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Short Communication

Increased *leptin* mRNA expression in the blood of dogs naturally infected by *Leishmania infantum*

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ABSTRACT

Canine leishmaniosis (CL) is a severe and potentially fatal zoonosis caused by the protozoan *Leishmania infantum*. Severe forms of CL are commonly associated with a non-protective, humoral immune-response and high parasitic loads. Leptin, a 16 kD hormone mainly secreted by adipocytes, regulates both the innate and adaptive immunity. The goal of this study was to evaluate *leptin* mRNA expression levels in blood samples from privately owned dogs with CL ($n = 11$) and healthy controls ($n = 10$) using quantitative, real-time polymerase chain reaction. Blood samples from dogs with CL expressed significantly higher *leptin* mRNA levels (two-fold) compared to healthy controls ($P = 0.018$). The results suggest a possible involvement of leptin in the pathophysiology of *Leishmania* infection in dogs and the possible use of leptin as a biomarker for CL. Future studies investigating the immunological role of leptin in dogs with CL are warranted.

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Canine leishmaniosis (CL) is a severe and potentially fatal zoonosis caused by the protozoan *Leishmania infantum*. Clinically CL is very polymorphic and may manifest as a subclinical or severe systemic disease (Bottero et al., 2006). Presentation can range from mild dermatitis associated with specific cellular immunity (Saridomichelakis and Koutinas, 2014), to a severe systemic disease characterized by renal failure, a non-protective humoral immune-response, and a reduced T helper (Th) cell-mediated immune response (Saridomichelakis and Koutinas, 2014).

Biological molecules and their ability to orchestrate the Th1/Th2 immune response have been widely investigated in the past few years for their potential use as therapies for infectious diseases. Leptin is a 16 kD hormone secreted primarily by adipose tissue, but also by the stomach, skeletal muscle, placenta, memory T cells and macrophages (Procaccini et al., 2014). Leptin has multiple physiological roles, including control of energy balance and body growth, renal function, haematopoiesis, reproduction, and immune function (Agarwal et al., 2009). Immunologically, leptin activates naïve ($CD4^+CD45RA^+$) T cells and inhibits memory ($CD4^+CD45RO^+$) and regulatory ($Foxp3^+CD4^+CD25^{high}$) T cells (Procaccini et al., 2014). Leptin also promotes the polarization of Th cells towards a Th1 lineage, thereby increasing the production of interferon (IFN)-γ and tumour necrosis factor (TNF)-α (Procaccini et al., 2014).

Regulatory T cells (Treg) secrete leptin, but also express high levels of leptin receptors regulating their own activation in an autocrine fashion (Procaccini et al., 2014). Leptin has also been shown to increase proinflammatory cytokines in macrophages exposed to *Leishmania* spp. and to stimulate phagocytosis of the protozoan (Gainsford et al., 1996). Overall, an increased inflammatory response corresponds with disease exacerbation and the remaining impaired Tregs are important in the regulation of disease pathology in leishmaniosis (Ehrlich et al., 2014). Indeed, Tregs, macrophage activation, and a proinflammatory state are all significant in the pathogenesis of CL.

The objective of this study was to evaluate the mRNA expression of leptin in leukocytes using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) on blood samples from dogs with CL and from healthy control dogs. The hypothesis was that dogs with CL will show altered transcription of the Leptin gene in leukocytes, consistent with the lower levels of circulating Treg in dogs with CL (Cortese et al., 2013) and the poor protective immunological response to the parasite in peripheral tissues with high Treg levels (Adalid-Peralta et al., 2011).

A total of 21 dogs were enrolled in this study, 11 diagnosed with (but not treated for) CL, and 10 healthy control dogs (CN). The diagnosis of CL was performed in accordance with current guidelines (Paltrinieri et al., 2010). In all dogs a body condition score (BCS) and a test for infectious diseases (Canine SNAP 4Dx, IDEXX laboratories) were also conducted. Peripheral blood was collected for a complete cell blood count (CBC) and for biochemical analysis. Total

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Table 1
Primers used.

Canine gene	Primer sequence	Amplicon length
Leptin	Forward: GTGGCACTGACGACTGTTTACTG	150
	Reverse: GACCATTCCAAGGCCCATACT	
GAPDH	Forward: TGTCCCCACCCCCAATG	68
	Reverse: TCGTCATATTTGGCAGCTTTCTC	

RNA was extracted from fresh blood and converted to complementary DNA (cDNA) by reverse transcription.

Specific primers from canine *leptin* and canine *glyceraldehyde-3-phosphate dehydrogenase-like* (*GAPDH*) were designed from published mRNA GenBank gene sequences (Table 1). PCR reactions were performed as previously described (Squillacioti et al., 2011). All variables, clinical, haematological, and ΔC_t ($C_t$leptin – C_tGAPDH) values, were subjected to normality testing. Student's unpaired *t* test and the Mann–Whitney test were used and $P \leq 0.05$ was considered significant. Statistical analysis was conducted using MedCalc 12.0 statistical software.

Dogs with CL included seven males (six intact) and four females (three intact), while healthy controls dogs (CN) included five males (all intact) and five females (all intact). The mean age (±standard deviation) at the point of enrolment was 6.36 ± 1.69 years and 5 ± 2.41 years for the CL and CN groups, respectively. The median BCS was 4/9 (range, 4–6) and 5/9 (range, 4–6) for the CL and CN groups, respectively. There was no difference in bodyweight ($P = 0.93$), age ($P = 0.24$), and sex ($P = 0.55$) distributions between the two groups. The more frequent clinical signs observed in CL were lymphadenopathy (72.7%), ocular disorders (45%), splenomegaly (45.5%), moderate to severe generalized exfoliative dermatitis (45.5%) and onychogryphosis (36.4%). The results of haematological parameters are summarized in Table 2. A significant increase in *leptin* mRNA levels (two-fold) was detected in the CL compared with the CN group ($P = 0.018$) (Fig. 1).

Our findings show that an increase in *leptin* mRNA expression levels is present in dogs with CL. There were no differences in bodyweight and nutritional status between the two groups, ruling out obesity as a confounding factor and confirming the possible involvement of leptin in the pathogenesis of protozoan diseases (Baltaci and Mogulkoc, 2012). In addition, although our study evaluated mRNA expression levels in leucocytes and not leptin circulating protein levels, the results could explain the systemic inflammatory status present in CL (La Cava and Matarese, 2004; La Cava, 2012).

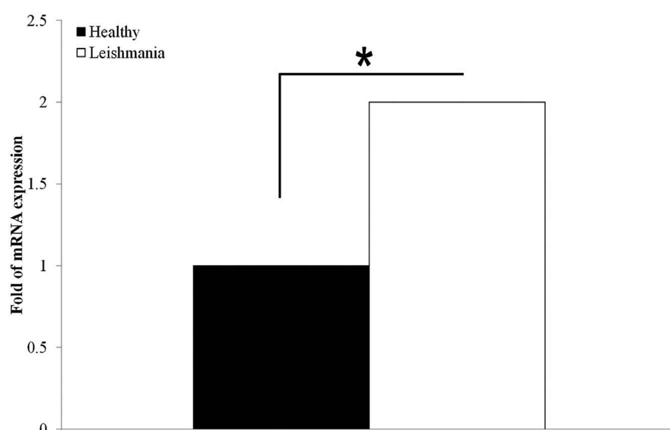

Fig. 1. Relative *leptin* mRNA expression in blood samples from dogs with leishmaniosis compared with healthy control dogs. Groups were compared using an unpaired Student's *t* test. *$P \leq 0.05$.

Table 2
Mean (±standard deviation, SD) of the haematological parameters evaluated in the 21 dogs enrolled in the present study.

Parameter	CN (±SD)	CL (±SD)	*P*
RBC ($\times 10^6$/dL)	7.3 ± 0.7	5.8 ± 1.2	0.0063
Hb (g/dL)	16.9 ± 1.8	12.8 ± 2.7	0.0009
Hct (%)	50.6 ± 4.1	38.3 ± 82	0.0006
MCV (fL)	69.0 ± 3.0	65.2 ± 2.1	0.007
MCH (pg)	23.0 ± 0.7	21.8 ± 0.8	0.0047
MCHC (%)	33.4 ± 1.2	33.5 ± 0.5	0.88
PLT ($\times 10^3$/mm^3)	266 ± 140	161 ± 93	0.045
WBC ($\times 10^3$/mm^3)	10 ± 2	9.6 ± 4.7	0.63
Urea (mg/dL)	42.2 ± 6.0	48.0 ± 17.6	0.38
Creatinine (mg/dL)	0.8 ± 0.2	1.4 ± 1.0	0.11
ALT (U/L)	41.2 ± 6.9	55.7 ± 33.6	0.18
AST (U/L)	43.0 ± 6.5	55.3 ± 11.6	0.036
TP (g/dL)	6.8 ± 0.2	8.9 ± 1.9	0.0042

CN, healthy dogs; CL, dogs with canine leishmaniosis; RBC, red blood cells; Hb, haemoglobin; Hct, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; Plt, platelet; WBC, white blood cells; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TP, total proteins.

Leptin is highly involved in the regulation of the immune system: it stimulates the production of proinflammatory cytokines such as interleukin (IL)-1 and IL-6, it is stimulated by these same cytokines, and it inhibits Tregs (La Cava and Matarese, 2004; Procaccini et al., 2014). These immunological effects may be relevant in severe forms of CL in which alterations of the immune response (reduced circulating and increased tissue Tregs) (Adalid-Peralta et al., 2011; Cortese et al., 2013) and subsequent persistent chronic inflammation could be the effect of a systemic increase in leptin expression.

Because circulating leucocytes include both monocytes and lymphocytes, it is difficult to discern exactly which cell source may be responsible for this increase in *leptin* mRNA expression. *Leptin* increased mRNA levels in circulating mononuclear cells could be due to either an increase in total mononuclear cells (increase in mRNA levels compared to controls) and/or to an increased *leptin* gene transcription in Tregs. This increased transcription in turn may lead to an increase of Tregs in peripheral tissues with lesions, resulting in higher tissue production of leptin reflecting the CL immune dysregulation in tissues.

In conclusion, although the results of this study are preliminary and limited to *leptin* mRNA expression in circulating mononuclear cells, they suggest an involvement of leptin in CL. Future studies investigating circulating leptin protein levels, the immunological role of leptin in dogs with CL, and the potential utility of leptin as a biomarker of the severity of CL are warranted.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

References

Adalid-Peralta, L., Fragoso, G., Fleury, A., Sciutto, E., 2011. Mechanisms underlying the induction of regulatory T cells and its relevance in the adaptive immune response in parasitic infections. International Journal of Biological Sciences 7, 1412–1426.

Agarwal, R., Rout, P.K., Singh, S.K., 2009. Leptin: A biomolecule for enhancing livestock productivity. Indian Journal of Biotechnology 8, 169–176.

Baltaci, A.K., Mogulkoc, R., 2012. Plasma leptin levels in rats with induced Toxoplasma gondii infection. Bratislava Medical Journal 113, 67–69.

Bottero, E., Poggi, M., Viglione, M., 2006. Lesioni papulari indotte da Leishmania spp. in cani giovani. Veterinaria 1, 33–36.

Cortese, L., Annunziatella, M., Palatucci, A.T., Rubino, V., Piantedosi, D., Di Loria, A., Ruggiero, G., Ciaramella, P., Terrazzano, G., 2013. Regulatory T cells, cytotoxic T lymphocytes and a T(H)1 cytokine profile in dogs naturally infected by Leishmania infantum. Research in Veterinary Sciences 95, 942–949.

Ehrlich, A., Moreno Castilho, T., Goldsmith-Pestana, K., Chae, W.J., Bothwell, A.L., Sparwasser, T., McMahon-Pratt, D., 2014. The immunotherapeutic role of regulatory T cells in *Leishmania* (*Viannia*) *panamensis* infection. Journal of Immunology 193, 2961–2970.

Gainsford, T., Willson, T.A., Metcalf, D., Handman, E., McFarlane, C., Nq, A., Nicola, N.A., Alexander, W.S., Hilton, D.J., 1996. Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. Proceedings of the National Academy of Sciences of the United States of America 93, 14564–14568.

La Cava, A., 2012. Proinflammatory activities of leptin in non-autoimmune conditions. Inflammation and Allergy Drug Targets 11, 298–302.

La Cava, A., Matarese, G., 2004. The weight of leptin in immunity. Nature Reviews. Immunology 4, 371–379.

Paltrinieri, S., Solano-Gallego, L., Fondati, A., Lubas, G., Gradoni, L., Castagnaro, M., Crotti, A., Maroli, M., Oliva, G., Roura, X., et al., 2010. Guidelines for diagnosis and clinical classification of leishmaniasis in dogs. Journal of American Veterinary Medical Association 236, 1184–1191.

Procaccini, C., Pucino, V., De Rosa, V., Marone, G., Matarese, G., 2014. Neuro-endocrine networks controlling immune system in healthy and disease. Frontiers in Immunology 5, 143.

Saridomichelakis, M.N., Koutinas, A.F., 2014. Cutaneous involvement in canine leishmaniosis due to Leishmania infantum (syn. L. chagasi). Veterinary Dermatology 25, 61–71.

Squillacioti, C., De Luca, A., Liguori, G., Paino, S., Mirabella, N., 2011. Expression of urocortin and corticotropin-releasing hormone receptors in the bovine adrenal gland. General Comparative Endocrinology 172, 416–422.