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Evaluation of mir-155 and mir-21 expression in the brain of dogs with Leishmaniosis

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Background

Canine Leishmaniosis (CanL) is a chronic and progressive disease, that may include neurological disorders. Due to systemic inflammation, meningitis and choroiditis are found in infected dogs, in addition to the presence of perivascular cuffs and a pro-inflammatory environment [1]. Dysregulation of microRNAs activity may be involved in several diseases since they regulate the expression of proteins that are fundamental in the immune system. The objective of this study was to characterize the expression of the mir155 and mir21 in the brain inflammatory response in 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 to 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. Brain total RNA was extracted using the AllPrep DNA/RNA/miRNA Universal Kit (Qiagen). Degrees of inflammation were assigned by evaluating HE-stained sections of the brain. The expression of mir-155 (Slope -3.755; Efficiency 0.85; R2 = 0.969) and mir-21 (Slope - 3.857; Efficiency 0.82; R2 = 0.997) in the brain were evaluated by RTqPCR and relative gene expression was determined using mir-103p as a normalizing gene (Slope -3.868; Efficiency 0.81; R2 = 0.997). Statistical analyzes were performed using GraphPrism.

Results

Seven dogs were classified as having a moderate (5/20) to severe (2/20) inflammation score, while the remaining dogs had no (8/20) or mild (4/20) degrees of inflammation. There was no difference in the relative gene expression of mir-21 or mir155 between the control and infected groups (Figure 1A and B). There was also no significant correlation between the expression of mir-21 or mir-155 and the inflammation score of the animals (Figure 1C). Interestingly, the dogs that showed the highest expression of mir-155 had the highest inflammation scores. A positive correlation was observed between the expression of mir-21 and mir-155 in infected dogs (Figure 1D). miRNA 155 is associated with promoting the reactivity of astrocytes and microglia. The TRL receptor pathway, activated in the nervous tissue of dogs with CanL [1], is possibly regulated by mir-155. In contrast, mir-21 acts by suppressing neuroinflammation. In CanL, the increase in its expression was related to a decrease in the oxidative response and consequent difficulty in eliminating the parasite [2].

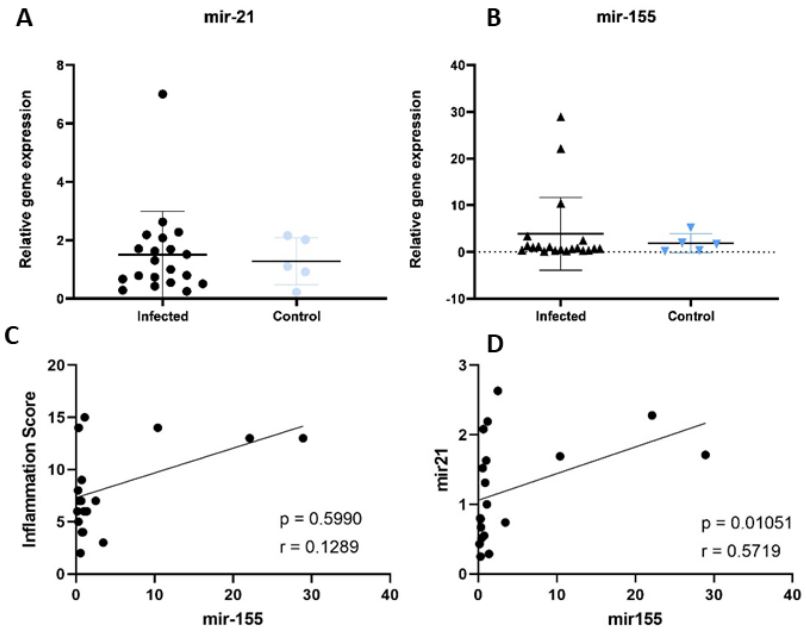

Figure 1. Relative gene expression of mir21 **(A)** and mir155 **(B)** in infected and control groups. Correlation graph demonstrating the tendency of positive correlation between the inflammation score and increased mir-155 expression **(C)**. Correlation graph demonstrating positive correlation between the expression of mir-21 and mir-155 (p = 0,0010; r = 0.5719) **(D)**.

Conclusion

The brain immune response in CanL is a complex process regulated by several factors. Our findings suggest that mir-155 and mir-21 are not related to the development of inflammatory changes in the brains of dogs with CanL. Thus, complementary studies with other relevant miRNAs in the CNS should be carried out to better understand their role in the disease's pathogenesis.

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

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