*Mem. Inst. Oswaldo Cruz*, Rio de Janeiro, Vol. 83(2): 145-151, abr./jun. 1988

# EXPERIMENTAL CANINE MUCOCUTANEOUS LEISHMANIASIS *(LEISHMANIA BRAZILIENSIS BRAZILIENSIS)*

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*Four mongrel dogs were intradermically inoculated with 3 x $10^6$* Leishmania braziliensis braziliensis *promastigotes. Three out of the four animals developed cutaneous lesions respectively 4, 7, and 8 months after. The fourth dog did not develop lesion at the inoculation site, but a mucosal ulcer was seen 16 months after the inoculum. Clinical, histopathological, and serological findings were similar to what is found in natural canine infection as well as in the human disease. These results suggest that dogs may be an useful model for* L. b. braziliensis *infection.*

Key words: *Leishmania braziliensis braziliensis* – canine experimental infection

The first endeavors to inoculate *Leishmania* in dogs were performed in the beginning of this century using biopsy samples collected from human lesions caused by *L. tropica* (Nicolle & Manceaux, 1910; Laveran, 1916).

Experimental infections of dogs with *L. braziliensis* were successfully obtained by Wenyon (1913), Strong (1915), and Pedroso (1923). The latter injected promastigotes isolated by culture from human or canine lesions. All ten inoculated dogs developed nodules in about two months, and spontaneous healing occurred six to 12 months after. Similar results were obtained by Aragão (1927), Fonseca (1928), and Pifano (1960).

Herrer & Battistini (1951) were able to demonstrate that dogs can be experimentally infected with *L. peruviana* isolated from lesions of human patients suffering from the cutaneous form of leishmaniasis which occurs in Peru – "uta". This may be considered as one of the most important works on experimental American Cutaneous Leishmaniasis (ACL), showing that uta canine lesions are usually slight and located on the snout. These findings led him to studies on natural canine cutaneous leishmaniasis (Herrer, 1951).

This work was partially supported by UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical diseases (TDR), FINEP and FIPEC.

Received October 19, 1987.
Accepted March 2, 1988.

The presence of naturally infected dogs in endemic areas of human ACL (Mayrink et al., 1979; Falqueto et al., 1985; Coutinho et al., 1985) demonstrates that dogs are susceptible to *L. b. braziliensis* infection. The present results, although preliminary, show that the outcome of the experimental infection produced in dogs by *L. b. braziliensis* is similar to those occurring in naturally infected animals. Moreover, canine and human leishmaniasis caused by *L. b. braziliensis* have similar clinical, histopathological, and immunological features as demonstrated in a previous paper from our group (Pirmez et al., 1987). This data suggests that dogs might be considered as a suitable animal model for experimental studies on host-parasite *(L. b. braziliensis)* relationship.

## MATERIAL AND METHODS

*Animals* – Two female and two male normal mongrel dogs were used in this study. All dogs came from non endemic areas of ACL or Chagas' disease of Rio de Janeiro, Brazil. These animals had good general health. One of them was two months old and the remaining animals were adults of unknown age.

*Inoculum* – The *Leishmania* parasite utilized for the experimental inoculation of dogs was isolated in NNN culture medium from biopsy samples of a cutaneous lesion present in a naturally infected dog. The isolated parasite was immunologically characterized by means of monoclonal antibodies in an indirect radioimmune binding assay (Grimaldi et al., 1987) and was identified as *Leishmania braziliensis braziliensis* (MCAN/BR/83/CAMPEIRO). Cultured

promastigotes were washed three times in RPMI 1640, counted in a hemocytometer chamber and the suspension was adjusted to contain 3 x $10^7$ promastigotes/ml. 0.1 ml of this suspension was then injected in the muco-cutaneous junction of the snout. Three dogs were inoculated with promastigotes forms obtained from the first passage (7 days) of the culture, and one with promastigotes harvested from the third passage (21 days).

*Serological test* – An indirect immunofluorescent test (IFAT) was used to detect anti-*Leishmania* antibodies. Promastigotes of a Brazilian *Leishmania* strain (MHOM/BR/76/JOF), phenotypically similar to *L. major* (Momen et al., 1985) were used as antigen. A rabbit anti-dog fluorescein labelled immunoglobulin was used as a conjugate at a 1:20 dilution. Titers were expressed as the reciprocal of the highest serum dilution giving positive promastigote fluorescence. Titers > 45 were considered as positive.

*Follow-up* – Dogs were clinically and serologically examined every month. Biopsy samples were taken from lesions as soon as they appeared and examined for parasites. The latter comprised of a) Giemsa-stained smears of imprints; b) isolation of the *Leishmania* parasite by cultivation in an enriched blood-agar medium (NNN) containing modified liver infusion (5 g/l) and tryptose (5 g/l) as a liquid phase (LIT medium), and c) microscopical examination of H & E-stained paraffin sections.

## RESULTS

Three out of the four experimentally inoculated dogs developed cutaneous lesions at the site of the injection respectively 4, 7 and 8 months after. Lesions appear as a papule of 0,5 cm of diameter (Fig. 1) which slowly grew in the following five months. At this time, the nodule ulcerated showing sharp borders, with a necrotic centre (Fig. 2). Ulcers persisted for three to five months after which time thus tended to be characterized by scaring and decreasing size (Fig. 3a, 3b).

Anti-*Leishmania* antibodies appeared only after the presence of visually detectable cutaneous lesions in these 3 dogs. Afterwards the IFAT titre was positive for the whole period of observation (Fig. 4).


Fig. 1: Initial papules 7 months after the parasite inoculation.


Fig. 2: Ulcerated nodule five months after the onset of the lesion.

The histopathological picture of the cutaneous lesions 3 months after the onset in these 3 dogs was characterized by a diffuse and chronic inflammatory reaction in the dermis with plasma cells, lymphocytes and grouped macrophages outlining granulomas (Fig. 5a). In addition, there were small and multiple foci of fibrinoid degeneration of the connective tissue. In spite of positive culture and smears in all three cases, amastigotes were found in only one dog by histopathological examination. Six months after the onset of the lesion, when it became clinically ulcerated, the histopathological examination showed a similar pattern of inflammatory reaction, but with more intense necrotic reaction of the connective tissue (Fig. 5b). However, when a clinically detectable regression of the lesion starts, the inflammatory infiltrate and the fibrinoid degeneration became restricted to small and discrete foci (Fig. 6).

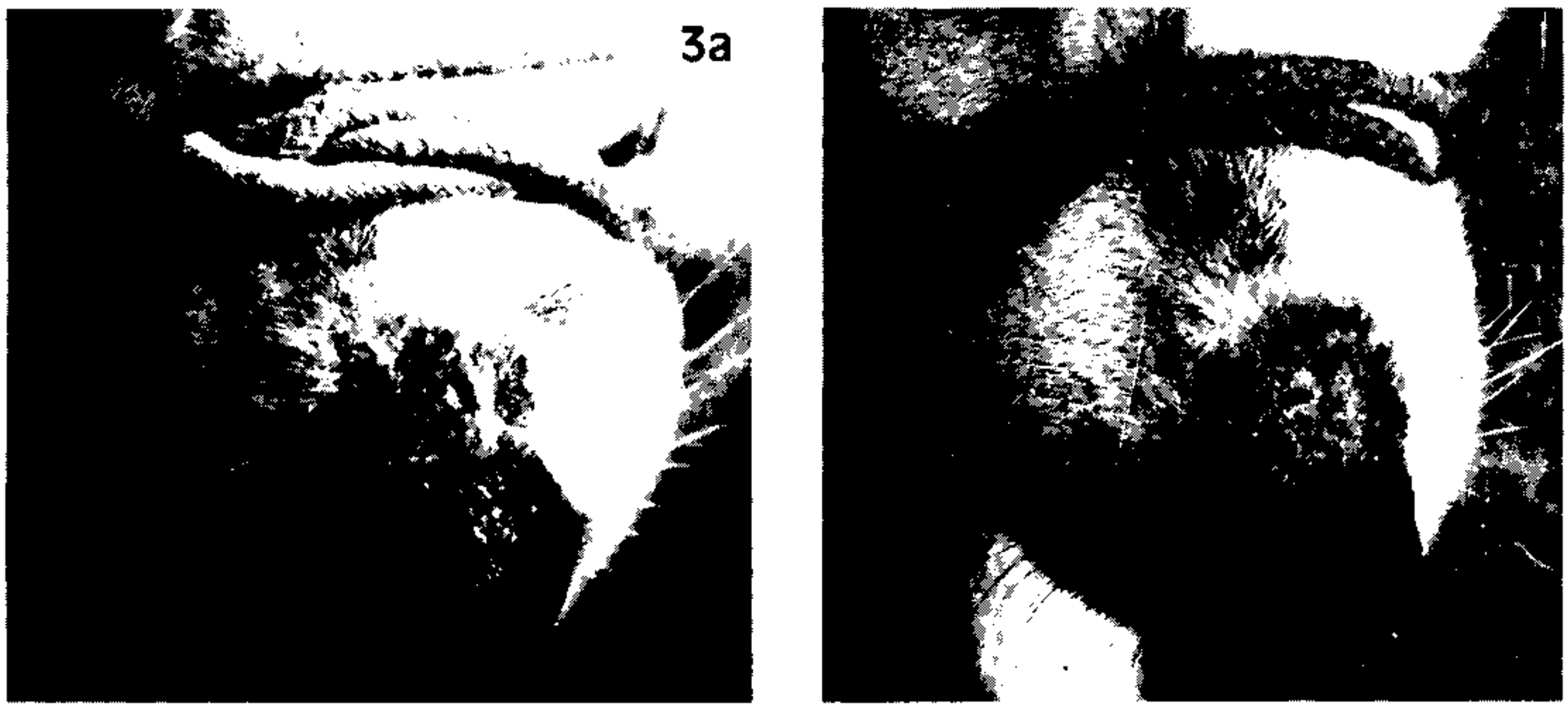


Fig. 3: Ulcerated lesion with six months of evolution (a) and three months after (b).

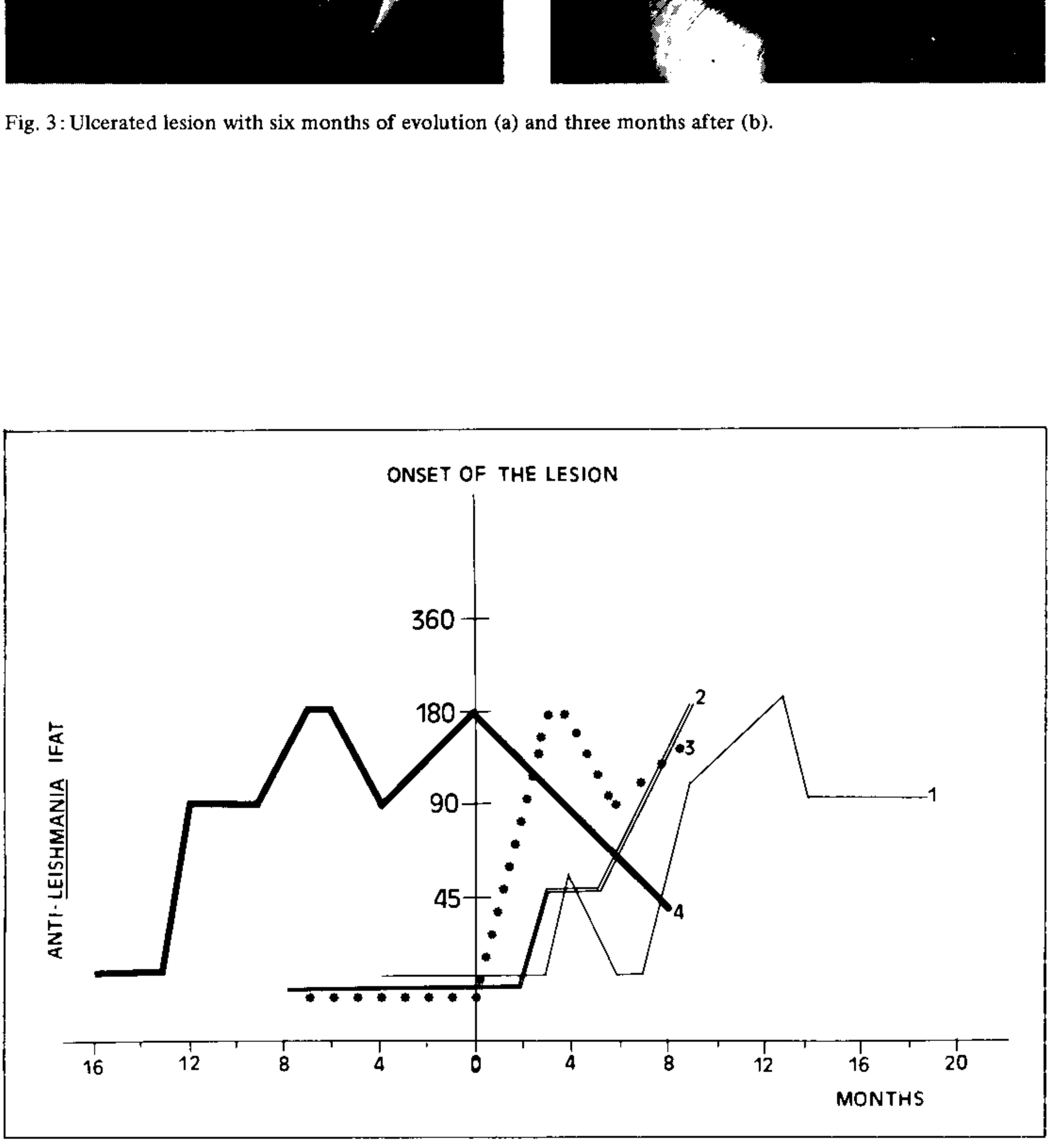


Fig. 4: Anti-*Leishmania* serum antibody titers found before and after the onset (point zero) of the lesion.

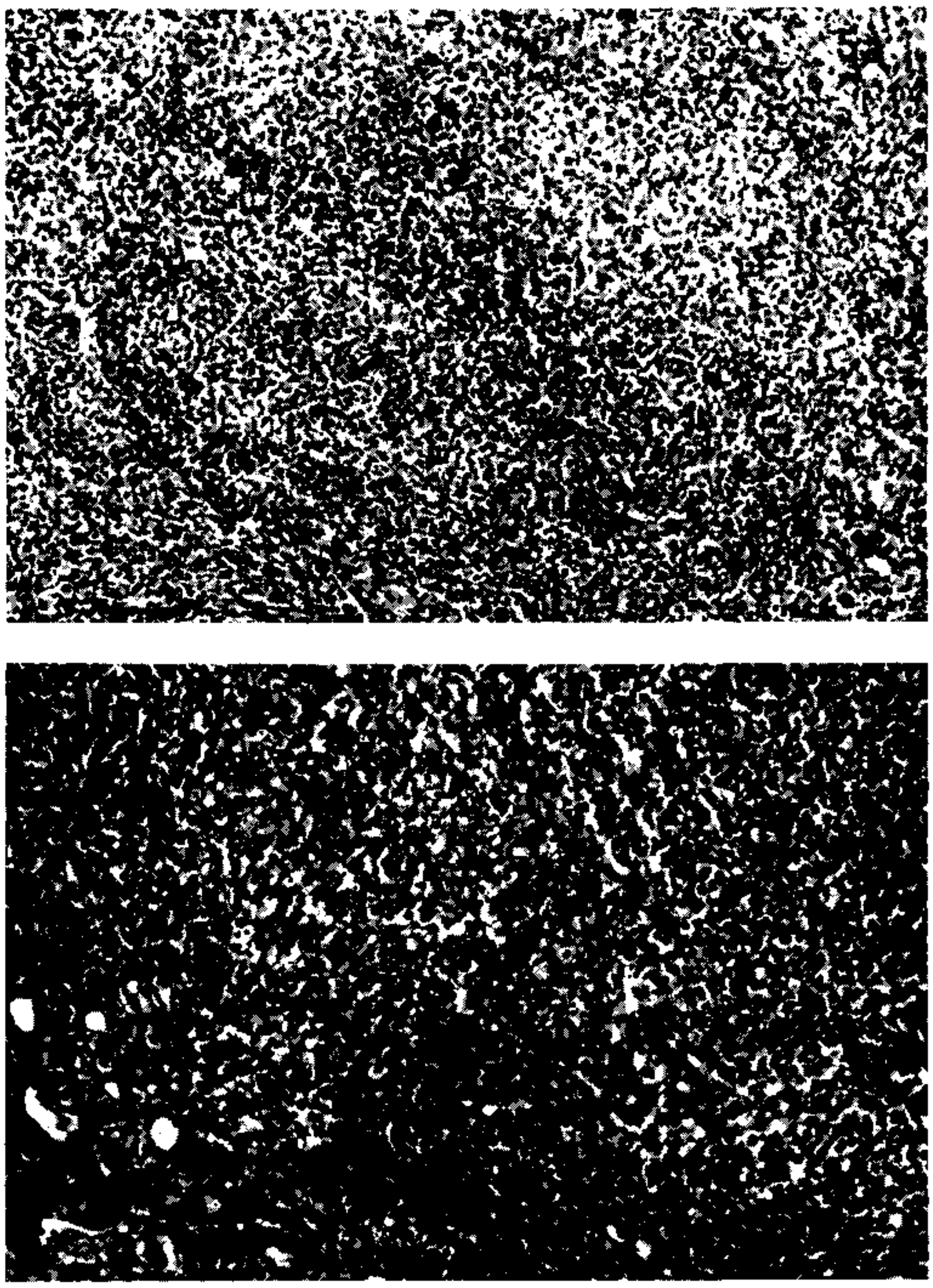

Fig. 5: (a) Diffuse inflammatory infiltrate on the dermis composed by grouped macrophages surrounded by lympho-plasmacyte cells three months after the onset of the lesion; (b) similar histopathological picture six months after the onset of the lesion showing extensive necrosis of the connective tissue.

After 17 months of evolution, two of the animals were sacrificed for necropsy. Cutaneous lesions were then partially regressed and macroscopical examination did not show any visceral abnormality. The remaining third dog was sacrificed 24 months after the onset of the lesion. In this case, a reactivation of the lesion was observed (Fig. 7); the lesion became ulcerated and increased in size. Necropsy findings were similar to the other dogs.

The fourth dog, aged of two months was inoculated with promastigotes harvested from 21 day old cultures. Despite the absence of a clinically detectable lesion at the inoculation site, culture of a biopsy sample collected at that time was positive. Histopathological examination showed a small and discrete inflammatory reaction of lymphocytes and macrophages around small vessels and dermal annexes. No lesion suggesting ACL could be detected at the inoculation site after 15 months of observation. On the 16th month, a small and shallow ulcer was observed in the inferior and internal border of the snout (Fig. 8), where parasites were isolated. Serological test showed rising *Leishmania* titers since 4 months after the inoculation. After 2 years of evolution, this dog was also sacrificed for necropsy. No macroscopical abnormalities were seen. The mucosal lesion, however, showed an intense inflammatory infiltrate composed of lymphocytes, plasmocytes and grouped macrophages with discrete fibrinoid degeneration of the connective tissue.


Fig. 6: Histopathological examination of a lesion spontaneously regressed showing discrete foci of perianexial mononuclear infiltrate.


Fig. 7: Reactivation of a lesion which was regressed two months before. Fig. 8: Mucosal lesion detected 16 months after the parasite inoculation. Any detectable gross alteration was found on the inoculation site.

In all four dogs, parasites were not detected in the peripheral blood, spleen, liver and lymphnodes by cultures, imprints, or by histopathological examination.

## DISCUSSION

Studies on canine experimental ACL infection are scanty, most of them without a precise characterization of the inoculated parasite or without a follow-up long enough to evaluate the course of the infection.

All inoculated dogs in this study developed cutaneous lesions due to *L. b. braziliensis*. The incubation period was respectively 4, 7 and 8 months in three animals, and 16 months in the fourth. The choice of *L. b. braziliensis* promas-

tigotes which had been recently isolated from lesions seems to be particularly important since variations in the infectivity may occur in the same strain, drastically decreasing in strains which have been maintained for a long time *in vitro* (Grimaldi et al., 1982). This phenomena might explain the long incubation period of one of the animals infected with parasites after 21 days in culture, as well as the unsuccessful experiments described by Migone (1913) and Fonseca (1928).

ACL canine experimental lesions developed in a similar manner as compared to human ACL lesions, starting with the formation of a papule which increases in size and finally ulcerates. Furthermore, canine lesions also have a tendency to heal spontaneously. However, cutaneous lesions tend to reappear several weeks or months after the spontaneous healing. This phenomenon was also observed in dogs naturally infected (Pirmez et al., 1987). In this respect canine ACL differs from the human disease caused by *L. b. braziliensis*, where severe secondary mucosal lesions can occur several years after healing of the primary cutaneous lesion.

Positive anti-*Leishmania* antibody titers were found only when clinically detectable lesions appears, lasting during the course of the disease and being present even when the lesion had clinically healed. Similar results have been found in naturally infected dogs (Coutinho et al., 1985; Pirmez et al., 1987) which confirms that the IF serological test is a valuable diagnostic tool.

One of the animals, however, did not develop a lesion at the site of the inoculation, but near it. Interestingly, although we could not find any macroscopical change at that site, culture of a fragment from that apparently normal skin was positive for *Leishmania*. It seems unlikely that the positivity was due to the same promastigotes which were inoculated since the culture was performed nine months after the parastite injection. Although no parasites had been found in normal skin of naturally infected dogs this observation could explain cases of "primary" mucosal lesions observed by Pirmez et al. (1987). However, it should be pointed out that this dog was inoculated with promastigotes which had been in culture for a longer time than the others, although of the same strain. That dog was the youngest one (two-month aged) that might also have an influence on the course of the infection.

The histopathological picture of the experimental canine ACL was similar to what has been observed in naturally infected dogs and also in human lesions. The intensity degree of fibrinoid degeneration consistently goes on parallel with the clinical activity of the lesion, thus suggesting (as previously stated by Ridley, 1979) that necrosis is the main mechanism for elimination of the parasite is correct.

These experimental results are still preliminary and it is necessary to study a greater number of experimentally infected dogs in order to obtain more accurate kinetic data on the infection. However, these results seem to be relevant since experimentally infected dogs reproduce the natural canine infection which is very similar to human disease. Thus, dogs may represent an adequate experimental model for *Leishmania braziliensis braziliensis* infection which is still not available.

RESUMO

**Leishmaniose cutâneomucosa canina experimental** *(Leishmania braziliensis braziliensis)* — Quatro cães foram inoculados por via intredérmica com $3 \times 10^6$ promastigotos de *Leishmania braziliensis braziliensis.* Três deles desenvolveram lesões cutâneas quatro, sete e oito meses após, respectivamente. O quarto cão não desenvolveu lesão no sítio do inóculo, mas uma úlcera mucosa foi detectada 16 meses após o inóculo. Os achados clínicos, histopatológicos e serológicos foram similares àqueles verificados na infecção canina natural, bem como na doença humana. Estes resultados sugerem que o cão pode representar um modelo experimental para a infecção causada pela *L. b. braziliensis.*

Palavras-chave: *Leishmania braziliensis braziliensis* — infecção canina experimental

ACKNOWLEDGEMENTS

We wish to thank Dr. Gabriel Grimaldi for the immunological characterization of the parasite; Dr. Deoclécio de Brito, director of the Municipal Institute of Veterinary, who kindly offered us all the facilities to maintain the

animals and also Dr. Vera Lúcia Ribeiro, Dr. Vanda Coutinho, Valmir Silva, Nilton F. Conceição, Marise P. Nunes and Dr. Fátima Conceição Silva who helped us in several steps of this work.

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