Parasite load in intact and ulcerative skin of dogs with leishmaniasis

Carga parasitária em fragmentos de pele intacta e ulcerada em cães com leishmaniose

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Received June 9, 2015
Accepted July 22, 2015

Abstract

The skin is the site of inoculation of Leishmania spp. in susceptible hosts, and consequently dermatopathies, especially ulcerative dermatitis, are the main clinical signs observed. The aim of this study was to assess parasitism of the skin (intact and ulcerated) among dogs that were naturally infected by Leishmania spp., through immunohistochemical analysis. Skin fragments (intact and ulcerated) were collected from 13 dogs with positive parasitological (bone marrow aspiration and exfoliative skin) and serological examinations (ELISA S7® Biogene) for Leishmania spp. These samples were processed using the immunohistochemical technique, involving the streptavidin-peroxidase complex. Ulcerative lesions were mainly observed on the elbows (53.84%; 7/13), nostrils (15.38%; 2/13), ears (23.07%; 3/13) and wings of the ilium (7.69%; 1/13). A severe parasite load was detected in 46.15% and 76.92% of the intact and ulcerated skin samples tested, respectively. The parasite load on ulcerated skin was statistically higher than on intact skin (p = 0.0221). These results indicate that the intact and ulcerated skin may host a high parasite load of amastigote forms of Leishmania spp., which can favor the transmission of the parasite.

Keywords: Skin, leishmaniasis, immunohistochemistry, reservoir, dog.

Resumo

A pele é o local de inoculação da Leishmania spp. nos hospedeiros susceptíveis e dessa forma, as dermatopatias, principalmente as dermatites ulcerativas são os principais sinais clínicos observados. O objetivo deste estudo foi avaliar o parasitismo na pele (íntegra e ulcerada) em cães naturalmente infectados por Leishmania spp. através da técnica de imunohistoquímica. Fragmentos de pele (íntegra e ulcerada) foram coletados de 13 cães com diagnóstico parasitológico (aspirado de medula óssea e esfoliação cutânea) e sorológico positivos (ELISA S7® Biogene) para Leishmania spp. Estas amostras foram processadas por imunohistoquímica pelo complexo estreptoavidina-peroxidase. As lesões ulcerativas foram observadas principalmente nas regiões do cotovelo 53,84% (7/13), narina 15,38% (2/13), orelha 23,07% (3/13) e wings of the ilium 7,69% (1/13). Uma intensa carga parasitária foi detectada 46,15% e 76,92% das amostras de pele íntegra e ulcerada, respectivamente. A carga parasitária na pele ulcerada foi estatisticamente superior à pele íntegra (p = 0,0221). Esses resultados indicam que a pele intacta e ulcerada pode albergar uma intensa carga parasitária de formas amastigotas de Leishmania spp., o que pode favorecer a transmissão do parasita.

Palavras-chave: Pelle, leishmaniose, imunohistoquímica, reservatório, cão.
region, calcaneus and ischial tuberosity (CAMILHA & SOTO-BLANCO, 2008; TORRES-NETO et al., 2008).

In this context, the skin represent an excellent biological sample for detection of amastigote forms in parasitological tests (e.g. exfoliative cytology), immunostaining (TAFURI et al., 2004; QUEIROZ et al., 2011) or parasite DNA detection (QUEIROZ et al., 2011; REIS et al., 2013, RAMOS et al., 2013). Indeed, assessment of the parasite load in both healthy and ulcerated skin fragments from dogs could contribute towards better understanding of their role as parasite reservoirs for *L. infantum* transmission to susceptible hosts (SOLANO-GALLEGO et al., 2004). Therefore, the aim of this study was to assess the parasite load in the intact and ulcerated skin of dogs positive for *Leishmania* spp.

This study was conducted using 13 positive dogs for *Leishmania* spp. of different ages, breeds and sex. The animals were being kept at the Zoonotic Disease Control Centers of the municipalities of Petrolina (9° 23' 39" S, 40° 30' 35" W) and Goiana (7° 34' 19" S, 35° 0’ 7”), both located in the state of Pernambuco, Brazil. All procedures performed in this study were approved by the Ethics Committee for Animal Use (ECAU) of the Federal Rural University of Pernambuco (protocol number 010/2011).

All the animals were clinically examined and the dermatological alterations presented were classified as previously proposed (FERRER et al., 1988). All dogs were diagnosed positive at enzyme-linked immunosorbent assay (ELISA S7* Biogene) and microscopic cytological examination of exfoliative skin tissue and bone marrow biopsy material. Intact skin fragments were collected from the scapular region, while ulcerated skin was obtained from any part of the body at the periphery of the skin lesion. All the fragments were collected using a biopsy punch (4 mm) and were then fixed in 10% buffered formalin for 48 hours. Following this, they were transferred to glass vials containing a solution of 70% ethanol until the time of immunohistochemical processing.

For the immunohistochemical examination, immunolabeling of the amastigote forms of *Leishmania* spp. was performed using the streptavidin-peroxidase technique (TAFURI et al., 2004). Skin from a dog with intense cutaneous parasitism due to *Leishmania* spp. was used as a positive control. In addition, skin from a negative dog confirmed by PCR examination of bone marrow and skin was used as negative control.

The intensity of parasitism was reported as the number of immunolabeled amastigote forms and was expressed as the mean number observed in five microscope fields at 400X magnification. The parasite load was defined as follows: - absent, (+) low, (+ +) moderate and (+ + +) high, corresponding to 0, 1-100, 101-300 and > 300 amastigote forms of *Leishmania* spp., respectively (GIUNCHETTI et al., 2006).

Differences among the parasite loads in the skin fragments were statistically analyzed through the Mann-Whitney test using the Biostat 5.0 software (AYRES et al., 2007). Differences were considered statistically significant when P ≤ 0.05.

All the animals examined here presented at least one cutaneous clinical sign. These dermatological alterations ranged from ulcerative to scaly alopecia. Ulcerative lesions were mainly observed on the elbows (53.84%; 7/13), nostrils (15.38%; 2/13), ears (23.07%; 3/13) and wings of the ilium (7.69%; 1/13).

Severe parasitism was observed in 46.15% (6/13) of the intact skin samples and in 76.92% (10/13) of the ulcerated skin samples (Figure 1). The overall results regarding the parasite load are reported in Table 1. Interestingly, the parasite load detected in ulcerated skin samples was higher than that recorded for intact skin samples (p = 0.0238).

In this study, the cutaneous parasitism of 13 dogs that were naturally infected by *Leishmania* spp. was assessed. The presence of skin lesions observed here is a common clinical finding in CVL cases and may occur in 45% of the infected dogs (GIUNCHETTI et al., 2006; COSTA et al., 2008). In the animals studied, the ulcerative lesions were observed mainly on the elbows. It is known that the presence of ulcerative dermatitis is normally associated with areas of bone projections (FERRER et al., 1988).

All the skin fragments (both the intact and the ulcerated skin samples) analyzed here through immunohistochemical examination scored positive. In fact, the immunohistochemical technique enables high contrast between amastigote forms of *Leishmania* spp. and the host tissue, thus enabling a more accurate diagnosis (ORDEIX et al., 2005; TAFURI et al., 2004; FIGUEIREDO et al., 2010). The parasite load in ulcerated samples was statistically higher than that in intact skin samples. These results differ from...
The present study demonstrates that intact and ulcerated skin samples may host a high number of amastigote forms of *Leishmania* spp., which can favor transmission of the parasite. Over the last years, the role of asymptomatic dogs in the life cycle of leishmaniasis has been extensively disputed. Recently, a study suggested that both symptomatic and asymptomatic animals are potentially infective to sand flies (LAURENTI et al., 2013). In addition, it has been demonstrated that only the cutaneous parasitism is not pivotal for successful transmissibility (TRAVI et al., 2001). Therefore, animals where cutaneous lesions are absent, may act as important source of infection by phlebotomine sand flies.

**References**


