# INTERSTITIAL PNEUMONITIS IN CANINE VISCERAL LEISHMANIASIS

M. I. S. DUARTE (1), M. D. LAURENTI (2), V. L. BRANDÃO NUNES (3), A. F. REGO JR. (4), E. T. OSHIRO (5) & C. E. P. CORBETT (1)

#### SUMMARY

Forty-one naturally infected dogs with visceral leishmaniasis from an urban area of Corumbá (Mato Grosso do Sul-BRAZIL) were studied and three types of lung involvement due to visceral leishmaniasis were characterized; a cellular, a cellular-fibrotic and a fibrotic type. These types seem to represent a sequential evolutive process. Visceral leishmaniasis frequently causes an interstitial pneumonitis in naturally infected dogs (80.5%) as well as in man and experimentally infected hamsters

**KEY WORDS:** Visceral leishmaniasis; Interstitital pneumonitis; Canine pathology.

# INTRODUCTION

Foci of canine visceral leishmaniasis in Brazil, together with human cases, suggest that the dog is an important host of this protozoan disease. In the Northeast of Brazil the disease is endemic (ALENCAR, 19593; ALENCAR & CUNHA, 1983 4; BRENER, 1957 10; DEANE & DEANE, 1954 16; DEANE & DEANE, 1955 17; ESPINOLA GUEDES et al., 1974 22 and SHER-LOCK & ALMEIDA, 1969 33). Nevertheless, outbreaks of the disease have also been well characterized in other regions (I Reunião Nacional sobre Pesquisa em Leishmaniose, 1984 32). Nowadays new human cases have been report. ed in Brazil. New cases have been reported in São Luis (Maranhão), in the outskirts of Maceió (Alagoas), in Paraíba, in Rio de Janeiro and in Corumbá (Mato Grosso do Sul) (I Reunião Nacional sobre Pesquisa em Leishmaniose, 1984 34).

The histopathology of canine visceral leishmaniasis has not been well characterized so far. Few references have been reported on the identification of parasites in the tissue along with infiltrates of mononuclear inflammatory cells in different organs (ADLER & TCHER-MONORETZ, 19462; ALENCAR, 19593; ALVA-RENGA, 1960 5; ANDERSON et al. 1980 6; BRENER, 1957 10; CORBIEL et al, 1976 13; GEORGE et al, 1976 24). Interstitial pneumonitis and considered to be highly suggestive of visceral leishmaniasis (DUARTE et al, 1985 21) described in man (ANDRADE, 1959 8 and RASO & SIQUEIRA, 1964 31) has been found in 62.5% of the autopsy material (CHASSOT et al, 1983 11).

The histopathology of the lung has not been characterized so far in canine visceral

Professor Assistente Doutor do Departamento de Patologia da Faculdade de Medicina da Universidade de São Paulo, Av. Dr. Arnaldo, 455, 01246, São Paulo, Brasil

<sup>(2)</sup> Biomédica — Laboratório de Patología de Moléstias Infecciosas da Faculdade de Medicina da Universidade de São Paulo

<sup>(3)</sup> Professora Titular de Parasiotlogia e Parasitologia Clínica do Departamento de Patologia do Centro de Ciências Biológicas e da Saúde da Universidade Federal do Mato Grosso do Sul.

<sup>(4)</sup> Médico Veterinário — IAGRO — Departamento de Vigilância Agropecuária de Corumbá, Mato Grosso do Sul, Brasil

<sup>(5)</sup> Médica Veterinária da Universidade Federal do Mato Grosso do Sul, Brasil

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leishmaniasis. Rare cases of lung involvement were described stressing mainly the parasite's identification and concomitant secondary infections. TRYPHONAS, 1977 <sup>34</sup>, described a marked septal thickening characterizing an interstitial pneumonitis in the lungs of a dog living in Spain for two years together with other visceral changes. In this case no parasites were found. In 1980, ANDERSON <sup>6</sup>, found a diffuse multifocal septal thickening in the lungs of a dog from Texas with infiltration of plasma cells and histiocytes, some of which containing leishmania.

An outbreak of visceral leishmaniasis in Corumbá involving both men and dogs stimulated us to study the lungs of naturally infected dogs in order to characterize the histopathological changes due to the disease. Efforts were also directed to identify a possible relation between lesions found in dogs and those seen in man and hamsters.

# MATERIAL AND METHODS

Forty-one dogs from Corumbá, presenting the usual clinical picture of the chronic phase of visceral leishmaniasis (ADLER & THEO-DOR, 1931 1; ALENCAR, 1959 3; ANOSA, 1983 9; BRENER, 1957 10; COVALEDA, HIVES & SO-LER DURALL, 1951 14; CUNHA, 1938 15; DONA-TIEN & LESTOQUARD, 1935 18; FAURE-BROC, 1936 23; JOYEX & SANDET, 1938 25; KEENAN et al, 1984 26; LANNOTTE et al, 1979 27; LEN-NOX, SMART & LITTLE, 1972 28; MENDONZA et al, 1983 29; QUEIROZ, 1959 30 and SHERLOCK & ALMEIDA, 1969 33) were studied. The animals presented wide-spread alopecia and furfuraceous scaling mainly around the nose and eyes, cutaneous ulcers, keratitis, long nails, low weight, high body temperatures, adenomegaly, arthritis and sometimes paralysis of the hindlegs. Identification of the agent was done by examination of skin lesions, spleen and liver smears; by parasite isolation in culture medium (NNN); by inoculation in hamsters followed in some cases by leishmania typing (isoenzymes and monoclonal antibodies - J. J. SHAW -The Wellcome Parasitology Unit. Belém, Pará, BR). Parasites were also found in histopathological specimens from liver, spleen, lymph nodes and skin. All organs showed histopathological changes compatible with visceral leishmaniasis.

Fragments from lungs and other organs were collected through autopsy of the dogs and fixed in phosphate buffered saline containing 10% formalin.

Lung fragments (4  $\mu$  thick) were stained by the hematoxylin-eosin, Masson's and Picrosirius methods (CONSTANTINE & MOURAY, 1968 <sup>12</sup>). The picro-sirius stained slides were observed under polarized light in an optical microscope.

Four dogs from the same place, without clinical signs of visceral leishmaniasis, showed neither lung nor any other organ changes which could be related to the infection. These dogs were used is controls.

#### RESULTS

Histopathological pulmonary changes were found in 33 out of 41 cases. The main lesion was septal thickening which intensity and distribution varied from case to case with no preference for any specific area of the lung.

Based on the characteristics of the septal thickening changes were classified in three types:

- "Cellular" (Figure 1): in which the septal thickening was due mainly to a mononuclear cells infiltrate (lymphocytes, plasma cells, macrophages and cells with fat-containing vacuolated cytoplasm). The septal capillaries showed congestion with no endothelial changes. Pneumocytes I and II of the alveolar lining were preserved. The intensity of the mononuclear cell infiltrate varied from area to area in the same cases and no correlation with any region was seen. There was at least one area of preserved parenchyma in all cases even in those with large involvement. Vicariant emphysema was found in the most severe cases.
- 2. "Fibrotic-cellular" (Figure 2): in which septal changes resulted from infiltration of mononuclear cells, as in the cellular type, associated with small bands of fibrous tissue. Septal capillaries and alveolar lining showed no changes. Vicariant emphysema was present in areas of higher involvement.

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Fig. 1 — Cellular type: septal thickening by mononuclear cell infiltrate, HE X 100.

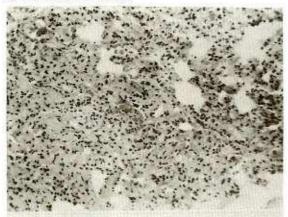


Fig. 2 — Cellular-fibrotic type: detail of the mononuclear cells infiltrate together with delicate bands of fibrous tissue within the septum. HE X 460.

3. "Fibrotic": bands of fibrous tissue thickening the intra-alveolar septum and involving large areas of the lung parenchyma were characteristic of this type. The alveolar lumen was preserved. Fibrosis was accompanied by discrete mono-uclear cell infiltrate without alterations of capillaries and pneumocytes. Vicariant emphysema was related with the more fibrotic areas. Fibrosis, even thinner ones, could be demonstrated through the Picrosirius stain method when examined under polarized ligh (Figure 3 and 4).

In the whole there were no epithelial bronchial changes but in 2 cases there were discrete mononuclear cells infiltrate in the corium.

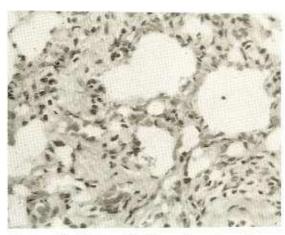


Fig. 3 — Fibrotic type: the fibrous tissue is much more evident than the mononuclear cell infiltrate. HE X 540.



Fig. 4 — Lung tissue treated with Picro-sirius stain and absorved under polarized light, showing septal thickening due to collagen fibers. X 100.

The alveolar lumen had neither edema or exsudation.

Amastigates were found within the cytoplasma of either septal or alveolar macrophages only in the lungs of 3 of the cases with interstitial pneumonitis. However, numerous amastigates were found in other tissues examined (skin, lymph nodes, spleen and liver). Eight of the cases showed no pulmonary histopathological changes.

### DISCUSSION

No systematical reference on the pulmonary involvement in dogs with visceral leishmaniasis was found in the literature. There DUARTE, M. I. S.; LAURENTI, M. D.; BRANDÃO NUNES, V. L.; REGO JR., A. F.; OSHIRO, E. T. & CORBETT, C. E. P. — Interstitial pneumonitis in canine visceral leishmaniasis. Rev. Inst. Med. trop. São Paulo, 28:431-436, 1986. 1986.

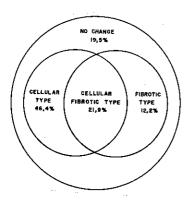


Fig. 5 — Distribution of the histopathological types of the septal thickening of the lung in 41 visceral leishmaniasis dogs.

are few reports describing bronchopneumonia as secondary infections and also few references on other pulmonary changes concurrent with this protozoan disease. GEORGE, in 1976 <sup>24</sup>, related "focal interstitial thickening" in one dog, TRYPHONAS, in 1977 <sup>34</sup> and ANDERSON in 1980 <sup>6</sup>, also described an "interstitial pneumonitis" in a dog, evading to suggest any relation to visceral leishmaniasis in spite of the identification of leishmania done by Anderson in the lung.

In this work we tried to define the pulmonary changes possible to occur in visceral leismaniasis by studying naturally infected dogs, caught during an urban epidemic outbreak of visceral leishmaniasis in Corumbá (Mato Grosso do Sul, BR). All animals had clinical signs of the disease and parasites were identified either in smears and histological tissue sections from their organs, by isolation in culture medium or by inoculation in hamsters.

It is important to point out that all animals had histopathological characteristics of visceral leishmaniasis showing no other pulmonary changes as bronchopneumonia or any other alveolar lumen involvement.

The pulmonary changes found in naturally infected dogs were frequent (80,5%) and characterized by an interstitial pneumonitis which we believe to be related to visceral leishmaniasis.

The different patterns of septal thickening were described according to the prominence of elements participating in each case. The cellular type (46,49%) is due mainly to a mononuclear cell infiltrate in the septum. The cellular-fibrotic type (21,9%) presents both mononuclear cell infiltrate and fibrosis. Finally, in the fibrotic type (12,2%), the septal changes originate from fibrosis. We believe that all three types represent evolutive phases of the same process. The fibrotic phase is subsequent to a previous septal inflammatory process.

In man, ANDRADE, 1958 7 and 1959 8, found interstitial pneumonitis in 3 out of 5 autopsies of patients with visceral leishmaniasis and considered it as part of the disease. RASO and SIQUEIRA, 1964 31, described one more case of interstitial pneumonitis. Interstitial pneumonitis was found in 62,5% of autopsies and foci of septal fibrosis in 30% (DUARTE, M.I.S. -unpublished data). Amastigotes were rarelly found (CHASSOT et al, 1983 141) in lungs. Using an immunoenzimatic method (PAP- peroxidase anti-peroxidase) and specific antibodies, particulate antigenic material and/or amastigotes was found in the interalveolar septum, wheneinterstitial pneumonitis was present (DUARTE et al, 1985 21). This fact confirms that septal changes are due to the disease. A few PAP tests in dog lung performed in our laboratory, showed the same results.

We have already demonstrated an interstitial pneumonitis developing in distinct stages in hamsters experimentally infected with L. donovani (DUARTE, 1979 <sup>19</sup>; DUARTE e CORBETT, 1984 <sup>20</sup>). There was a exsudative stage of short duration followed by cellular proliferation and later on, by fibrosis. All sequential changes between cellular proliferation and fibrosis could be characterized as part of the same process.

Therefore visceral leishmaniasis often determines an interstitial pneumonitis, either in man, or in naturally infected dogs, or even experimentally infected hamsters. The histopatological changes seen in each case depend on the evolution of the process. There is also a similarity of histopathological changes in the three species: man, dog and hamster. Interstitial pneumonitis seems to be the pulmonary representation of an interstitial pathology of visceral leishmaniasis, also seen in others organs with interstitial lesions such as liver and kidneys.

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#### RESUMO

# Pneumonia intersticial na leishmaniose visceral canina

Estudamos 41 cães naturalmente infectados com leishmaniose visceral, provenientes da área urbana de Corumbá (Mato Grosso do Sul, BR) e caracterizamos 3 tipos de acometimento pulmonar devido à leishmaniose visceral: celular, fibrótico-celular e fibrótico. Estes tipos pa recem representar um processo evolutivo seqüencial. A leishmaniose visceral causa uma pneumonite intersticial em 80.5% dos cães naturalmente infectados à semelhança do que ocorre no homem e em hamsteres experimentalmente infectados.

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