Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 151 (2008) 86–90
veterinary parasitology
www.elsevier.com/locate/vetpar

Short communication

Genital lesions and distribution of amastigotes in bitches naturally infected with *Leishmania chagasi*

Fabiana L. Silva, Antonio A.M. Rodrigues, Isabela O.P. Rego, Raquel L.H. Santos, Raquel G. Oliveira, Teane M.A. Silva, Mariana N. Xavier, Ernane F. Nascimento, Renato L. Santos *

Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270-901 Belo Horizonte, MG, Brazil

Received 5 June 2007; received in revised form 24 September 2007; accepted 24 September 2007

Abstract

Recent reports indicate that *Leishmania chagasi* has tropism to the male canine genital system, which is associated with shedding of the organism in the semen, supporting the hypothesis of venereal transmission. The aim of this study was to describe the lesions and assess parasite load in the genital system of bitches with canine visceral leishmaniasis (CanL). Symptomatic ($n = 5$) and asymptomatic ($n = 5$) bitches seropositive for CanL were randomly selected at the Center for Zoonosis Control (Belo Horizonte, State of Minas Gerais, Brazil). Five serologically negative, healthy, adult bitches also from the CZC were used as controls. Samples from genital organs (vulva, vagina, cervix, uterine body, uterine horns, uterine tubes, and ovaries), liver, and spleen were histologically evaluated and processed for immunodetection of *Leishmania* sp., and PCR. The most significant histological change was a mild to moderate vulvar dermatitis, characterized by a histio-plasma-lymphocytic infiltrate. This change was detected in all asymptomatic, four symptomatic, and three uninfected control bitches. In one symptomatic and one asymptomatic bitch intracytoplasmic amastigotes were observed within macrophages in the inflammatory infiltrate. Samples from all the segments of the genital tract were positive in at least one infected animal, in the absence of detectable amastigotes in the tissue. These findings support the notion that *L. chagasi* does not have genital tropism in the bitch, which is in contrast to our previous findings in naturally infected male intact dogs.

Keywords: *Leishmania*; Genital tract; Bitches; Immunohistochemistry; Polymerase chain reaction

1. Introduction

Visceral leishmaniasis (VL) is a zoonotic disease that affects man, dogs and several wildlife species, with wide geographic distribution in tropical and subtropical areas. This disease is predominantly acquired through invertebrate vectors, but transmission in the absence of the vector has been reported (Harris, 1994; Owens et al., 2001; Bosch et al., 2002; Gaskin et al., 2002). In the Americas, VL is caused by *Leishmania (Leishmania) chagasi*, which is transmitted by the sand fly *Lutzomyia longipalpis*, and the dog is the most important reservoir for human infections (Deane and Deane, 1962; Chang, 1979).

The infection results in variable clinical manifestations in dogs, ranging from unapparent sub-clinical infections to a systemic disease characterized by fever, anemia, progressive weight loss, hepatomegaly, splenomegaly, generalized lymphadenopathy, and cutaneous

* Corresponding author. Tel.: +55 31 3499 2239; fax: +55 31 3499 2230.
E-mail address: rsantos@vet.ufmg.br (R.L. Santos).

0304-4017/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2007.09.032

lesions (Slappendel and Greene, 1990; González et al., 1990; Ciaramella et al., 1997). The most important microscopic lesion is accumulation of macrophage-containing amastigotes in the spleen, lymph nodes, liver, and skin (Tryphonas et al., 1977; Keenan et al., 1984; Tafuri et al., 1996).

Genital lesions associated with *Leishmania* amastigotes have been more often described in male human patients, affecting the glans penis (Schubach et al., 1998; Cabello et al., 2002), prepuce (Aste et al., 2002), and scrotum (Cabello et al., 2002). These lesions are characterized by a chronic proliferative and ulcerative inflammation (Cabello et al., 2002; Schubach et al., 1998). Both muco-cutaneous and visceral manifestations of the disease may be associated with genital lesions. *Leishmania* amastigotes has been found in the testis of a boy with acute lymphoblastic leukemia in the absence of apparent gross lesions (Kapila et al., 1994). Importantly, Symmers (1960) reported an ulcerative lesion containing macrophages parasitized with *Leishmania* in the vulva of a woman who contracted the infection venereally.

Lesions in the testis and epididymis have been described in dogs with canine visceral leishmaniasis (CanL) (Diaz et al., 1982). Additionally, there is a report of *Leishmania infantum* amastigotes in the glans penis of a dog with transmissible venereal tumor (Catone et al., 2003). Recently, a comprehensive study of lesions in the genital system of male dogs with CanL demonstrated a very high frequency of lesions particularly in the epididymis, glans penis, and prepuce. Furthermore, dogs with CanL often shed *Leishmania* in the semen (Diniz et al., 2005). These findings support the hypothesis of venereal transmission of CanL, as previously documented in human VL (Symmers, 1960).

Considering the high frequency of genital lesions and shedding of *Leishmania* in the semen of dogs with CanL (Diniz et al., 2005) and consequently the potential for venereal transmission of CanL, as well as the absence of studies assessing parasitism and lesions in the genital tract of bitches with CanL, the goal of this study was to characterize genital lesions in bitches naturally infected with *Leishmania* and to detect the parasite in canine female genital organs by immunohistochemistry and PCR.

2. Material and methods

Ten adult crossbreed bitches (excluding senile and pre-pubertal animals), seropositive for leishmaniasis were randomly selected at the Center for Zoonosis Control (CZC) in Belo Horizonte (State of Minas Gerais, Brazil), and divided into two groups: (i) asymptomatic ($n = 5$) and (ii) symptomatic animals ($n = 5$). Five serologically negative adult bitches also from the CZC were used as controls. All animals were submitted to euthanasia as part of the official program for zoonosis control, and were immediately necropsied. Fragments of the genital tract (vulva, vagina, cervix, uterine body, uterine horns, uterine tubes, and ovaries), liver, and spleen were collected for histopathology and immunohistochemistry. Tissue samples were fixed in 10% neutral buffered formalin for 24–48 h and then processed for paraffin embedding and stained with hematoxylin and eosin (HE) or further processed for immunodetection of amastigotes according to the method described by Tafuri et al. (2004). Canine hyperimmune serum was used as primary antibody and a commercially available streptavidin–peroxidase complex (LSAB$^+$ kit, Dako USA) was employed as detection system. The reactions were revealed by diaminobenzidine (DAB) and the slides counter-stained with hematoxylin. Samples of the same tissues were collected for DNA extraction using the isothiocyanate-guanidine protocol as previously described (Pitcher et al., 1989).

Amplification of *Leishmania* DNA was performed as previously described (Lachaud et al., 2002). This protocol amplifies kinetoplast DNA from *Leishmania* species belonging to the donovani complex. Briefly, approximately 100 ng of DNA extracted from frozen tissue samples was added to 15.6 μl of a solution containing 5.1 μl of 10× PCR buffer, 5.1 μl of a 200 μM dNTP solution, 0.2 μM primer A (5′-CTTTTCTGGTCCCGCGGGTAGG-3′), 0.2 μM primer B (5′-CCACCTGGCCTATTTTACACCA-3′), 3 mM $MgCl_2$, and 1.5 U of Taq polymerase. Cycling parameters were denaturation at 94 °C for 4 min; 49 cycles of denaturation (94 °C for 30 s), annealing (59 °C for 30 s), and extension (72 °C for 30 s), and a final extension at 72 °C for 10 min. PCR products (145 bp) were resolved by agarose gel electrophoresis. DNA samples from tissues that tested positive by immunohistochemistry as well as purified *Leishmania* DNA were used as positive controls.

3. Results and discussion

None of the animals (either uninfected controls or infected) had any gross lesions in the genital system. Indeed, no microscopic lesions were observed in the ovaries, uterine tubes, uterus, and cervix from all animals, including uninfected controls and infected. The most significant histological change was a mild to