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**UNIVERSIDADE ESTADUAL PAULISTA**  
**“JÚLIO DE MESQUITA FILHO”**  
**FACULDADE DE MEDICINA VETERINÁRIA**  
**CÂMPUS DE ARAÇATUBA**

**RELAÇÃO ENTRE A EXPRESSÃO DE miRNAs EM**  
**CÉLULAS MONONUCLEARES DE SANGUE**  
**PERIFÉRICO E CARGA PARASITÁRIA NA**  
**LEISHMANIOSE VISCERAL CANINA**

**Jaqueline Poletto Bragato**  
Médica Veterinária

ARAÇATUBA – SP  
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**Orientadora: Prof<sup>a</sup>. Adjunto Valéria Marçal Felix de Lima**

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## EPÍGRAFE

*“A única maneira de fazer um bom trabalho é amando o que você faz. Se você ainda não encontrou, continue procurando. Não se desespere. Assim como no amor, você saberá quando tiver encontrado.”*

**Steve Jobs**

## **DEDICATÓRIA**

**Aos meus pais Luiz e Neusa, que  
nunca mediram esforços para que  
eu pudesse realizar todos os meus  
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**RELAÇÃO ENTRE A EXPRESSÃO DE miRNAs EM CÉLULAS  
MONONUCLEARES DE SANGUE PERIFÉRICO E CARGA PARASITÁRIA  
NA LEISHMANIOSE VISCERAL CANINA**

**RESUMO** - A Leishmaniose Visceral (LV) no homem é uma doença crônica e frequentemente fatal se não tratada. A doença possui alta taxa de mortalidade e a região de Araçatuba concentra grande número de casos no estado de São Paulo. A Leishmaniose Visceral Canina (LVC) constitui um grave problema de Saúde Pública, pois os animais infectados são potentes transmissores do parasita para humanos pelo vetor flebotomíneo. O cão é, portanto, um alvo importante nas medidas de controle. A progressão da infecção canina é acompanhada por falha na imunidade celular com redução de linfócitos circulantes e citocinas que suprimem a função dos macrófagos. A função da célula T na indução da resposta celular é determinante para a eliminação do parasita no interior dos macrófagos. Embora a supressão imunológica já esteja caracterizada, os fatores determinantes são pouco conhecidos. Recentes estudos mostraram que a regulação da função efetora dos macrófagos e células T parece depender de microRNAs (miRNAs). Existem muitas evidências da função dos miRNAs na regulação da expressão de proteínas que são fundamentais para o desenvolvimento, função e diferenciação de vários tipos celulares do sistema imunológico. Uma desregulação da atividade dos miRNAs está envolvida em diversas doenças, incluindo a LV. Na LVC, devido a supressão imunológica celular ser determinante para a progressão da doença, o conhecimento de miRNAs associados a regulação imunológica pode ser importante para a mudança do padrão de resposta.

**Palavras-chave:** cão, Leishmaniose Visceral, microRNA, zoonose



## **RELATIONSHIP OF PERIPHERAL BLOOD MONONUCLEAR CELLS miRNA EXPRESSION AND PARASITIC LOAD IN CANINE VISCERAL LEISHMANIASIS**

**SUMMARY** - Visceral Leishmaniasis (VL) in humans is a chronic and often fatal disease if left untreated. The disease has high mortality rate, and the region of Araçatuba concentrates a large number of cases in São Paulo state. Canine VL (CVL) is a serious public health problem, because infected animals are potent transmitters of the parasite to humans by the vector phlebotomine. Therefore, dogs are important targets in the control measures. The progression of canine infection is accompanied by a failure of cellular immunity with reducing of circulating lymphocytes and cytokines that suppress the macrophage function. The role of T cells in the induction of cellular response is crucial to the elimination of the parasite in macrophages. Although immunosuppression is already characterized, the determining factors are not well known. In the last decade studies have shown that regulation of effector function of macrophages and T cells appears to depend on microRNAs (miRNAs). There are many evidences of function of miRNA in regulating of the expression of proteins that are primordial for the development, function and differentiation of various cell types of the immune system. A deregulation of miRNA expression is involved in a variety of disorders including VL. In CVL, due to cell immune suppression be determinant of disease progression, knowledge of miRNAs associated with immune regulation may be important for change the response pattern.

**Keywords:** dog, Visceral Leishmaniasis, microRNA, zoonosis

### **OBJETIVO GERAL**

Comparar o padrão de atividade de miRNA em células mononucleares de sangue periférico de cães saudáveis e cães naturalmente infectados por *L. infantum*, sendo assim, melhorar a nossa compreensão da patogênese da LV no hospedeiro e revelar novos caminhos que possam constituir alvos terapêuticos.

### **OBJETIVOS ESPECÍFICOS**

Identificar perfis específicos de miRNA associados a infecção por *L. infantum* cujos alvos sejam proteínas associadas a regulação da resposta imunológica.

Correlacionar o perfil específico de miRNA das células mononucleares do sangue periférico de cães naturalmente infectados com a carga parasitária.

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## **CAPÍTULO 2**

## RELATIONSHIP OF PERIPHERAL BLOOD MONONUCLEAR CELLS miRNA EXPRESSION AND PARASITIC LOAD IN CANINE VISCERAL LEISHMANIASIS

**SUMMARY** - Visceral leishmaniasis (VL) in humans is a chronic and often fatal disease if left untreated. Dogs are powerful reservoirs of the parasite which allows for transmission to humans by a phlebotomine vector. Immune response can be modulated by microRNAs (miRNAs). Here, we evaluated the expression of miRNAs in peripheral blood mononuclear cells (PBMC) of symptomatic dogs naturally infected with *Leishmania (L.) infantum* (n=10) and compared to healthy dogs (n=5). Microarray analysis revealed that miR 21, miR 424, miR 194 and miR 451 had a 3-fold increase in expression, miR 192, miR 503, and miR 371 had a 2-fold increase in expression, whereas a 2-fold reduction in expression was observed for miR 150 and miR 574. Real-time PCR validated the differential expression of miR 21, miR 150, miR 451, miR 192, miR 194, and miR 371. Parasite load of PBMC was measured by real-time PCR and correlated to differentially expressed miRNA, showing a strong positive correlation with expression of miR 194, a regular positive correlation with miR 371 expression and a moderate negative correlation with miR 150 expression in PBMC. These findings suggest that the immune response in dogs infected with *L. infantum* may be post-transcriptionally modulated by miRNAs. We suggest that their role in immune regulation and their correlation to parasite load may help in the identification of therapeutic targets in Canine Visceral Leishmaniasis (CVL).

**Keywords:** Canine Visceral Leishmaniasis, Immune response, MicroRNAs, Zoonosis

**AUTHOR SUMMARY** - VL is a chronic disease that affects humans, dogs, cats and other mammalian animals. The disease is caused by a protozoan called *Leishmania infantum* and can lead to death if left untreated. Dogs are the main reservoirs of the parasite, which is then transmitted to humans by the phlebotomine vector. Dogs cannot form an effective immune response against this infection, and this could be modulated by small non-coding RNAs, called microRNAs, responsible for post-transcriptional control of gene expression. In this study, we investigate the miRNAs modulated in canine visceral leishmaniasis and the correlation of these miRNAs with the parasite load of the dogs. Furthermore, we identified in silico the target genes of these miRNAs. These findings can help in the identification of therapeutic targets for this disease.

## **Introduction**

VL is a zoonosis caused by the protozoan *Leishmania infantum* and is the most fatal form of this parasitic disease [1]. VL is still one of the most neglected diseases in the world and, of the human cases reported in America, 95.1% are in Brazil [2]. Dogs are considered the main domestic reservoirs of *L. Infantum* [3]. Once in the vertebrate host, the parasite may cause lesions and symptoms that are characteristic of CVL, although some infected dogs may be oligo or asymptomatic [4], others may evolve to spontaneous cure [5]. The most frequent signs of VL are lymphadenopathy, onychogryphosis, cutaneous lesions, weight loss, cachexia, fever and locomotor abnormalities [6].

In dogs infected with *L. infantum*, a reduction in the number of T-lymphocytes in the peripheral blood occurs [7,8], as well as a disorganization of the splenic white pulp [9]. Protective immunity in dogs has generally been associated with a cellular immune response manifested by a positive lymphoproliferative response to *Leishmania* spp antigens [10] and cytokine production, such as IFN $\gamma$  and TNF- $\alpha$ , which are necessary for macrophage activation and parasite death [11]. The role of T cell in the induction of the

that their role in immune regulation and their correlation to parasite load may help in the identification of therapeutic targets in Canine Visceral Leishmaniasis (CVL).

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