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Thyroid Function in Dogs with Visceral Leishmaniasis

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Abstract: Several non-thyroid factors such as age, breed, concomitant diseases and use of certain drugs might influence the diagnosis of hypothyroidism in dogs. Changes in the concentrations of thyroid hormones due to non-thyroid illnesses are named euthyroid sick syndrome. Among the diseases with potential to provoke a decrease in thyroid hormones there are infecto-contagious diseases such as parvovirosis, babesiosis and leishmaniasis. Canine visceral leishmaniasis is a severe systemic disease which may cause kidney and liver diseases, cardiac injuries and injuries to other organs. The aim of this study was to evaluate the influence of visceral leishmaniasis on thyroid function in dogs without signs of hypothyroidism with or without azotemia. Positive animals for leishmaniasis were divided in six groups regarding: absence of hypoalbuminemia, presence of hypoalbuminemia, normal creatinine, increased creatinine, normal urea and increased urea. The effect of these groups was evaluated on the thyroid-stimulating hormone, total thyroxine and free thyroxine concentrations. Dogs that were positive for leishmaniasis (0.63 ng mL^{-1}) presented thyroid-stimulating hormone serum concentrations higher than seronegative animals (0.43 ng mL^{-1}) while total thyroxine (1.28 μg dL^{-1}) and free thyroxine (1.49 ng dL^{-1}) in dogs with leishmanisis were lower ($p<0.01$) when compared to healthy dogs (total thyroxine for 2.31 μg dL^{-1} and free thyroxine 1.84 ng dL^{-1}) however, within the values for euthyroid animals. In dogs seropositive for leishmaniasis, total thyroxine means in the group with the presence of hypoalbuminemia (1.01 μg dL^{-1}) were lower ($p<0.04$) when compared to the means in the group with absence of hypoalbuminemia (1.4 μg dL^{-1}). Based on this study, it can be concluded that dogs that were positive to visceralleishmaniasis did not develop euthyroid sick syndrome, although compared to normal thyroid hormones were reduced.

Keys words: Euthyroid, sick syndrome, leishmania, hypthyroidism, thyroxine, hypoalbuminemia

INTRODUCTION

Alterations in thyroid hormones concentrations in response to Non-Thyroid Illnesses (NTIs), often termed the Euthyroid Sick Syndrome (ESS) is well recognized in both animals and human. Several non-thyroid factors such as age, breed, concomitant diseases and use of certain drugs might influence the diagnosis of hypothyroidism in dogs (Ferguson, 1997; Scott-Moncrieff *et al*., 1998; Kantrowitz *et al*., 2001; Cardoso *et al*., 2007; Fialkovicova *et al*., 2012). Also, the stress induced by non-thyroid illnesses might cause an increase in the circulation of glucocorticoids and alter the thyroid function (Mooney *et al*., 2008).

Alterations in thyroid hormone concentrations due to Non-Thyroid Illnesses (NTIs) are well-known among humans and animals (Mooney *et al*., 2008). Non-thyroid illnesses arealso named Euthyroid Sick Syndrome (ESS). In humans, there is a decrease in Total Triiodothyronine (TT3) concentration with Total Thyroxine (TT4) serum concentrations continuing within normality levels. However, in severe illnesses, a reduction in TT3 and TT4 might happen. In these cases, the free portion of the hormone remain within normal levels (Yildizdas *et al*., 2004; Dagan *et al*., 2006; Golombek, 2008).

Nonetheless in dogs, a decrease only in TT3 concentrations is not common. Most of the time there is a reduction both in TT3 and TT4 and in a few cases, only

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in TT4. Nonetheless, Free Thyroxin (FT4) is less affected by NTIs with only mild decreases in severe systemic diseases (Kantrowitz *et al*., 2001). Among the diseases with potential to provoke a decrease in thyroid hormones there are infecto-contagious diseases such as leishmaniasis.

Canine visceral leishmaniasis is a severe systemic disease which may cause kidney and liver diseases, cardiac injuries and injuries to other organs. Parvovirosis, babesiosis, leishmaniosis and other severe systemic diseases can cause euthyroid sick syndrome (Elliot *et al*., 1995; Schoeman *et al*., 2007; Mooney *et al*., 2008; Zygner *et al*., 2012). Since, they cause an increase in the production of post-inflammatory citokines such as TNF-α and IL-6 which contribute to the inhibition of the hypothalamus-hypophysis-thyroid axis, resulting in a decrease in the production, secretion and circulation of thyroid hormones (T3 and T4) (De Groot, 1999; Van den Berghe, 2001; Adler and Wartofsky, 2009). The diagnosis of leishmaniosis is basead of clinical sinal and clinicopathological abnormalities, Complete Blood Count (CBC) biochemical profile and urinalysis can be both wide and non-specific. The diagnosis can be made by the detection of specific serum antibodies (IgG) using preferably quantitative serological techniques, such as the Immunofluorescence Antibody Test (IFAT) and Enzymelinked Immunosorbent Assay (ELISA) immunochromatography and PCR.

The aim of this study was to evaluate the influence of visceral leishmaniasis on the thyroid function of dogs without signs of hypothyroidism with or without azotemia.

MATERIALS AND METHODS

Animals: One hundred thirty-eight adult, mixed breeds, eighty-five female (forty-nine sexually intact) and fifty-three male (thirty-five sexually intact) were enrolled in the study. All animals were originated from the Zoonozis Center from the City Hall in Campo Grande, Mato Grosso do Sul, Brazil, March to December 2012. Most sick animals had signs which were compatible with visceral leishmaniosis. Animals with signs compatible with hypothyroidism such as lethargy, apatia, obesity and dyslipidemia; history of hormone replacement, usage of drugs that interfere with the thyroid function were excluded from the study.

This study was approved by the Ethics board of the animal experiments, State University of Northern Parana, School of Veterinary Medicine, Department of Production and Veterinary Medicine, Bandeirantes-Parana, Brazil and is in accordance with the ethical principles of animal experimentation (COBEA).

Analytical method: The samples for serum biochemical analyses and ELISA were collected in test tubes and were kept at room temperature for 30 min to coagulate and then were centrifuged. Sera were prepared and immediately analysed or stored at 4°C for analysis in a period of up to 24 h from collection using a Semi-Automatic Biochemical Analyzer (TP-Analyzer Plus, Brazil) and appropriate commercial kits for albumin, urea, creatinine and urea. Normal serum values for the study were considered as creatinine between 0.5-1.5 mg dL^{-1}; urea between 21.4-59.9 mg dL^{-1} and albumin between 2.6-33.3 g dL^{-1}.

Leishmaniasis diagnostic for the 138 samples for serum were tested using ELISA (Leishmania chagasi kit®, Biomanguinhos, Brazil) technique. A total of 95 positive and 43 negative dogs for *Leishmania* sp. were identificados.

Positive animals for leishmaniasis were divided in six groups regarding: absence of hypoalbuminemia (Group 1-G1) presence of hypoalbuminemia (Group 2-G2, normal creatinine (Group 3-G3, increased creatinine (Group 4-G4), normal urea (Group 5-G5) and increased urea (Group 5-G6).

Total T4 was measured by use of a commercially available solid-phase radioimmunoassay kit (Clinical Assays Gammacoat M Total T4 RIA kit; DiaSorin Inc., Stillwater, MN, USA) that has been validated for canine serum. Free T4 was measured by use of a commercially available kit (Free T4 by equilibrium dialysis; Nichols Institute Diagnostics, San JuanCapistrano, CA, USA) that has been validated in that laboratory for canine serum. Canine TSH was measured by use of a commercially available immunoradiometric assay (Coat-A-Count canine TSH IRMA; Diagnostic Products Corp., Los Angeles, CA, USA) that has been validated in that laboratory for canine serum. Normal values for Thyroid-Stimulating Hormone (TSH) were 0.18-1 ng dL^{-1}, for TT4 1.5-4 ug dL^{-1} and for FT4 1.0-4.0 ng dL^{-1}.

Statical analysis: The experimental design was entirely randomized, weighing the effect of Leishmaniasis on the variables TSH, TT4 and FT4. Variance analysis weighed the effect of these groups on the concentrations of TSH, TT4 and FT4. All variables were previously submitted to Lilliefors and Levene tests. Distributions that did not present variance homogeneity but presented $p<0.05$ in the variance analysis had means compared by Scheffe test which is more robust in face of violations in variance equalities. All analyses were performed in Statica 7.0 program for Windows® (SPSS, USA).

RESULTS

One hundred thirty eight dogs evaluated 95 for were positive for leishmaniasis and and 43 negative dogs. Dogs that are seropositive for leishmaniasis presented TSH serum concentration higher than seronegative animals while TT4 and FT4 in dogs with leishmaniasis were lower when compared to healthy dogs (Table 1) without significant difference among groups. However, both groups presented values within normal range that is of euthyroid animals.

The values TSH, TT4 and FT4 of the groups regarding the values of albumin (G1 and G2), creatinine (G3 and G4) and urea (G5 and G6) are shown in Table 2.

In dogs that are seropositive for leishmaniasis, no significant differences were observed among animals with increased urea and creatinine and those with values within normal levels (Table 2). In dogs seropositive for leishmaniasis, TT4 mean in the G5 is lower ($p<0.05$) when compared to the mean in the G6. The remaining groups did not present statistic differences among each other (Table 2).

Table 1: Results of serum TSH, TT4 and FT4 concentrations in the two groups of dogs with (95 dogs) or without (43 dogs) leishmaniasis (Leishmania chagasi)

Parameteres	Dogs without leishmaniasis	Dogs with leishmaniasis	Reference values
TSH (ng mL^{-1})			
Mean±SD (n)	0.43±0.23 (43)	0.63±0.32 (95)	(0.18-1)
Range	0.13-0.94	0.14-1.20	p<0.01
TT4 (µg dL^{-1})			
Mean±SD (n)	2.31±0.81 (43)	1.28±0.85 (95)	(1.5-4.0)
Range	0.67-3.96	0.29-4.37	p<0.01
FT4 (ng dL^{-1})			
Mean±SD (n)	1.84±0.51 (43)	1.49±1.81 (95)	(1.0-4.0)
Range	1.01-2.46	0.39-1.50	p<0.01

Means followed by different letters present significance according to Scheffe test. SD: Standard Deviation, n: number for animals; TSH: Thyroid-Stimulation Hormone; TT4: Total Thyroxine; FT4: Free Thyroxine

Table 2: Results of serum TSH, TT4 and FT4 concentrations in the six groups of 95 dogs with leishmaniasis (Leishmania chagasi)

	Groups					
Parameteres	G1	G2	G3	G4	G5	G6
TSH (ng mL^{-1})						
Mean (n)	0.62 (53)	0.62 (39)	0.62 (53)	0.63 (42)	0.61 (53)	0.65 (42)
SD	0.31	0.34	0.31	0.34	0.31	0.35
Range	0.18-1.2	0.14-1.2	0.18-1.2	0.14-1.2	0.17-1.2	0.14-1.2
P	0.97		0.84		0.57	
TT4 (ug dL^{-1})						
Mean (n)	1.43 (53)	1.11 (49)	1.37 (53)	1.15 (42)	1.4(63)^{a}	1.01(33)^{b}
SD	0.96	0.66	0.97	0.66	0.93	0.6
Range	0.29-4.37	0.43-2.8	0.29-4.4	0.43-2.8	0.29-4.4	0.34-2.8
P	0.07		0.21		0.04	
FT4 (ng dL^{-1})						
Mean (n)	1.39 (53)	1.92 (49)	1.38 (53)	1.63 (42)	1.39 (63)	1.67(33)
SD	1.52	2.77	1.52	2.13	1.40	2.41
Range	0.39-1.5	0.89-1.5	0.39-1.2	0.89-1.5	0.39-1.2	0.89-1.5
P	0.24		0.50		0.48	

Means followed by different letters present significance ($p<0.05$) according to ScheffE test. Significant difference between G5 and G6 ($p<0.05$). SD: Standard deviation; n: number for animals; TSH: thyroid-stimulation hormone; TT4: Total Thyroxine; FT4: Free Thyroxine; G1: absence of hypoalbuminemia; G2: presence of hypoalbuminemia; G3: normal creatinine; G4: increased creatinine; G5: normal urea; G5: increased urea (Group 5-G6)

DISCUSSION

The decrease in FT4 concentrations is probably due to the increase in the circulation of interleukines such as TNF-α that can be increased in leishmaniasis since this cytokine promotes hypotension and decrease in the kidney filtration rate, contributing therefore to the development of ESS. The decrease in FT4 concentrations poses a positive feedback on the hypophysis, stimulating the production and secretion of TSH which could justify the increase in TSH serum concentrations. Nonetheless, probably these levels of cytokine are not enough to cause ESS such as the one described in animals with babesiosis and parvovirosis (Elliot *et al*., 1995; Schoeman *et al*., 2007; Mooney *et al*., 2008; Zygner *et al*., 2012). The decrease in thyroid hormones in dogs with leishmaniosis was not enough to produce ESS. It is known that the level of parasitism and the severity of the disease can also influence the concentrations of thyroid hormones (Bohm *et al*., 2006).

Probably, ESS occurs due to an increase in the production of interleukines such as TNF-α and IL-6, inhibiting the functioning of the hypothalamus-hypophysis-thyroid axis and consequently decreasing the production and secretion of thyroid hormones (TT3 and TT4) as well as their serum concentrations (De Degroot, 1999).

Leishmaniasis causes several systemic alterations, including liver and kidney alterations. These alterations are well-described as causes of Euthyroid Sick Syndrome (ESS) since hypoalbuminemia and azotemia/uremia caused by liver disease change the function of the hypothalamus-hypophysis-thyroid axis (Kantrowitz *et al*., 2001; Gommeren *et al*., 2009). In dogs that are seropositive for leishmaniasis, no significant differences were observed among animals with increased urea and creatinine and those with values within normal levels (Table 2). The decrease in free and total thyroxin concentrations was described in babesiosis (Schoeman *et al*., 2007; Zygner *et al*., 2012) without significant differences between azotemic and non-azotemic animals. In babesiosis studies, TSH and serum albumin concentrations were not evaluated (Schoeman *et al*., 2007; Zygner *et al*., 2012).

Hypoalbuminemia might occur in liver, kidney and enteric diseases with loss of proteins and chronic

inflammations. Probably, hypoalbuminemia in these animals with leishmaniasis is due to liver and kidney injuries and also due to chronic inflammation. In dogs seropositive for leishmaniasis, TT4 mean in the G5 is lower ($p<0.05$) when compared to the mean in the G6. The remaining groups did not present statistic differences among each other (Table 2). Albumin, together with other proteins is an important thyroxin transporter. The decrease in albumin and probably in other proteins such as transthyretin (Piechotta *et al*., 2012) probably has influenced the decrease in TT4 concentrations.

The decrease in albumin in seropositive dogs could have also been due to kidney diseases however, no urinalysis was performed to investigate the presence of proteinuria. And in a few cases, hypoalbuminemia could be caused due to intestinal protein loss or malnutrition since these dogs were from zoonosis centers.

Despite the decrease in TT4 and FT4 concentrations and the increase in TSH, the animals in this study would not have been confused with hypothyroid animals since the concentrations of thyroid hormones were within the range of euthyroidism. The mild decrease in TT4 concentrations, Hypoalbuminemia might occur in liver, kidney and enteric diseases with loss of proteins and chronic inflammations. Probably, hypoalbuminemia in these animals with leishmaniasis is due to liver and kidney injuries and also, due to chronic inflammation. In dogs seropositive for leishmaniasis, TT4 mean in the G5 is lower ($p<0.05$) when compared to the mean in the G6. The remaining groups did not present statistic differences among each other (Table 2). Albumin, together with other proteins is an important thyroxin transporter. The decrease in albumin and probably in other proteins such as transthyretin (Piechotta *et al*., 2012) probably has influenced the decrease in TT4 concentrations. The decrease in albumin in seropositive dogs could have also been due to kidney diseases, however, no urinalysis was performed to investigate the presence of proteinuria. And in a few cases, hypoalbuminemia could be caused due to intestinal protein loss or malnutrition since these dogs were from zoonosis centers. Despite the decrease in TT4 and FT4 concentrations and the increase in TSH, the animals in this study would not have been confused with hypothyroid animals since the concentrations of thyroid hormones were within the range of euthyroidism. The mild decrease in TT4 concentrations, despite hypoalbuminemia, indicates that other transporting proteins were not decreased. However, this was not investigated in this study.

The presence of chronic dermatologic alterations and even neuropathies are differential diagnosis both for hypothyroidism and leishmaniasis. Due to this, thyroid function tests can be requested and can be compatible with hypothyroidism. Nonetheless, in areas with high prevalence of leishmaniosis, the researchers recommend serology and PCR of this disease since it might cause decrease in FT4 and TT4 and increase in TSH, findings which are compatible with hypothyroidism.

Also, the increase in the production of anti-Thyroglobulin (TG) antibodies in several systemic diseases (Diaz-Espineira *et al*., 2008) in important transport protein for thyroid hormones which might intensify the decrease in TT4 has already been described. However, in these cases, normal or increased FT4 is expected. Production and/or circulation of anti-TG antibodies were not analyzed in this study.

Based on this study, it is possible to state that animals that were positive for visceral leishmaniasis did not develop ESS. However, further studies are necessary in order to evaluate thyroid functions in animals with severe signs of leishmaniasis. Moreover, the presence or absence of azotemia has not interfered in the thyroid function and only a reduction in albumin decreases TT4 concentrations.

CONCLUSION

Based on this study, it can be concluded that dogs that were positive to visceral leishmaniasis did not develop euthyroid sick syndrome, although, compared to normal thyroid hormones were reduced.

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