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UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
FACULDADE DE MEDICINA VETERINÁRIA
CAMPUS DE ARAÇATUBA

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**MIR-150 REGULA A CARGA PARASITÁRIA DE
LEISHMANIA INFANTUM E OS NÍVEIS DE GZMB NAS
PBMCS DE CÃES COM LEISHMANIOSE VISCERAL
CANINA**

Araçatuba
2022

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Dissertação apresentada à Faculdade de Medicina Veterinária de Araçatuba da Universidade Estadual Paulista “Júlio de Mesquita Filho” – UNESP, como parte dos requisitos para a obtenção do título de Mestre em Ciência Animal (Área de Medicina Veterinária Preventiva e Produção Animal).

Orientadora: Prof.^a Associada Valéria Marçal Felix de Lima

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CERTIFICADO DE APROVAÇÃO

Título: **MIR-150 REGULA A CARGA PARASITÁRIA DE LEISHMANIA INFANTUM E OS NÍVEIS DE GZMB NAS PBMCS DE CÃES COM LEISHMANIOSE VISCERAL CANINA**

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***“Bem sei que tudo podes, e nenhum
dos teus planos pode ser frustrado.”***

Jó 42:2

SOARES, M.F. et al. **miR-150 regula a carga parasitária de *Leishmania infantum* e os níveis de GZMB nas PBMCs de cães com Leishmaniose Visceral Canina.** 2022. 100 f. Dissertação (Mestrado) - Faculdade de Medicina Veterinária, Universidade Estadual Paulista, Araçatuba, 2022.

RESUMO

A *Leishmania infantum* causa a leishmaniose visceral, uma doença tropical negligenciada que pode modular a resposta imune do hospedeiro por meio de pequenos RNAs não codificantes chamados microRNAs (miRNAs). Alguns miRNAs são expressos diferencialmente em células mononucleares do sangue periférico (PBMCs) de cães com leishmaniose visceral canina (LCan), incluindo o miR-150, cuja expressão está diminuída. Embora o miR-150 esteja negativamente correlacionado com a carga parasitária de *L. infantum*, não está claro se o miR-150 afeta diretamente a carga parasitária de *L. infantum* e (em caso afirmativo) como esse miRNA contribuiria para a infecção. Aqui, isolamos as PBMCs de 14 cães naturalmente infectados (grupo LCan) e seis cães saudáveis (grupo controle) e as tratamos *in vitro* com o mimetizador ou o inibidor do miR-150. Medimos a carga parasitária de *L. infantum* usando o qPCR e comparamos os tratamentos. Também medimos os níveis de proteína-alvo do miR-150 preditas *in silico* (STAT1, TNF- α , HDAC8 e GZMB) usando citometria de fluxo ou ensaios imunoenzimáticos. O mimetizador do miR-150 diminuiu a carga parasitária de *L. infantum* nas PBMCs de cães do grupo LCan. Também descobrimos que a inibição do miR-150 reduziu os níveis de GZMB. Esses achados demonstram que o miR-150 exerce uma função importante na infecção por *L. infantum* nas PBMCs caninas, o que poderia ser usado para mais estudos visando o desenvolvimento de drogas.

Palavras-chave: LCan. *Leishmania infantum*. miRNA. miR-150. GZMB

SOARES, M.F. et al. **miR-150 regulates the *Leishmania infantum* parasitic load and GZMB levels in peripheral blood mononuclear cells of dogs with canine visceral leishmaniasis.** 2022. 100 f. Dissertação (Mestrado) - Faculdade de Medicina Veterinária, Universidade Estadual Paulista, Araçatuba, 2022.

ABSTRACT

Leishmania infantum causes visceral leishmaniasis, a neglected tropical disease that can modulate the host immune response by altering the expression of small non-coding RNAs called microRNAs (miRNAs). Some miRNAs are differentially expressed in peripheral blood mononuclear cells (PBMCs) of dogs with visceral canine leishmaniasis (CanL), like the down-regulated miR-150. Even though miR-150 is negatively correlated with *L. infantum* parasitic load, it is unclear if miR-150 directly affects *L. infantum* parasitic load and (if so) how this miRNA would contribute to infection. Here, we isolated PBMCs from 14 naturally infected dogs (CanL group) and six healthy dogs (Control group) and treated them *in vitro* with miR-150 mimic or inhibitor. We measured *L. infantum* parasitic load using qPCR and compared treatments. We also measured miR-150 *in silico* predicted target protein levels (STAT1, TNF- α , HDAC8, and GZMB) using flow cytometry or enzyme-linked immunosorbent assays. Increasing miR-150 activity diminished *L. infantum* parasitic load in CanL PBMCs. We also found that inhibition of miR-150 reduced GZMB levels. These findings demonstrate that miR-150 plays an important role in *L. infantum* infection in canine PBMCs, and these findings merit further studies aiming at drug development.

Key-words: CanL. *Leishmania infantum*. miRNA. miR-150. GZMB.

APÊNDICE - REFERÊNCIAS DA INTRODUÇÃO GERAL

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ANEXO B - CERTIFICADO DO COMITÊ DE ÉTICA



UNIVERSIDADE ESTADUAL PAULISTA
"JULIO DE MESQUITA FILHO"



CAMPUS ARAÇATUBA
FACULDADE DE ODONTOLOGIA
FACULDADE DE MEDICINA VETERINÁRIA

CEUA - Comissão de Ética no Uso de Animais
CEUA - Ethics Committee on the Use of Animals

CERTIFICADO

Certificamos que o Projeto de Pesquisa intitulado "**Papel do miR-150 na indução de resposta imune contra Leishmania infantum em cães**", Processo FOA nº 00259-2020, sob responsabilidade de Valéria Marçal Félix de Lima apresenta um protocolo experimental de acordo com os Princípios Éticos da Experimentação Animal e sua execução foi aprovada pela CEUA em 01 de Dezembro de 2020.

VALIDADE DESTE CERTIFICADO: 31 de Dezembro de 2022.

DATA DA SUBMISSÃO DO RELATÓRIO FINAL: até 31 de Janeiro de 2023.

CERTIFICATE

We certify that the study entitled "**The role of miR-150 in the immune response against Leishmania infantum in dogs**", Protocol FOA nº 00259-2020, under the supervision of Valéria Marçal Félix de Lima presents an experimental protocol in accordance with the Ethical Principles of Animal Experimentation and its implementation was approved by CEUA on December 01, 2020.

VALIDITY OF THIS CERTIFICATE: December 31, 2022.

DATE OF SUBMISSION OF THE FINAL REPORT: January 31, 2023.

Prof. Associado Guilherme de Paula Nogueira
Coordenador da CEUA
CEUA Coordinator