; the specific tests are of high monetary value, overwhelming the owner by indicating treatment of higher costs, which need to be monitored continuously. They are mostly immunosuppressed patients susceptible to opportunistic agents. The response to the results observed in this study may not be in the alterations due to the hormonal disorder, but in the concomitant infections. The presence of haemoparasites and hematological changes, especially of the white series with neutrophils with left-shift deviation, may be related to hyperadrenocorticism, which leads to the clinician to count on all the variables observed to reach the best result.

REFERENCES

- Ayres M., Ayres Jr M., Ayres D.L. & Santos A.A.S. *BioEstat 5.3: Aplicações Estatísticas nas Áreas das Ciências Biológicas e Médicas*. Publicações Avulsas do Mamirauá, Belém, 2007.
- Bastos E.M.D., Leal P.D.S.A. & Lopes C.W.G. Importância da avaliação morfológica de neutrófilos como marcadores de infecção sistêmica em cães. Relato de dois casos. *Revista Brasileira de Medicina Veterinária*, 38:195-202, 2016.
- Behrend E.N., Kooistra H.S., Nelson R., Reusch C.E. & Scott-Moncrieff J.C. Diagnosis of spontaneous canine hyperadrenocorticism: 2012 ACVIM consensus statement (small animal). *Journal of Veterinary Internal Medicine*, 27: 1292-1304, 2013.
- Bhavani M.S., Thirunavukkarasu P.S., Kavitha S., Nagarajan B., Baranidaran G.R. & Bhat A.A. Steroid induced hyperadrenocorticism in dogs-A Short study. *Journal of Animal Research*, 5: 203, 2015.
- Bryden S.L., Burrows A.K. & O'hara, A.J. Mycobacterium goodii infection in a dog with concurrent hyperadrenocorticism. *Veterinary Dermatology*, 15: 331-338, 2004.

- Burns M., Kelly A.B., Hornof W.J. & Howerth E.W. Pulmonary artery thrombosis in three dogs with hyperadrenocorticism. *Journal of the American Veterinary Medical Association*, 178: 388-393, 1981.
- Chalker V.J. Canine mycoplasmas. *Research in Veterinary Science*, 79:1-8, 2005.
- Chen H.Y., Lien Y.H. & Huang H.P. Association of Renal Resistive Index, Renal Pulsatility Index, Systemic Hypertension, and Albuminuria with Survival in Dogs with Pituitary-Dependent Hyperadrenocorticism. *International Journal of Endocrinology*, ID 3814034, 2016.
- Çolakoglu E.Ç., Haydardedeoğlu A.E., Alihosseini H. & Kurtdede A. Mitotane-Induced Hypoadrenocorticism in a Dog with Hyperadrenocorticism. *Kafkas Universitesi Veteriner Fakültesi Dergisi*, 21: 135-137, 2015.
- Frank L.A., Henry G.A., Whittemore J.C., Enders B.D., Mawby D.I. & Rohrbach B.W. Serum Cortisol Concentrations in Dogs with Pituitary-Dependent Hyperadrenocorticism and Atypical Hyperadrenocorticism. *Journal of Veterinary Internal Medicine*, 29: 193-199, 2015.
- Goldston R.T. & Hoskins J.D. *Geriatrics e Gerontologia do Cão e do Gato*. São Paulo, Ed. Roca. 1999. 551 p.
- Jericó M.M., Otsuka M., Maganin Jr, A. & Larsson C.E. Métodos de imunoenensaio não-radiométricos [fluoroimunoensaio (FIE) e enzimaimunoensaio (EIE) e o radioimunoensaio (RIE) na avaliação da função adrenaç de cães normais e cães com hiperadrenocorticismo. *Ciência Rural*, 32: 259-262, 2002.
- Kang M.H., Heo R.Y. & Park H.M. Evaluation of D-Dimer Concentrations in Clinically Ill Dogs with High Risk of Thromboembolic Disease. *Pakistan Veterinary Journal*, 36: 219-223, 2016.
- Kittrell D. & Berkwitt L. Hypercoagulability in dogs: pathophysiology. *Compendium: Continuing Education for Veterinarians*, 34: E1-E5, 2012.
- Klose T.C., Creevy K.E. & Brainard B.M. Evaluation of coagulation status in dogs with naturally occurring canine hyperadrenocorticism. *Journal of Veterinary Emergency and Critical Care*, 21: 625-632, 2011.
- Koenig A. Endocrine emergencies in dogs and cats. *Veterinary Clinics of North America: Small Animal Practice*, 43: 869-897, 2013.
- Kol A., Nelson R.W., Gosselin R.C. & Borjesson D.L. Characterization of thrombelastography over time in dogs with hyperadrenocorticism. *Veterinary Journal*, 3: 675-681, 2013.
- Leal P.D.S., Moraes M.I.M.R., Barbosa L.L. de O. & Lopes C.W.G. Infecção por hematozoários nos cães domésticos atendidos em serviço de saúde animal, Rio de Janeiro, Brasil. *Revista Brasileira de Medicina Veterinária*, 37(Supl.1): 55-62, 2015.
- Leal P.D.S., Paes de Almeida E.C. & Lopes C.W.G. Diagnóstico de dermatopatia piogranulomatosa por *Nocardia* spp. numa cadela Labrador Retriever com hipotireoidismo, Rio de Janeiro, Brasil - Relato de caso. *Revista Brasileira de Medicina Veterinária*, 38: 203-209, 2016.
- Ling G.V., Stabenfeldt G.H., Comer K.M., Gribble D.H. & Schechter R.D. Canine hyperadrenocorticism: pretreatment clinical and laboratory evaluation of 117 cases. *Journal of the American Veterinary Medical Association*, 174: 1211-1215, 1979.
- Mawby D.I., Whittemore J.C. & Fecteau K.A. Canine Pancreatic-Specific Lipase Concentrations in Clinically Healthy Dogs and Dogs with Naturally Occurring Hyperadrenocorticism. *Journal of Veterinary Internal Medicine*, 28: 1244-1250, 2014.
- Midence J.N., Drobatz K.J. & Hess R.S. Cortisol Concentrations in Well-Regulated Dogs with Hyperadrenocorticism Treated with Trilostane. *Journal of Veterinary Internal Medicine*, 29: 1529-1533, 2015.
- Nagata N. & Yuki M. Long-term outcome of myotonia associated with hyperadrenocorticism in 2 dogs. *Canadian Veterinary Journal*, 56: 931, 2015.
- O'Neill D.G., Scudder C., Faire J.M., Church D.B., McGreevy P.D., Thomson P.C. & Brodbelt D.C. Epidemiology of hyperadrenocorticism among 210,824 dogs attending primary-care veterinary practices in the UK from 2009 to 2014. *Journal of Small Animal Practice*, 57: 365-373, 2016.
- Pace S.L., Creevy K.E., Krimer P.M. & Brainard B.M. Assessment of coagulation and potential biochemical markers for hypercoagulability in canine hyperadrenocorticism. *Journal of Veterinary Internal Medicine*, 27: 1113-1120, 2013.
- Park F.M., Blois S.L., Abrams-Ogg A.C.G., Wood R.D., Allen D.G., Nykamp S.G. & Downie A. Hypercoagulability and ACTH-Dependent Hyperadrenocorticism in Dogs. *Journal of Veterinary Internal Medicine*, 27: 1136-1142, 2013.
- Parry N. Diagnosis of hyperadrenocorticism in dogs. *Companion Animal*, 17: 14-18, 2012.
- Peterson M.E. Diagnosis of hyperadrenocorticism in dogs. *Clinical Techniques in Small Animal Practice*, 22: 2-11, 2007.
- Pöppel Á.G., Coelho I.C., da Silveira C.A., Moresco M.B. & de Carvalho G.L.C. Frequency of endocrinopathies and characteristics of affected dogs and cats in southern Brazil (2004-2014). *Acta Scientiae Veterinariae*, 44: 01-09, 2016.
- Pruett S.B. Quantitative aspects of stress-induced immunomodulation. *International Immunopharmacology*, 1: 507-520, 2001.
- Ramsey I., & Ristic J. Diagnosis of canine hyperadrenocorticism. *Practice-London-British Veterinary Association*, 29: 446, 2007.
- Romão F.G., Campos E.F., Mattoso C.R.S. & Takahira R.K. Hemostatic profile and thromboembolic risk in healthy dogs treated with prednisone: a randomized controlled trial. *BMC Veterinary Research*, 9: 268, 2013.
- Rose L., Dunn M.E. & Bedard C. Effect of canine hyperadrenocorticism on coagulation parameters. *Journal of Veterinary Internal Medicine*, 27: 207-211, 2013.
- Sampaio I.B.M. *Estatística aplicada à experimentação animal*. 3ª ed. Belo Horizonte, FEPMVZ, 2007. 274p.
- Sasaki M., Ohta K., Matsuu A., Hirata H., Ikadai H. & Oyamada T. A molecular survey of *Mycoplasma haemocanis* in dogs and foxes in Aomori Prefecture. *Japan Journal of Protozoology Research*, 18:57-60, 2008.
- Scudder, C., Kenny, M.P. & Niessen, S. Treatment of canine and feline hyperadrenocorticism: trilostane and the alternatives. *Companion Animal*, 20: 230-238 2015.
- Soares F.A.C., Matheus J.P., Carvalho G.L., Neuwald E.B., Pöppel Á.G., Valle S.F. & González F.H.D. Cardiocirculatory, biochemical and hemostatic evaluation of dogs with hyperadrenocorticism at diagnosis and after treatment. *Korean Journal of Veterinary Researsch*, 56: 1-6, 2016.
- Solter, P., Hoffmann, W., Hungerford, L., Peterson, M. & Dorner, J. Assessment of corticosteroid-induced alkaline phosphatase isoenzyme as a screening test for hyperadrenocorticism in dogs. *Journal of the American Veterinary Medical Association*, 203(4), 534-538, 1993.
- Stoppelaar S.F., Van't Veer C. & Van der Poll T. The role of platelets in sepsis. *Thrombosis and Haemostasis*, 112: 666-677, 2014.
- Tall A.R. & Yvan-Charvet L. Cholesterol, inflammation and innate immunity. *Nature Reviews: Immunology*, 15: 104-116, 2015.
- Teske E., Rothuizen J., De Bruijne J.J. & Rijnberk A. Corticosteroid-induced alkaline phosphatase isoenzyme in the diagnosis of canine hypercorticism. *Veterinary Record*, 125: 12-14, 1989.
- Thrall M.A. *Hematologia e Bioquímica Clínica Veterinária*, 1ª ed. São Paulo: Roca, 2007, 582p.