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Increased S100 protein immunoexpression in the brain of dogs with Leishmaniosis

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Background
S100 protein is actively secreted by astrocytes during cellular stress and is responsible for astroglial proliferation and maturation. Although low concentrations of S100 are considered neuroprotective, higher concentrations of S100 have been shown to activate microglia *in vitro*. We aimed to investigate the immunoexpression of the S100 protein in the brains of dogs with Canine Leishmaniosis (CanL).

Materials and Methods
Twenty dogs diagnosed with CanL were used, euthanized at the Zoonosis Control Center, in accordance with the Brazilian law for untreated animals (Ethics Committee CEUA-FOA 739/2022) and immediately submitted for necropsy. To compose the control group, samples were collected from five dogs whose death was not related to infectious causes and which did not present histopathological changes in the nervous tissue (ex. abdominal trauma). Infected animals had inflammatory changes classified from mild to severe in the nervous tissue, including meningitis, choroiditis, and the presence of perivascular cuffs, evaluated in HE-stained sections. Immunohistochemistry (IHC) was performed using primary antibodies to S100, GFAP (Glial Fibrillary Acidic Protein) and IBA-1, a marker for activated microglia. The IHC assessment was carried out by capturing images with a 40x objective of 5 fields (hotspots) of the subependymal and subpial regions of each animal. Using ImageJ software, the percentage of marked area for each antibody was determined. The data was statistically analyzed using GraphPad Prism.

Results
A significant increase in immunolabeling for the S100 protein was observed in the brains of dogs with CanL compared to the control group, in the subpial (p = 0.0000719) and subependymal (p = 0.0009185) regions (Figure 1A). S100 labelling was mainly located in the cytoplasm of astrocytes, with little evidence of its extensions, and sometimes co-localized with GFAP labelling. Although it is believed that the increase in S100 may be related to the activation of microglia, no statistical correlation was found between the expression of S100 and IBA-1, however, there was a positive correlation between the expression of S100 and GFAP (r = 0.44, p = 0.0388) (Figure 1B).

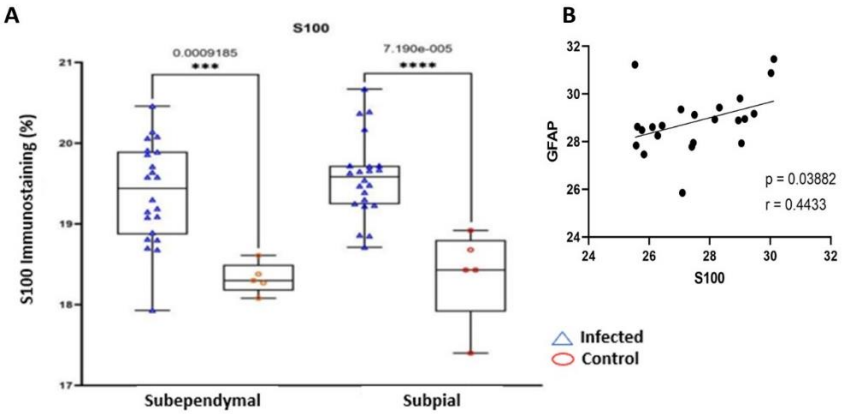

Figure 1. (A) Boxplot graph representing the percentage of marked area of S100 in the subpial and subependymal regions, in infected and control groups. The average marked area of the infected group was higher than the control group in both regions analyzed, with a significant difference (p =0.0009185)

between the subependyma and (p = 7.190e-005) in the subpial region. **(B)** Correlation graph demonstrating the positive correlation between the percentage of immunostaining for GFAP and S100 (r = 0.443; p = 0.03).

Conclusion

The increased expression of S100 in astrocytes in the brains of dogs with CanL may play a role in modulating a pro-inflammatory environment in the brains of these animals.

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