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Acute and subclinical *Ehrlichia canis* infection and cortisol response to ACTH stimulation test

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Abstract

Adrenocortical disturbances are associated with canine ehrlichiosis due to the immunological changes caused by infection and consequent inflammation. Thus, this study aimed to evaluate the occurrence of adrenocortical hormonal changes in dogs naturally infected with *Ehrlichia canis* (n=21) as confirmed by the presence of anti-*E. canis* antibodies (Dot-ELISA) and nested PCR (nPCR). Serum cortisol concentrations were assessed by radioimmunoassay before and one hour after ACTH stimulation. Ten healthy dogs were subjected to the same stimulation protocol and used as controls. The results revealed that the dogs with naturally acquired acute and subclinical ehrlichiosis secreted cortisol following ACTH stimulation in similar concentrations to those of healthy dogs.

Key words: adrenal, dogs, canine ehrlichiosis, hormones.

Introduction

Canine monocytic ehrlichiosis is caused by *Ehrlichia canis*. It is a disease with a worldwide distribution that represents a major and severe canine health problem (11). Generally, this disease leads to death due to hematological changes and other factors that can be explained by the multifactorial catabolic effects associated with the disease (24). These changes might be related to the well-known immune-inflammatory responses (3, 9, 20, 22) and possibly to hormonal responses (10).

Adrenocortical hormonal secretion is often increased during inflammatory reactions and experimentally induced infections. Therefore, inflammation or infection induces a hormone-modulated defense, but the exact mechanisms involved in this process are poorly known (5). 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 ACTH and glucocorticoid secretion (1). Directly or indirectly, cytokines stimulate the hypothalamic-pituitary-adrenal axis, which inhibits or modulates inflammation via the immunosuppressive effects of glucocorticoids (12). However, some authors have observed that at low concentrations, endogenous glucocorticoids exert immunostimulatory actions on macrophages (25). Thus, the secretory activities of the adrenal glands appear to be essential for adaptations to internal and external alterations (8).

Changes in cortisol and other hormones have been described in humans with paracoccidioidomycosis (12), malaria (13), and irritable bowel syndrome (19), whereas in dogs, these conditions have been described in cases of trypanosomiasis (23), babesiosis (18, 24), parvoviral diarrhea (17), and chronic ehrlichiosis (10). Severe illness and inflammation induce cytokines release, as IL-1, IL-6, and TNF-alpha, which are responsible for pituitary stimulation and cortisol secretion (18), as described in malaria infection (13). This inflammatory response has

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already been described in experimentally induced ehrlichiosis, during its acute phase of development (3, 9, 20, 22), although the endocrine profile has not been investigated.

Studies with human patients or dogs, with different disorders, suggest that the adrenal response might depend on the species, and pathogenesis of the related illness. Also, the response in either acute or chronic illness seems to be controversial (12, 13, 17, 18).

Adrenocortical involvement may occur in dogs naturally infected with *E. canis* due to the changes in the immune system that have been previously observed in humans (12, 13) and canine patients (17, 18) with other diseases. Thus, the aim of this study was to assess adrenocortical hormonal involvement before and after ACTH stimulation in acute or subclinical naturally occurring canine monocytic ehrlichiosis by quantifying serum cortisol.

Materials and methods

Dogs

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.

The dogs included in this study were selected from the clinical routine of the Veterinary Teaching Hospital of Faculdade de Ciências Agrárias e Veterinárias, Unesp – Campus of Jaboticabal - SP, Brazil, after free and informed consent was provided by the owners. Adult dogs with ages ranging from one to 10 years, 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 in the 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 (13 males and 8 females, with mean body weight of 17.4 kg and mean age of 4.8 years) 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). Another 10 healthy dogs (4 males and 6 females, with mean body weight of 18.2 kg and mean age of 3 years) 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).

Experimental protocol

The dogs in Groups 1 and 2 were subjected to the adrenocorticotropic hormone (ACTH) stimulation test in the morning after remaining 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 -20ºC until cortisol was measured. Cortisol concentrations were assessed by radioimmunoassay using kit Coat-a-Count (Siemens), and the reference values for the species were as follows: pre-ACTH cortisol: 5 to 60 ng/mL; and post-ACTH cortisol: 60 to 170 ng/mL.

After obtaining the blood samples, all dogs in Group 1 were started on doxycycline hyclate treatment (5 mg/kg every 12 hours for 28 days orally) according to the Veterinary Teaching Hospital protocol (adapted from 15, 21).

Statistical analyses

The data were analyzed with Statistical Analysis System (SAS, 1996) (16) software and were examined for the normality (Cramér-von-Mises). The Tukey-Kramer test was used to analyze the pre- and post-ACTH cortisol, and the Student´s T-test was used to examine delta cortisol. Significance was set at $P<0.05$.

Results

The cortisol concentrations were different between the baseline and post-ACTH measurements in Groups 1 ($P<0.0001$) and 2 ($P<0.0001$). In both groups, the hormonal values were greater at the post-ACTH time points than at the pre-ACTH time points (Fig. 1). However, there were no differences when the cortisol concentrations at each time point were compared between groups ($P>0.05$). Moreover, there was no difference in delta cortisol values between the studied groups ($P>0.05$; Fig. 2).

Discussion

All dogs included in the study were carefully selected, following inclusion and exclusion criteria presented before. Leptospirosis and babesiosis were ruled out in these dogs because these infectious diseases are frequently diagnosed in the region where the study was developed and also, because cortisol changes have already

been described in dogs infected with *Babesia canis* (18, 24).

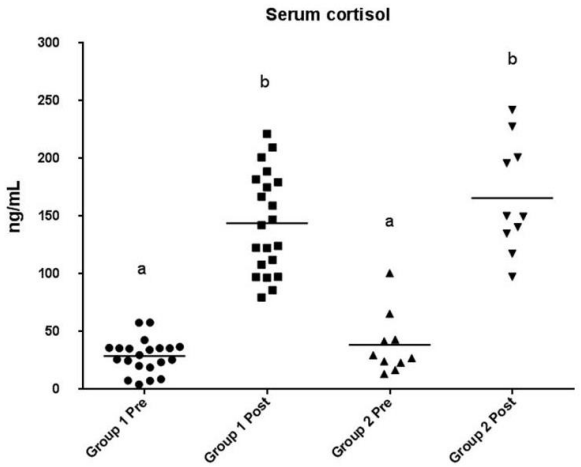

Figure 1. Point plots of the baseline and post-ACTH stimulation serum cortisol concentrations in Groups 1 and 2. The horizontal bars indicate the mean values, and the different letters indicate significant differences (P<0.05).

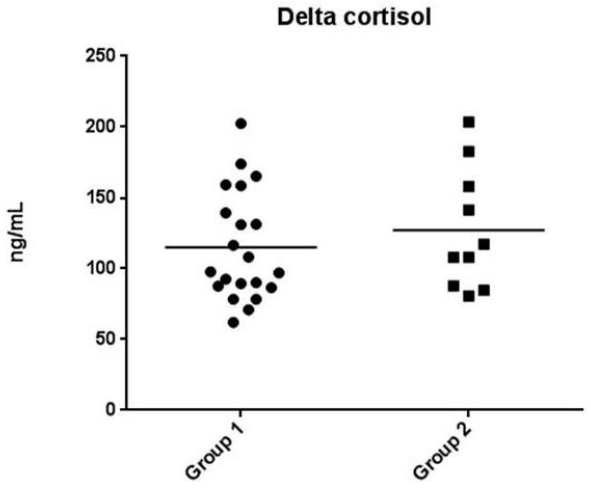

Figure 2. Point plots of the delta cortisol measures of Groups 1 and 2. The horizontal bars indicate the mean delta cortisol values of each group.

Importantly, in our study, only dogs with naturally occurring ehrlichiosis were included, which made the classification of the disease according to its phase of development inappropriate, since we could not properly inform the infection progression period. However, no dog presented with myelosuppression, which is common at the chronic phase of ehrlichiosis, which, together with the clinical signs that were most frequently observed in the selected dogs (i.e., splenomegaly, tick infestation, hyporexia/anorexia, apathy, uveitis and fever in addition to thrombocytopenia, anemia and/or leukopenia or thrombocytopenia in the absence of other clinical signs), suggests that these dogs had either the acute or subclinical phase of *E. canis* infection. Additional studies examining cortisol concentrations following ACTH administration in dogs with myelosuppression due to chronic ehrlichiosis would be relevant to determine whether the hormonal concentration would differ from our observations in this study.

Changes in cortisol concentration have been reported in chronic severe ehrlichiosis, in a study where punctual plasma cortisol was assessed from blood samples obtained at the time of the presentation at the Veterinary Hospital (10). Mean cortisol concentrations were higher in dogs with ehrlichiosis when compared to dogs with concurrent babesiosis and healthy dogs. The authors agreed that cortisol values have varied widely between dogs and groups, and assumed that the extremely low or high values in some individual dogs with ehrlichiosis could be due to the variations in the clinical severity of the disease. In order to minimize the hormonal fluctuation, which might happen even due to the blood collection, we proposed to evaluate serum cortisol in dogs with ehrlichiosis using ACTH stimulation test, as reported in healthy dogs (4) and in dogs with portosystemic shunt (7).

Our results revealed that baseline cortisol concentrations were not useful for adrenocortical evaluation in dogs with naturally acquired ehrlichiosis. This conclusion can be reached by observing the baseline cortisol concentrations of Groups 1 and 2. One sick dog had a baseline cortisol level that was below the minimum reference value (4.2 ng/mL), and two control dogs had baseline cortisol concentrations above the maximum reference value (65.6 ng/mL and 100.7 ng/mL). Researchers have discouraged the use of baseline cortisol as a unique evaluation because the concentrations of this hormone fluctuate during the day, in response to stressful stimuli and even at rest (2, 6). Therefore, baseline cortisol variations of the sick and healthy dogs in this study were considered individual.

Regarding the post-ACTH cortisol concentrations, one dog in Group 1 (221.4 ng/mL) and two dogs in the control group (227.7 ng/mL and 242.1 ng/mL) exhibited post-ACTH cortisol concentrations that were above the maximum reference value, although they were not different from the upper limit for the species in this study (P>0.05). These alterations might have been due to individual variation. They do not seem to have been indicative of spontaneous hypercortisolism and were possibly not due to infection with *E. canis*.

One hypothesis for this study was that dogs with naturally acquired ehrlichiosis have either excessive cortisol secretion, that could be related to the intense inflammatory response *E. canis* induces in dogs (3, 9, 20, 22) or have adrenocortical hypofunction, also known as relative adrenal insufficiency, characterized as inadequate production of cortisol in critical illness (14). Our

interpretations of the cortisol responses to the ACTH stimulation test were based on criteria described in the literature (7) and considered the ideal response to be a 50% increase in the post-ACTH cortisol concentration over the baseline value. Thus, the dogs in both groups exhibited adequate cortisol responses to the ACTH stimulation test. This finding was corroborated by the results of the evaluations of the pre/post-ACTH changes in cortisol (i.e., the delta cortisol); this change is a parameter that is used to evaluate adrenocortical reserve (18). In our study, the lack of significance of the delta cortisol measures between groups and the elevated post-ACTH cortisol concentrations observed in both groups suggest that dogs with naturally acquired ehrlichiosis, in either acute or subclinical phase, maintain the ability to secrete cortisol in adequate concentrations following ACTH stimulation and therefore have adequate hormonal adrenocortical reserves.

Conclusions

In conclusion, this study demonstrates that dogs with naturally acute and subclinical acquired ehrlichiosis maintain the ability to secrete cortisol after ACTH stimulation.

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