

Serum DHEA-S increases in dogs naturally infected with *Ehrlichia canis*



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ABSTRACT

Adrenocortical disturbances are expected in canine ehrlichiosis due to the immunological challenges caused by infection and consequent inflammation. Thus, this study aimed to evaluate the occurrence of adrenocortical hormonal alterations in dogs naturally infected with *Ehrlichia canis* (n = 21) as positively confirmed by the presence of anti-*E. canis* antibodies (Dot-ELISA) and nested PCR (nPCR). Serum dehydroepiandrosterone sulfate (DHEA-S) concentrations were assessed via ELISA before and one hour after ACTH stimulation. Another 10 healthy dogs were subjected to the same stimulation protocol and used as controls. The results revealed that baseline and post-ACTH DHEA-S concentrations were significantly greater in sick dogs, regardless of gender, and this finding illustrates the stress induced by naturally acquired ehrlichiosis in dogs.

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Canine monocytic ehrlichiosis is caused by *Ehrlichia canis* and is a disease with a worldwide distribution that represents a major canine health problem (Lappin, 2006). Death may occur due to both its associated hematological alterations and other consequent factors, related to the well-known immune-inflammatory responses (Faria et al., 2011; Kim and Rikihisa, 2002; Tajima and Rikihisa, 2005; Unver et al., 2006) and possibly to hormonal responses (Kumar et al., 2006), which explain the disease's multifactorial catabolic effects (Varshney et al., 2003).

Adrenocortical hormonal secretion is increased during the development of inflammatory or infectious reactions, suggesting that they induce the response of hormone-modulated defense (Garcia-Leme, 1989). Furthermore, infection or inflammation invariably activates the immune system and consequently activates the hypothalamic-pituitary-adrenal axis via cytokines that are released by the immune system that induce, directly or indirectly, the secretion of ACTH and adrenocortical hormones (Buckingham et al., 1992).

Dehydroepiandrosterone sulfate (DHEA-S) is a dehydroepiandrosterone (DHEA) metabolite and a sexual androgen precursor that is synthesized from cholesterol and secreted by the reticulate zone adrenal cortex primarily in response to ACTH (Boonstra et al., 2008; Fischli et al., 2008). The gonads and brain also make minor contributions to the synthesis of DHEA-S (Boonstra et al., 2008). DHEA-S is an immunomodulator hormone that stimulates the production of T helper lymphocytes and cytokines (Boonstra et al., 2008; Kass and Finland, 1953; Morgan et al., 2009). There is evidence that DHEA-S stimulates immune system function (Mitchell et al., 2002) and is increased in stressful disorders (Morgan et al., 2009; Sugaya et al., 2012, 2015).

Adrenocortical involvement may be expected in dogs infected with *E. canis* due to the challenges to the immune system that have previously been observed in both critical human (Leal et al., 2003; Libonati et al., 2006) and canine patients (Schoeman and Herrtage, 2008; Schoeman et al., 2007) with other diseases. Thus, this study was developed to assess the adrenocortical involvement before and after ACTH stimulation in naturally occurring canine monocytic ehrlichiosis by quantifying serum DHEA-S.

This study was approved by local welfare committee of Faculdade de Ciências Agrárias e Veterinárias/Universidade Estadual Paulista, Campus of Jaboticabal – SP, Brazil, under protocol n. 003940/10.

Thirty-one dogs included in this study were selected from the clinical routine of the Veterinary Teaching Hospital of Faculdade de

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Ciências Agrárias e Veterinárias, Unesp – Campus of Jaboticabal – SP, Brazil, after free and informed consent was provided by the owners. Adult dogs from 1 to 10 years old of both sexes and different breeds were included in the study. Bitches in estrus; dogs with any other concomitant endocrine, infectious, neoplastic, renal or hepatic diseases; and all of those that had received systemic or topic corticotherapy 60 days prior to clinical admission were excluded. Screening tests (complete blood count, serum biochemistry, urinalysis and serological tests) were performed to guide the inclusion or exclusion in each experimental group.

Group 1 was comprised of 21 dogs, with mean body weight of 17.4 kg and mean age of 4.8 years old (14 males – 2 neutered and 12 intact males; and 7 females – 3 spayed and 4 intact females), with thrombocytopenia and clinical histories suggestive of ehrlichiosis in the acute or subclinical phase with possible coexisting anemia and/or leukopenia and evidence of the presence of ticks that were positive for anti-*E. canis* antibodies (Dot-ELISA) and nPCR for *E. canis*. Additionally, the dogs were required to be negative for anti-*Babesia canis* antibodies (RIFI), PCR for *B. canis* and anti-*Leptospira* spp. antibodies (microscopic agglutination test), since leptospirosis and babesiosis are frequently diagnosed in the region where the study was developed and also, because adrenocortical hormonal changes have already been described in dogs infected with *Babesia canis* (Schoeman and Herrtage, 2008; Varshney et al., 2003). Another 10 healthy dogs, with mean body weight of 18.2 kg and mean age of 3 years old (4 males – 2 neutered and 2 intact males; and 6 females – one spayed and 5 intact females) were selected from the Veterinary Teaching Hospital kennel; these dogs exhibited no alterations in the physical examinations, screening tests or infectious agent tests that were described for Group 1, and comprised Group 2 (control group).

The dogs in Groups 1 and 2 were subjected to the ACTH stimulation test in the morning after remaining in the appropriate kennel at the Veterinary Teaching Hospital for an overnight fast with water available *ad libitum*. Jugular venipuncture was performed immediately before and one hour after the intravenous administration of 5 µg/kg synthetic ACTH (Synacthen®, 1 mg/ml; Biofutura Pharma, Italy).

Aliquots of sera taken before and after the administration of ACTH were cryopreserved at –20 °C until DHEA-S was measured. DHEA-S concentrations were assessed via ELISA tests that were performed with a commercial kit (Human Diagnostics®, catalog n. 55060, Germany).

After obtaining the blood samples, all dogs in Group 1 were started on doxycycline hyclate treatment (5 mg/kg every 12 h for 28 d orally) according to the Veterinary Teaching Hospital protocol (adapted from Munhoz et al., 2012; Troy and Forrester, 1990).

The data were analyzed with Statistical Analysis System (SAS, 1996) software and were examined for the normality of the residuals (Cramér-von-Mises). The Tukey–Kramer test was used to analyze the pre- and post-ACTH DHEA-S concentrations and significance was set at $p < 0.05$.

There were significant differences between the pre- and post-ACTH DHEA-S concentrations in both groups ($p < 0.0001$; Table 1 and Fig. 1). Comparison of the hormonal concentrations between the groups revealed that the DHEA-S concentrations were three times higher in Group 1 at both timepoints. By analyzing the disease

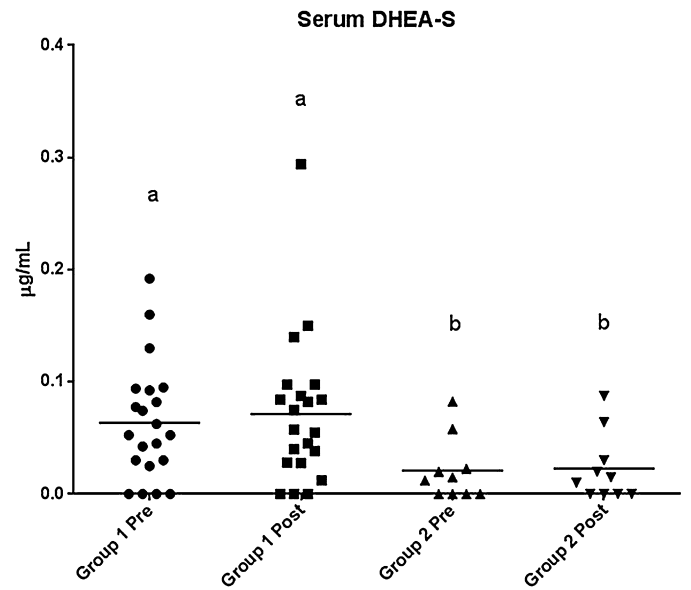


Fig. 1. Point plots of the baseline and post-ACTH stimulation serum DHEA-S concentrations of Groups 1 and 2. The horizontal bars indicate the mean values, and the different letters indicate significant differences ($p < 0.05$).

condition, we found significant differences between sick and healthy male dogs pre- ($p = 0.0018$) and post-ACTH ($p = 0.0004$) serum DHEA-S. This difference was also seen in DHEA-S concentrations between sick and healthy female dogs at pre- ($p = 0.0001$) and post-ACTH ($p = 0.0001$) timepoints. When comparing gender, we have observed that male dogs of Group 1 have greater pre- and post-ACTH DHEA-S concentrations than female dogs of the same group ($p = 0.0001$; $p < 0.0001$, respectively). The same way, healthy male dogs also have greater pre- and post-ACTH DHEA-S in comparison to healthy female dogs ($p = 0.0023$; $p = 0.0059$, respectively). The mean serum DHEA-S concentrations did not increase following ACTH administration in Groups 1 ($p > 0.05$) and 2 ($p > 0.05$) (Table 1).

At both timepoints, greater DHEA-S concentrations were detected in Group 1. In a general view, both male and female dogs of Group 1 have greater serum DHEA-S than male and female dogs of Group 2. It is remarkable that sick male dogs have more increased DHEA-S than sick female dogs or healthy male and female dogs. However, sick female dogs have greater pre- and post-ACTH DHEA-S concentrations than healthy male or female dogs, although not more than the male dogs with ehrlichiosis. These findings illustrate the stress involved in either the acute or subclinical phases of naturally acquired canine monocytic ehrlichiosis, since dogs with ehrlichiosis included in this study have greater hormone concentrations than the healthy ones, regardless of gender. Our results indicated that ehrlichiosis provoked stress that was capable of altering the DHEA-S concentrations of sick dogs as has been reported previously (Boonstra et al., 2008; Kroboth et al., 1999; Libonati et al., 2006) in different species and clinical situations; however, we found nothing in the literature specifically related to DHEA-S concentrations in dogs with ehrlichiosis.

Table 1

DHEA-S concentrations of dogs naturally infected with *Ehrlichia canis* (sick) and healthy dogs according to gender. Different letters indicate significant differences ($p < 0.05$).

Treatment	Male				Female			
Condition	Sick		Healthy		Sick		Healthy	
Variable	Pre-ACTH	Post-ACTH	Pre-ACTH	Post-ACTH	Pre-ACTH	Post-ACTH	Pre-ACTH	Post-ACTH
DHEA-S (µg/ml)	0.083 ± 0.052a	0.092 ± 0.070a	0.027 ± 0.037b	0.028 ± 0.040b	0.032 ± 0.038c	0.034 ± 0.037c	0.017 ± 0.023d	0.019 ± 0.025d

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