P-23

Inflammation of the adenohypophysis in dogs infected with *Leishmania* spp.

Edenilson D Frigerio[1*], Cecilia C Guizelini[2], Giulia G Jussiani[2], Gisele F Machado[2]

1. Department of Biological and Health Sciences, University Center of Adamantina, Adamantina, São Paulo, Brazil. 2. Department of Veterinary Clinics, Surgery and Reproduction, Faculty of Veterinary Medicine, São Paulo State University, UNESP, Araçatuba, São Paulo, Brazil.
*edenilsonfrigerio@fai.com.br

Background

Morphological involvement of endocrine glands, such as the pituitary gland, remains uninvestigated in dogs with canine leishmaniosis (CanL). The adenohypophysis is responsible for controlling physiological metabolic, reproductive and growth functions, contributing to the maintenance of homeostasis.

Materials and methods

We evaluated the pituitary gland of dogs naturally infected with *Leishmania spp.* (G1, n=25) euthanized at the Zoonosis Control Center, in accordance with Brazilian law for untreated animals (Ethics Committee CEUA-FOA 0354/2021). The dogs of the uninfected group (G2, n=6) had no history of neurological impairment, and euthanasia was performed "*in extremis*" after acute trauma by a car accident. Histological sections of the pituitary gland were stained with hematoxylin and eosin (HE) and subjected to immunohistochemistry (IHC) for *Leishmania spp*., T $CD3^+$ lymphocytes and B $Pax5^+$ lymphocytes. For this study, due to the better availability and quality of the tissue, areas of the adenohypophysis (*pars distalis* and *pars intermedia*) were evaluated. Inflammation was classified by intensity in a score from 0 to 3, absent (0), mild (1), moderate (2), and marked (3). The IHC evaluation was performed in five high-power fields (*hot spot*) in a 40x objective of each region with manual counting (Image J1.52ª) of the T $CD3^+$ lymphocytes and B $Pax5^+$ lymphocytes and for amastigotes analyzed in 40x and 100x objectives in the entire field. The Shapiro-Wilk test was used to assess the normality of the data. Differences between groups were determined by the Mann-Whitney test. The correlation between variables was assessed by Sperman's correlation test. P values <0.05 were considered statistically significant.

Results

In HE, group G1 showed an inflammatory infiltrate composed of lymphocytes, plasma cells and macrophages in the regions evaluated, different from group G2 which showed rare cells with a diffuse distribution. IHC enabled better identification and quantification of T $CD3^+$ lymphocytes. There was a significant difference in the number of $TCD3^+$ lymphocytes between groups G1 and G2 in the *pars distalis* (P = 0.0121). This difference showed a positive correlation with the inflammation score (P=<0.0001) (Figure 1). B $Pax5^+$ lymphocytes were immunolabeled in the *pars distalis* of three animals (Figure 2). Amastigotes in the adenohypophysis of four infected dogs were identified using IHC.

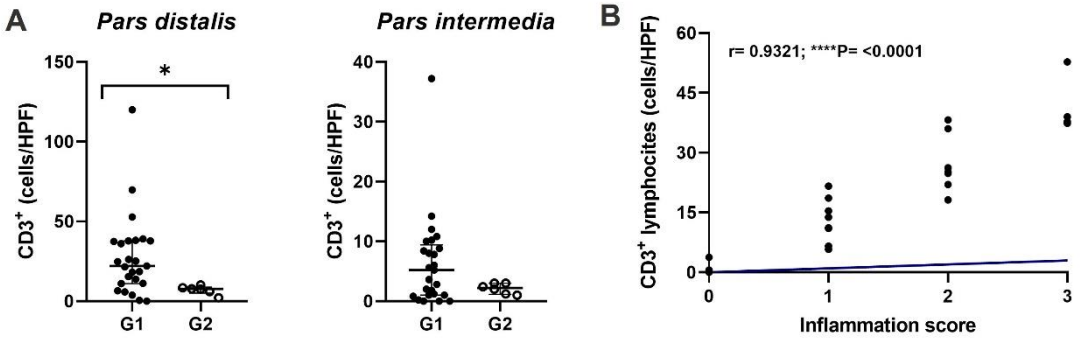

Figure 1. (A) Dispersion graphs showing the number of T $CD3^+$ lymphocytes in the adenohypophysis regions of dogs. The horizontal lines represent the median and interquartile range values. **(B)** Dispersion graph showing a high positive correlation between inflammation score and median T $CD3^+$ lymphocyte count in the *pars distalis*.

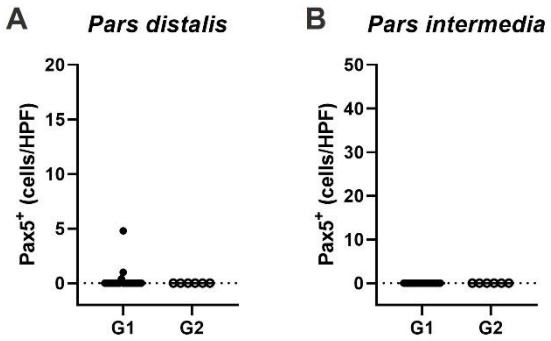

Figure 2. Dispersion graphs showing the number of B Pax5$^+$ lymphocytes in the adenohypophysis regions of dogs.

Conclusions

These data indicate the predominance of T CD3$^+$ lymphocytes in the inflammation of the adenohypophysis observed in CanL, which differs from the inflammation observed in other organs, where the presence of B lymphocytes is evident. We presuppose that the presence of the parasite and inflammation in the adenohypophysis may contribute to the gland's dysfunction in CanL.

Funding: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; #2022/07046-5).

Conflict of interest: All authors declare that they have no conflicts of interest.