Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 81(3) :303-309, jul./set. 1986

ACTIVE CUTANEOUS LEISHMANIASIS IN BRAZIL, INDUCED BY *LEISHMANIA DONOVANI CHAGASI*

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L.d. chagasi *was isolated from active cutaneous leishmaniasis in both human and canine infections in an endemic area in Rio de Janeiro, Brazil. Both isolates were identified by molecular and immunological characterization of the parasite using three different methods: electrophoretic mobility of isoenzymes; restriction endonuclease fragment analysis of kDNA and serodeme analysis using monoclonal antibodies. This seems to be the first well documented case in the New World of a "viscerotropic"* Leishmania *inducing a case of cutaneous leishmaniasis. This observation emphasizes that the diagnosis of the etiologic agent of human or canine visceral leishmaniasis based solely upon clinical and epidemiological criteria may lead to erroneous conclusions.*

Key words: leishmaniasis – cutaneous – *Leishmania donovani chagasi*

The leishmaniasis comprise a group of diseases caused by different species of parasitic protozoa belonging to the genus *Leishmania.* The identification and taxonomy of the morphologically similar species of *Leishmania* have traditionally been based mainly upon the clinical manifestations in man along with epidemiological features and geographical distribution (Chance, 1979). The difficulties involved with parasite identification based on the classical parameters are obvious since there is a wide range of host responses that can mask the parasite characteristics. However, there are now well established biochemical and immunological means of characterizaion that, by comparing with reference strains, may give species or subspecies identification of all recognized parasites of this genus infective to man (Chance & Walton, 1982). By means of some of these modern methods it has been demonstrated that a single *Leishmania* species or subspecies can induce a variety of pathological manifestations within an individual and/or among members of a population in a limited geographical region (Chance, 1979; Chance & Walton, 1982).

In general, patients with clinical active visceral leishmaniasis will suffer the same symptoms all over the world, but in different regions where the disease occurs, visceral manifestations may be preceeded, accompanied or followed by cutaneous lesions. For instance, Manson-Bahr (1955) referred to a report of a primary skin lesion in visceral leishmaniasis in southern USSR. A mild, dermatotropic and inapparent form of kala-azar also occurs in East Africa (Hoogstraal & Heyneman, 1969). Confirming these previous results, it was recently suggested that *L. donovani* was the etiological agent of all 7 cases of human sudanese leishmaniasis in which either mucosal, cutaneous or visceral disease was manifested (Veress, Abdalla & El-Hassan, 1980). Similarly, characterization of *Leishmania* spp. from Kuwait by isoenzyme electrophoresis demonstrated that an isolate from a patient with "oriental sore" had an isoenzyme pattern similar to that of a visceral parasite (Al-Taqi & Evans, 1978). *L. infantum* infection can also result in cutaneous pathological manifestations in adults (Bellazzoug et al., 1985; Rioux et al., 1980) and children (Schiliro et al., 1978) in different endemic areas of mediterranean visceral leishmaniasis.

In Brazil, some authors have reported cutaneous alterations during active diasease or after treatment of American visceral leishmaniasis (see Deane & Grimaldi, 1985). However, although such cutaneous or mucocutaneous lesions have been ascribed to *L. donovani* (Deane & Deane, 1955; Neves, Bezerra & Martins, 1963; Rodrigues da Silva, 1957; Veronesi et al., 1955) no parasite identification was done from the isolates and we have recently demonstrated the possibility of concommitant infection, having isolated *L. donovani* from bone marrow and *L.b. braziliensis* from a cutaneous ulcer in a patient with double infection (Oliveira Neto et al., 1986). We now report, for the first time, *L. donovani chagasi* (as defined by molecular characterization using monoclonal antibodies, isoenzyme electrophoresis and restriction-endonuclease fragment analysis of kDNA) inducing primary and active cutaneous leishmaniasis in both, human and canine infections, in the New World.

This work received financial support from CNPq and PADCT (Brazil) and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

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Received for publication Janyary 6th and accepted February 17th, 1986.

MATERIALS AND METHODS

Parasite isolation and culture – The primary isolation was made by *in vitro* culture of samples from the cutaneous lesions obtained by punch biopsies from both, the human and canine cases. The infected tissue was washed several times in a balanced salt solution containing relatively high amounts of antibiotics (penicillin, 1,000 U/ml and streptomycin, 1,000 Mg/ml) and then cut into small pieces and the epidermis removed. The pieces were then re-washed in the same solution and finally introduced aseptically in tubes containing the culture medium. The isolates were initially cultivated in brain-heart infusion agar (Difco), 5,2% (w/v), to which 15% rabbit blood was added. An overlay of enriched brain-heart infusion-liver infusion-trypticase liquid medium containing 10% heat inactivated fetal bovine serum (Flow Laboratories) (Grimaldi, quoted by Jaffe Grimaldi Jr. & McMahon-Pratt, 1984) was used. When promastigotes appeared the parasites were transferred to an enriched liquid medium, the Schneider's Drosophila medium (Hendricks, Woods & Hajduk, 1978), supplemented with 20% heat-inactivated fetal bovine serum, and incubated at 24°C.

Preparation of samples – The parasites (promastigotes in late phase of growth in culture) were harvested by centrifugation (1,500 r dor 10 minutes at 4°C) and washed twice in phosphate-buffered saline (PBS), pH 7.3. The final pellet was re-suspended in lysis buffer containing 0.04 M sodium chloride, 0.01 M sodium ethylenediamine tetra-acetate (EDTA), 0.001 M phenylmethylsulfonylfluoride, 0.001 M iodoacetamide, 0.01 M Tris, pH 8.0, for analysis with monoclonal antibodies and in 100 mM EDTA in saline pH 8.0, for enzyme electrophoresis and schizodeme analysis.

Monoclonal antibodies – The 30 monoclonal antibodies used for parasite characterization, specific for members of the *L. donovani, L. mexicana* and *L. braziliensis* complexes have already been described (Jaffe et al., 1984; McMahon-Pratt, Bennett & David, 1982; McMahon-Pratt et al., 1985; Oliveira Neto et al., 1986). The specificity of these monoclonal antibodies has been verified against a large panel of *Leishmania* strains and isolates by means of radioimmune binding assay (Grimaldi & McMahon-Pratt, 1984). The above mentioned technique, together with the specific monoclonals, were used for the immunological characterization and identification of both *Leishmania* stocks isolated from the patient (MHOM/BR/81/HCG5) and from a dog (MCAN/BR/81/CCG1).

Zymodeme and Schizodeme analysis – Zymodeme analysis (by isoenzyme characterization) and schizodeme analysis (by restriction-endonuclease fragment analysis of kDNA) of the isolates were carried out using the enzymes and conditions described previously (Momen et al., 1985).

Epidemiological data – The municipality of Rio de Janeiro has long been known as an endemic area for cutaneous leishmaniasis (Aragão, 1922; Marzochi et al., 1980), while more recently cases of visceral leishmaniasis have been reported (Marzochi et al., 1985). The two forms of the disease occur in general in different suburbs of the city. However, in the suburb of Campo Grande, the locality inhabited by the patient and where the infected dog was captured, situated about 30 km of the city center, the distribution of visceral and cutaneous diseases overlap (Coutinho et al., 1985). The physical geography of the endemic area and a study of phlebotomine population in the region have been described previously (Lima, Marzochi & Sabroza, 1982; Souza et al., 1981). The parasites involved in human and canine leishmaniasis in this area have recently been identified as *L. donovani* and *L. braziliensis* (Lopes et al., 1984; Grimaldi & McMahon-Pratt, 1984).

Case Report – The patient was a white female of 35 years of age. She consulted the doctor for the first time in July, 1981, stating that at the end of April she noticed on her leg the appearance of a papular lesion, of firm consistence and with an erythematous surface. This lesion rapidly increased in size and become ulcerated. On physical examination, the patient showed no clinical alterations, without any involvement of the various organs and systems, and was in a good state of general health. However, on dermatological examination, an ulcerated skin lesion of about 3 cm in diameter with elevated and infiltrated borders was noted on the posterior face of her left leg (Fig. 1). Regional lymph nodes were apparently not involved. On admission to the hospital, routine laboratory examination were normal, but Montenegro's skin test was positive and anti-leishmania antibodies were detected by indirect immunofluorescence immediatly before treatment (at a titre of 1:90) which became negative 4 months after conclusion of treatment. No attempt was done to isolate parasites from the bone marrow.

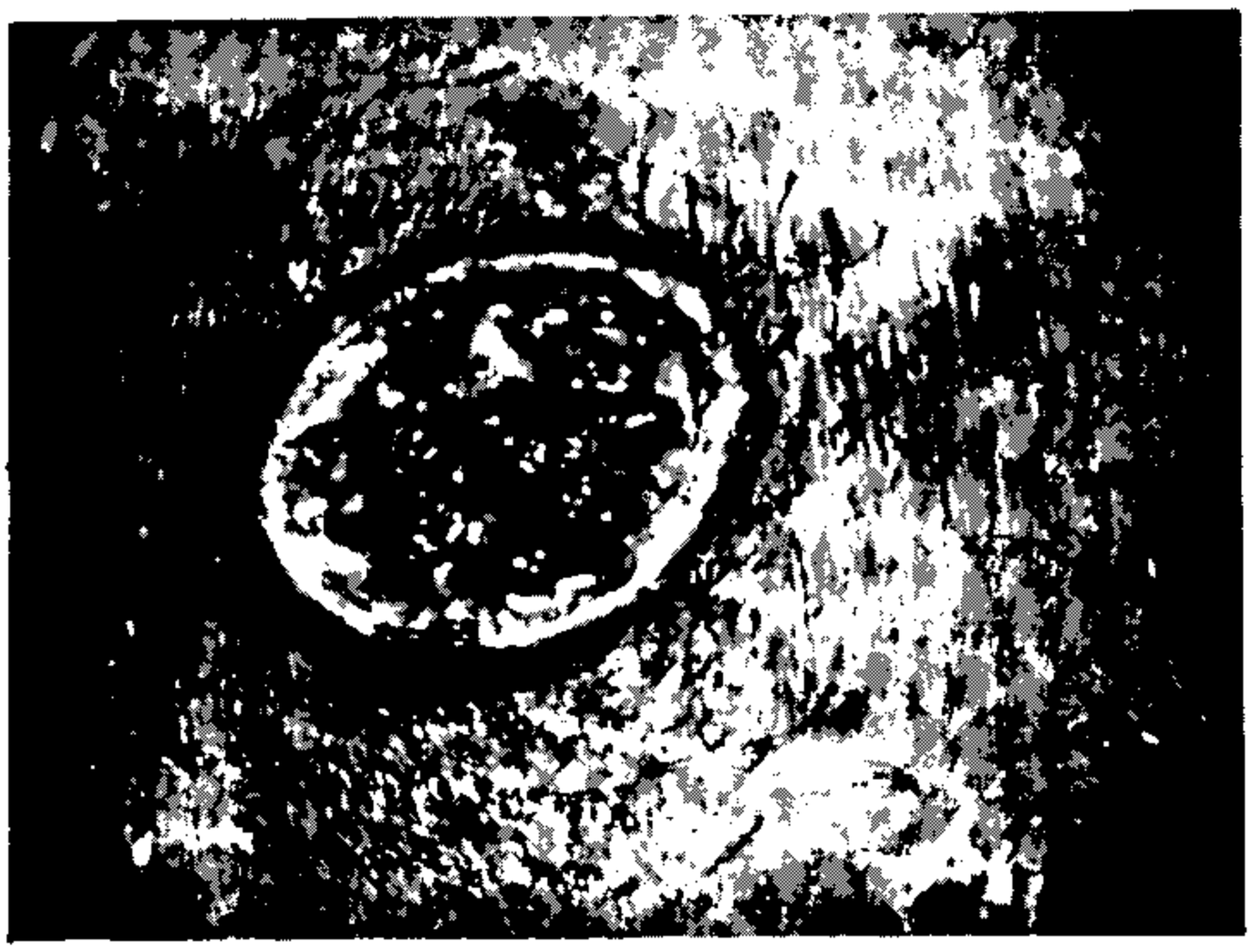


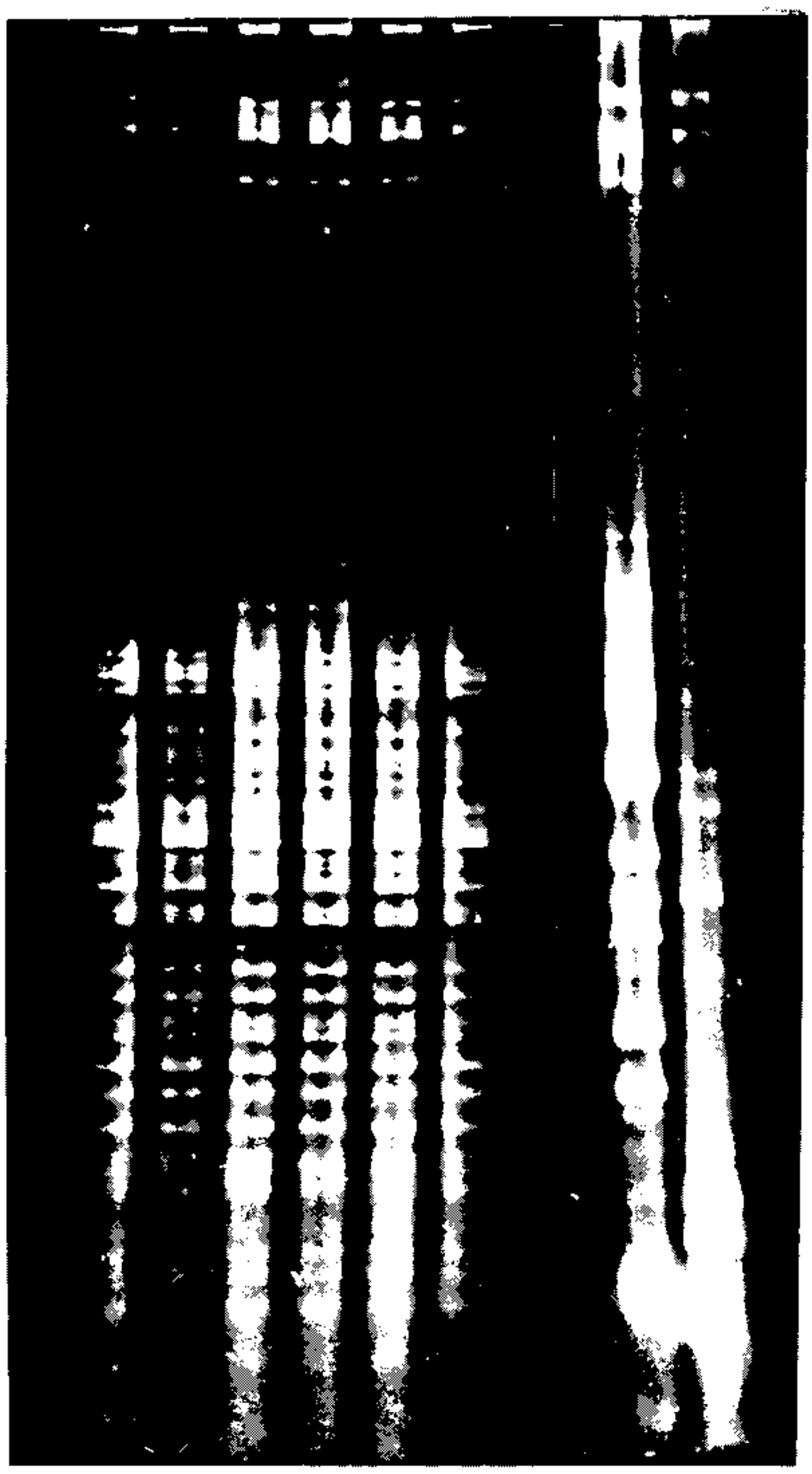
Fig. 1: photograph of the skin lesion in a patient caused by the *L. donovani chagasi* isolate MHOM/BR/81/HCG5.

A dog from the same locality, captured during the same period, had a skin ulcer on the ear. This animal was sacrificed by the health authorities, before the results of parasite characterization and no necropsy or attempts to isolate parasites from viscera was performed.

Parasite identification – The isolated parasites from both, human and canine cutaneous lesions were identified initially as *L. donovani* by isoenzyme characterization using the technique of agarose gel electrophoresis and six different enzymes: aspartate amino-transferase E.C. 2.6.1.1., alanine amino-transferase E.C. 2.6.1.2, phosphoglucomutase E.C. 2.7.5.1, glucose-6-phosphate dehydrogenase E.C. 1.1.1.49, glucose-phosphate isomerase E.C. 5.3.1.9, and malate dehydrogenase E.C.1.1.1.37. No enzymatic differences were noticed between these cutaneous *L. donovani* isolates and other strains isolated from cases of visceral leishmaniasis.

These observations were confirmed using the technique of restriction-endonuclease fragment analysis of kDNA or schizodeme analysis. Thus comparing the kDNA fingerprints produced by restriction enzymes MspI and MboI of both *Leishmania* stocks with reference strains, they were identified as *L. donovani chagasi.* Furthermore, comparison of kDNA fingerprints of these stocks together with other stocks isolated from human and canine cases of visceral leishmaniasis from the same locality showed a homogenous pattern (Fig. 2).

Moreover, based on the characteristic reactivities of *L. donovani* species and/or subspecies-specific monoclonal antibodies we were able to confirm again the identification of these isolates as *L.d. chagasi.* No cross-reaction was observed between any of these parasites and a panel of *L. braziliensis* and *L. mexicana* species specific monoclonal antibodies.



1 2 3 4 5 6 7 8

Fig. 2: polyacrylamide gel gradient (4 to 10%) separation of MspI endonuclease digested kDNA purified from cutaneous and visceral isolates of *L. donovani.*

1.	*L.d. chagasi*	MCAN/BR/82/CCG3	Visceral lesion
2.	*L.d. chagasi*	MCAN/BR/82/DOB19	Visceral lesion
3.	*L.d. chagasi*	MHOM/BR/81/HCG5	Cutaneous lesion
4.	*L.d. chagasi*	MHOM/BR/81/AC	Visceral lesion
5.	*L.d. chagasi*	MHOM/BR/81/HCG7	Visceral lesion
6.	*L.d. chagasi*	MHOM/BR/82/ETS(M)	Visceral lesion
7.	*L.d. donovani*	MHOM/IN/00/LRC-L52	Visceral lesion
8.	*L.d. infantum*	MHOM/FR/00/LRC-L47	Visceral lesion

DISCUSSION

In this report we have demonstrated the presence of *L.d. chagasi* in active cutaneous leishmaniasis, in both human and canine infections in Brazil.

We are confident about the parasite identification of both these isolates because: first, there was a concordance in the typing results using three well established molecular criteria at the genotype (restriction-endonuclease fragment analysis of kDNA) and phenotype (isoenzyme characterization and immunological identification with monoclonal antibodies) levels; second, the possibility of error by contamination with other strains is excluded, since these analyses were performed on recent isolates with clear origins and well controlled labels.

Although this is the first well documented report on *L.d. chagasi* inducing primary cutaneous leishmaniasis in the New World, this phenomenon is probally not so unusual, since some authors have reported cutaneous alterations during active state or after treatment of American visceral leishmaniasis in Brazil. Thus leishmaniae have been detected in macroscopically normal, though histologically altered skin (Bogliolo, 1956; Chagas et al., 1938; Deane & Deane, 1955; Prata & Piva, 1956) and cutaneous and mucocutaneous lesions have also been described in this country (Deane & Deane, 1955; Neves, Bezerra & Martins, 1963; Rodrigues da Silva, 1957; Veronesi et al., 1955).

The current thinking is that the "dermatotropic" *L. braziliensis, L.b. guyanensis* or *L. mexicana amazonensis* are the causative agents of American cutaneous leishmaniasis in Brazil, and the American visceral leishmaniasis is caused by the "viscerotropic" *L.d. chagasi* (Lainson, 1983). Against this established view there are recents reports demonstrating that kala-azar can be caused by "dermatotropic" species of *Leishmania* in the Old World (Schnur et al., 1981; 1985) as well as in the New World (Barral et al., submitted). On the other hand, it has also been reported that cutaneous or mucocutaneous leishmaniasis may be induced by different species or strains of *L. donovani* in the Old World (Al-Taqi & Evans, 1978; Bellazzoug et al., 1985; Rioux et al., 1980; Schiliro et al., 1978; Veress et al., 1980) besides this present report in the New World.

Although a new enzymatic variant of *L. infantum* was demonstrated as the causative organism of cutaneous leishmaniasis (Bellazzoug et al., 1985), our results showed homogenous patterns by schizodeme and zymodeme analyses, for the strains of *L.d. chagasi* isolated from human cutaneous and visceral leishmaniasis in the same endemic area (Fig. 2), suggesting that cutaneous leishmaniasis induced by *L.d. chagasi* is not related to different intrinsic characteristics of the parasite but, probably, with different host responses.

From this evidence, there is no doubt that a diagnosis of the etiologic agent of human or canine leishmaniasis based only upon clinical-pathological manifestations and epidemiological considerations will often be in error and should be based on the intrinsic characters of these organisms, which can now be detected by several molecular, biochemical and immunological techniques (Chance & Walton, 1982).

RESUMO

Em lesões cutâneas, de um caso humano e de um cão, procedente de área endêmica de leishmaniose tegumentar no Rio de Janeiro, foi isolada *L.d. chagasi.* Ambas as culturas foram identificadas por caracterização molecular e immunológica do parasito utilizando três diferentes métodos: mobilidade eletroforética de isoenzimas, análise do kDNA e anticorpos monoclonais. Este parece ser o primeiro caso humano bem documentado, no Novo Mundo, de uma *Leishmania* "viscerotrópica" induzindo lesões cutâneas e demonstra que o diagnóstico do agente etiológico baseado somente na observação clínica e dados epidemiológicos pode levar a conclusões errôneas.

ACKNOWLEDGEMENTS

We are grateful to Dr. Sergio Coutinho who carried out the immunofluorescent assay, Valeria da Silva Trajano and Andiaria R. da Silva for technical assistance and Marlene Braga for typing the manuscript.

REFERENCES

AL-TAQI, M. & EVANS, D.A., 1978. Characterization of *Leishmania* spp. from Kuwait by isoenzyme electrophoresis. *Trans. R. Soc. Trop. Med. Hyg., 72* :56-65.

ARAGÃO, H.B., 1922. Transmissão da leishmaniose no Brasil pelo *Phlebotomus intermedius. Brasil Médico, 36* :129-130.

BARRAL, A.M.P.; BADARO, R.; BARRAL NETTO, M.; GRIMALDI, Jr. G.; MOMEN, H. & CARVALHO, E.M., 1985. Isolation of *Leishmania mexicana amazonensis* from the bone marrow in a case of American visceral leishmaniasis (submitted).

BELLAZZOUG, S.; LANOTTE, G.; MAAZOUN, R.; PRATLONG, F. & RIOUX, J.A., 1985. A new enzymatic variant of *Leishmania infantum* (Nicolle, 1908) causative organism of cutaneous leishmaniasis. *Ann. Parasitol., 60* :1-3.

BOGLIOLO, L., 1956. Nova contribuição ao conhecimento da anatomia patológica da leishmaniose visceral. A propósito de um caso brasileiro e com especial referência à fibrose hepática leishmaniótica. *O Hospital, 50* :393-440.

CHAGAS, E.; CUNHA, A.M. da; FERREIRA, L.C.; DEANE, L.; DEANE, G.; GUIMARÃES, F.N. & PAUMGARTEN, M.J. VON, 1938. Leishmaniose visceral americana (Relatório dos trabalhos realizados pela comissão encarregada do estudo da leishmaniose visceral americana em 1937). *Mem. Inst. Oswaldo Cruz, 33* :89-229.

CHANCE, M.L., 1979. The identification of *Leishmania.* In: Problems in identification of parasites and their vectors (Taylor, A.E.R. & Muller, R. Eds.). Blackwell Scientifics Publications, Oxford pp 31-53.

CHANCE, M.L. & WALTON, B.C. (Eds.), 1982. Biochemical characterization of *Leishmania.* UNOP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. Geneva. p. 280.

COUTINHO, S.G.; NUNES, M.P.; MARZOCHI, M.C.A. & TRAMONTANO, N.C., 1985. A survey for cutaneous and visceral leishmaniasis among 1,342 dogs from areas in Rio de Janeiro (Brazil) where the human disease occurs. *Mem. Inst. Oswaldo Cruz, 80* :17-22.

DEANE, L.M. & DEANE, M.P., 1955. Observações preliminares sobre a importância comparativa do homem, do cão e da raposa (Lycalopex vetulus) como reservatório de *Leishmania donovani* em área endêmica de calazar no Ceará. *O Hospital, 48* :61-76.

DEANE, L.M. & GRIMALDI, Jr. G., 1985. Leishmaniasis in Brazil. In: Leishmaniasis (Chong, K.P. & Broy, R.S. Eds) Amsterdam, Elsevier Sci. Publ., Ch 14, pp 247-281.

GRIMALDI, Jr., G. & MCMAHON-PRATT, D., 1984. Immunological characterization of New World *Leishmania* stocks by means of monoclonal antibodies. XI Reunião Anual de Pesquisa Básica em Doença de Chagas. Caxambu (Brazil), abstract 155.

HENDRICKS, L.D.; WOOD, D.E. & HAJDUK, M.E., 1978. Haemoflagellates commercially available liquid media for rapid cultivation. *Parasitology, 76* :309-316.

HOOGSTRAAL, H. & HEYNEMAN, D., 1969. Leishmaniasis in Sudan Republic. Final epidemiological report. *Am. J. Trop. Med. Hyg., 18* :1091-1210.

JAFFE, C.L.; BENNETT, E. GRIMALDI, Jr., G. & MCMAHON-PRATT, D., 1984. Production and characterization of species specific monoclonal antibodies against *Leishmania donovani* for immunodiagnosis. *J. Immunol., 133* :440-447.

JAFFE, C.L.; GRIMALDI, Jr., G. & MCMAHON-PRATT, D., 1984. The cultivation and cloning of *Leishmania. In:* Genes and Antigens of parasites. A Laboratory Manual (Morel, C.M. ed.) 2nd. Edit. Fundação Oswaldo Cruz, Rio de Janeiro, pp 121-135.

LAINSON, R., 1983. The American leishmaniasis: some observations on their ecology and epidemiology. *Trans. R. Soc. Trop. Med. Hyg., 77* :569-596.

LIMA, L.C.R.; MARZOCHI, M.C.A. & SABROZA, P.C., 1982. Flebotomídeos em área de ocorrência de leishmaniose tegumentar no bairro de Campo Grande, Rio de Janeiro. *Rev. Bras. Malar. Doen. Trop., 33* :64-74.

LOPES, V.G.; MOMEN, H.; GRIMALDI, Jr. G.; MARZOCHI, M.C.A.; PACHECO, R.S. & MOREL, C.M., 1984. Schizodeme and zymodeme characterization of *Leishmania* in the investigation of foci of visceral and cutaneous leishmaniasis. *J. Parasitol., 70* :89-98.

MANSON-BAHR, P.E.C., 1955. A primary skin lesion in visceral leishmaniasis. *Nature,* London, *175* :433-434.

MARZOCHI, M.C.A.; COUTINHO, S.G.; SABROZA, P.C. & SOUZA, W.J.S., 1980. Reação de imunofluorescência indireta e intradermoreação para Leishmaniose tegumentar americana em moradores na área de Jacarepaguá (Rio de Janeiro). Estudo comparativo dos resultados observados em 1974 e 1978. *Rev. Inst. Med. Trop. S. Paulo, 22* :149-155.

MARZOCHI, M.C.A.; SABROZA, P.C.; TOLEDO, L.M.; MARZOCHI, K.F.B.; TRAMONTANO, N.C. & RANGEL, F.B., 1985. Leishmaniose visceral na cidade do Rio de Janeiro. *Cadernos de Saúde Pública, 1* :5-17.

MCMAHON-PRATT, D.; BENNETT, E. & DAVID, J.R., 1982. Monoclonal antibodies that distinguish subspecies of *Leishmania braziliensis. J. Immunol., 129* :926-927.

MCMAHON-PRATT, D.; BENNETT, E.; GRIMALDI Jr., G., & JAFFE, C.L., 1985. Subspecies and species specific antigens of *Leishmania mexicana* characterized by monoclonal antibodies. *J. Immunol., 134* :1935-1940.

MOMEN, H.; GRIMALDI, Jr. G.; PACHECO, R.S.; JAFFE, C.L.; MCMAHON-PRATT, D. & MARZOCHI, M.C.A., 1985. *Brazilian Leishmania* stocks phenotypically similar to *Leishmania major. Am. J. Trop. Med. Hyg. 34* :1076-1084.

NEVES, J.; BEZERRA, A.A. & MARTINS, N.R.L.L., 1963. Leishmaniose visceral associada a lesões cutaneas. *O Hospital* (Rio de Janeiro), *63* :337-347.

OLIVEIRA NETO, M.P.; MARZOCHI, M.C.A.; GRIMALDI, Jr., G.; PACHECO, R.S.; TOLEDO, L.M. & MOMEM, H., 1986. Concurrent human infection with *Leishmania donovani* and *Leishmania braziliensis. Ann. Trop. Med. Parasit.,* (in press).

PRATA, A. & PIVA, N., 1956. Presença de leishmanias em pele aparentemente normal de pacientes com calazar. *O Hospital* (Rio de Janeiro), *49* :481-486.

RIOUX, J.A.; LANOTTE, G.; MAAZOUN, R.; PERELLO, R. & PRATLONG, R., 1980. *Leishmania infantum* (Nicole, 1908), agent du bouton d'Orient autochtone. A propos de l'identification biochimique de deux sources isolées dans les Pyrénées-Orientales. *Comp. Rends. Acad. Sci.*, (Paris) *291* :701-703.

RODRIGUES DA SILVA, J., 1957. Leishmaniose visceral (calazar). Thesis – *Fac. Med. Univ. Brasil.* Rio de Janeiro.

SCHILIRO, G.; RUSSO, A.; MUSUMECI, S. & SCIOTTO, A., 1978. Visceral leishmaniasis following a skin lesion in a six year old sicilian girl. *Trans. R. Soc. Trop. Med. Hyg., 72* :656-657.

SCHNUR. L.F.; CHANCE, M.L.; EBERT, F.; THOMAZ, S.C. & PETERS, W., 1981. The biochemical and serological taxonomy of visceralizing *Leishmania. Ann. Trop. Med. Parasit., 75* :131-144.

SCHNUR, L.F.; MORSY, T.A.; FEINSOD, F.M. & EL MISSIRY, A.G., 1985. Is *Leishmania major* the cause of infantile kala-azar in Alexandria, Egypt? *Trans. R. Soc. Trop. Med. Hyg., 79* :134-135.

SOUZA, M.A.; SABROZA, P.C.; MARZOCHI, M.C.A.; COUTINHO, S.G. & SOUZA, W.J.S., 1981. Leishmaniose visceral no Rio de Janeiro. I. Flebotomídeos de área de procedência de caso humano autoctone. *Mem. Inst. Oswaldo Cruz., 76* :161-168.

VERESS, B.; ABDALLA, R.E. & EL HASSAN, A.M., 1980. Electron microscope investigations on leishmaniasis in the Sudan. I. Morphometric studies on *Leishmania* amastigotes in various forms of human leishmaniasis. *Ann. Trop. Med. Parasit., 74* :421-426.

VERONESI, R.; CASTRO, R.M.; MARQUES, J.C.; FIORILLO, A.M.; ZUCOLLOTO, M.; CZAPSKI, J.; SALLES, H.L.B. & AMATO NETO, V., 1955. Leishmaniose visceral (Kala-azar) no Brasil. Estudo do quadro clínico e humoral em 15 novos casos. *Rev. Hosp. Clin.* (S. Paulo), *1* :86-111.