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Isotype patterns of immunoglobulins: Hallmarks for clinical status and tissue parasite density in brazilian dogs naturally infected by *Leishmania* (*Leishmania*) *chagasi*

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Abstract

The role of anti-leishmanial immune response underlying the susceptibility/resistance during canine visceral leishmaniasis (CVL) has been recognized throughout ex vivo and in vitro investigations. Recently, we demonstrated that immunoglobulin levels (Igs), as well as the parasite load are relevant hallmarks of distinct clinical status of CVL. To further characterize and upgrade the background on this issue, herein, we have evaluated, in *Leishmania* (*Leishmania*) *chagasi* naturally infected dogs, the relationship between tissue parasitism (skin, bone marrow, spleen, liver and lymph node), the CVL clinical status (asymptomatic (AD), with no suggestive signs of the disease; oligosymptomatic (OD), with maximum three clinical signs—opaque bristles; localized alopecia and moderate loss of weight; symptomatic (SD), serologically positive with severe clinical signs of visceral leishmaniasis), and the humoral immunological profile of anti-*Leishmania* immunoglobulins (IgG, IgG1, IgG2, IgM, IgA and IgE). Our major statistically significant findings revealed distinct patterns of tissue parasite density

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within *L. chagasi*-infected dogs despite their clinical status, pointing out the spleen and skin as the most relevant sites of high parasitism during ongoing CVL. Parasite density of bone marrow and spleen were the most reliable parasitological markers to decode the clinical status of CVL. Moreover, the parasite density of bone marrow better correlates with most anti-*Leishmania* Igs reactivity. Additionally, a prognostic hallmark for canine visceral leishmaniasis was found, highlighting strong correlation between IgG1 and asymptomatic disease, but with IgA, IgE and IgG2 displaying better association with symptomatic disease. The new aspects of this study highlighted pioneer findings that correlated the degree of tissue parasite density (low (LP), medium (MP) and high (HP) parasitism) with distinct patterns of anti-*Leishmania* Igs reactivity. In this scope, our data re-enforce the anti-*Leishmania* IgG but with IgA reactivity as the better marker for overall tissue parasitism. The association between clinical status, Ig profile and the tissue parasitism support a novel investigation on the impact of humoral immune response and susceptibility/resistance mechanism during ongoing CVL.

Keywords: Canine visceral leishmaniasis; Clinical forms; Tissue parasitism; Ig profile; *Leishmania chagasi*

1. Introduction

Visceral leishmaniasis caused by *Leishmania (Leishmania) infantum* syn. *Leishmania* (*Leishmania*) *chagasi* affects wild and domestic animals as well as humans in several parts of the Old and New World. Peridomestic sand flies acquire the etiological agent by feeding on infected wild/domestic reservoirs leading to transmission to humans causing severe disease fatal if not treated immediately after the onset of early symptoms (WHO, 2000). The major prophylactic practice to control this human disease, as recommended by the World Health Organization, involves a systematic treatment of human cases besides vector control by insecticide and elimination of the domestic reservoir, mainly seropositive infected dogs (Tesh, 1995).

Canine visceral leishmaniasis (CVL) is one of the most important emerging diseases with high prevalence in Latin American countries (Tesh, 1995). The major signs of CVL include hepatosplenomegaly, lymphadenopathy, cutaneous lesions, keratoconjunctivitis, opaque bristles, alopecia, apathy, onychogriphosis, anorexia and severe weight loss (Bettini and Gradoni, 1986). Hypergammaglobulinemia is also one of the classic signs of CVL as the disease progresses and it is accompanied by a suppression of cellular immune response, both mitogen-triggered and antigen-specific as well as a strong up-regulation humoral response (Pinelli et al., 1994; Cabral et al., 1998). According to Mancianti et al. (1988), CVL can be categorized into three distinct clinical forms, based on major features observed for infected dogs, which can be classified as asymptomatic (AD), with no suggestive signs of the disease; oligosymptomatic (OD), with maximum three clinical signs including opaque bristles and/or localized alopecia, and/or moderate loss of weight and symptomatic (SD), with characteristic clinical signs of visceral leishmaniasis, such as opaque bristles, severe loss of weight, onychogriphosis, cutaneous lesions, apathy and keratoconjunctivitis, showing the most severe signs of CVL.

Several reports have focused attention on the relationship between distinct clinical forms of CVL, disease progression and the IgG isotype levels, in both, experimental and natural *L.* (*L.*) *infantum* and *L.* (*L.*) *chagasi* infections (Solano-Gallego et al., 2001; Leandro et al., 2001; Quinnell et al., 2003; Cordeiro-da-Silva et al., 2003; Vercammen et al., 2002). Although the majority of these investigations have being performed based on well-established ELISA and Western-blotting protocols, controversial data on immunoglobulin isotype profiles are frequently documented.

Increased levels of IgG and IgG2 have been indiscriminately reported for AD and SD as described by Bourdoiseau et al. (1997) and Vercammen et al. (2002). However, according to Deplazes et al. (1995), Nieto et al. (1999) and Solano-Gallego et al. (2001), SD showed considerably higher anti-*Leishmania* IgG1 antibodies in comparison to asymptomatic carriers. Additionally, Courtenay et al. (2002) and Quinnell et al. (2003) reported that higher levels of anti-*Leishmania* IgG/IgG1 and lower levels of IgG2 were also observed in SD. However, Leandro et al. (2001) and Cordeiro-da-Silva et al. (2003) have documented