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## LEISHMANIA MEXICANA IN PROECHIMYS IHERINGI DENIGRATUS MOOJEN (RODENTIA, ECHIMYIDAE) IN A REGION ENDEMIC FOR AMERICAN CUTANEOUS LEISHMANIASIS<sup>1</sup>

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Three isolates of *Leishmania* were recovered from five of 27 specimens of the rodent *Proechimys iheringi denigratus* Moojen captured near Três Braços in the Atlantic Forest region of Bahia, Brazil. Two of these isolates were recovered from hamsters inoculated with a pooled triturate of liver, spleen and skin tissue from apparently healthy *P. i. denigratus*. The third isolate was recovered from a triturate of only skin tissue from another. Metastasis was observed in the inoculated hamsters, the parasites grew abundantly in artificial media and a typical suprapylarial pattern of infection in *Lutzomyia longipalpis* was produced indicating that the parasites belong to the *Leishmania mexicana* complex. All isolates reacted with *Leishmania mexicana mexicana* and *Leishmania mexicana amazonensis* monoclonal antibodies. The isoenzyme analysis differentiated these isolates from standard isolates of *L. m. mexicana*, *L. m. amazonensis*, *L. m. aristedesii*, *L. m. pifanoi*, *L. m. garnhami* and *L. m. ssp. (Goiás-W. Barbosa)*. These isolates seem to be a subspecies of *L. mexicana* very closely related to *L. m. amazonensis* from which they differ by decreased electrophoretic mobility of GPI, PEP and ALAT.

This is the first record of the isolation of a parasite of the genus *Leishmania* in a rodent captured in the State of Bahia.

Key words: *Leishmania mexicana* ssp. Natural reservoir. Field study. Bahia, Brazil.

In Três Braços, Bahia, an area in which cutaneous leishmaniasis is endemic, the parasite most commonly isolated from skin lesions of man<sup>4</sup> and the dog<sup>1,2,4</sup> has been biologically, biochemically (isoenzymes) and immunologically (monoclonal antibodies) identified as *Leishmania braziliensis braziliensis*. There have been only two exceptions: one isolation of *L. mexicana amazonensis* (MHOM/BR/76/LTB-16) and another of *L. mexicana* ssp. (MHOM/BR/78/LTB-55). Both were from cutaneous lesions of humans<sup>4,5</sup>.

In an attempt to find the primary reservoirs of these parasites, more than 600 wild animals, principally rodents, marsupials, rabbits and edentates, have been examined (unpublished data). This paper describes three isolates which were recovered from rodents.

## MATERIALS AND METHODS

The rodents were live trapped in the tall forest habitat of the cacao growing region of Três Braços, in the municipalities of Ubaira and Wenceslau Guimarães, Bahia, Brazil. This area is located 150 km southwest of Salvador, latitude 13° 32' south and longitude 39° 45' west. The animals were anesthetized with chloroform and necropsied. All mammals necropsied were preserved either as skin and skull specimens or in formalin for future positive identification. Portions of skin tissue from the nose and base of tail, as well as liver and spleen, were removed for imprint smears and later triturated with saline solution and inoculated into the foot pads of hamsters. In some cases the skin and viscera were both inoculated into one hamster, in other cases the skin was inoculated into one hamster and the viscera into another. When the appearance of a cutaneous lesion indicated an inoculated hamster was harboring an infection, samples were taken for imprint smears, inoculations in new hamsters and isolation attempts in Difco blood agar culture media<sup>13</sup>.

Specimens of *Lutzomyia longipalpis*, from the colony maintained in our laboratory, were fed on the lesions and dissected following oviposition (4 or 5 days after feeding) to observe the development pattern of the parasites in the digestive tube<sup>11</sup>.

A battery of seven monoclonal antibodies, species and subspecies specific for the *L. mexicana* and *L. braziliensis* complexes, were employed to analyze the three isolates of *Leishmania* by indirect

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immunofluorescence<sup>12</sup>. The antibodies were from clones VI 4B9D10, XIII 3F4F6, XIII 3E6B11, XIII 2A5A10, IX 1F9D8, LXVII 1D7B8 and LXVIII 4E12D8, being one specific for *L. braziliensis*, three subspecific for *L. b. braziliensis*, one subspecific for *L. mexicana amazonensis* and two subspecific for *L. m. mexicana*. Promastigotes cultivated in Schneider's medium<sup>8</sup> were used as antigen. Fluorescent IgG anti-mouse conjugate (Cappel Laboratories, USA) was used at a 1:20 dilution. The isolates used as controls were MHOM/BR/83/LTB-363 (*L. b. braziliensis*) and MHOM/BR/75/L-3 (Josefa) (*L. m. amazonensis*), both of human origin. Preliminary data have been previously reported<sup>3</sup>.

The isoenzyme analysis of the isolates, using the thin starch gel electrophoresis technique, was conducted at the Unidade de Estudos Especiais, Instituto Evandro Chagas, Belém, by R.S. Braga. The following enzymes were used: G6PD, MDH, MPI, ASAT, 6PGDH, PGM, GPI, ALAT and PEP.

## RESULTS

Although none of the imprint smears of skin and visceral tissue contained amastigotes, three isolates of *Leishmania* (MPRO/BR/83/MTB-581/583, MPRO/BR/83/MTB-584/585 and MPRO/BR/83/MTB-574) were recovered from five of 27 specimens of the rodent *Proechimys iheringi denigratus* Moojen (identified by Dr. Alfred L. Gardner, National Museum of Natural History, Washington, D.C., USA). MTB-581/583 was isolated when the pooled triturate of liver and spleen tissue and skin from the nose and tail of two apparently healthy rodents was inoculated into the foot pads of a hamster. MTB-584/585 was isolated in the same manner from two other animals. MTB-574 was isolated when a triturate of apparently normal skin from the nose and base of the tail of a rodent was injected into the feet of a hamster. The injection of triturate of liver and spleen tissue of this same animal into another hamster produced no apparent infection, and after a year of observation the hamster was necropsied. The skin, liver and spleen showed no parasites upon microscopic examination and culture. These results indicate that the infection with at least one of these isolates was from the skin of the rodent.

The incubation period observed in the hamsters inoculated with tissue from these *Proechimys* (MTB-581/583, MTB-584/585 and MTB-574) varied from 2.5 to 6 months. In subsequent inoculations, this period decreased to less than 1 month. The lesions were similar to those which occur in animals inoculated with parasites of the *L. mexicana* complex with large, non-ulcerated histiocytomas rich in large amastigote forms. Metastasis to the nose, feet and testes was observed in the animals (Figure 1), but no visceralization occurred. No infection was obtained in Swiss 44 white mice inoculated intracutaneously.

The parasites grew abundantly in both media used. After an initial failure to infect *Lutzomyia*



Figure 1. Hamster inoculated subcutaneously (first passage) into the two hind feet with the strain MPRO/BR/83 MTB-581/583 (*L. mexicana* ssp.) showing large non-ulcerated histiocytomas (5 months post-inoculation). Positive smears and metastasis were first observed 30 days after inoculation.

*longipalpis* (0/30 positive), a typical suprapylaria pattern of infection was observed in 15/18, 25/26 and 12/17 of this phlebotomine species with the isolates MTB-581/583, MTB-584/585 and MTB-574 respectively. None of the three isolates from the *Proechimys* reacted with the specific or subspecific *L. braziliensis* monoclonal antibodies. All isolates reacted with antibodies of *L. m. amazonensis* as well as *L. m. mexicana*, and could not be differentiated subspecifically. One of the *L. m. mexicana* antibodies (LXVIII 4E12D8) did not react with either the isolates from the rodents, or those of human origin, (Table 1).

The isoenzyme analysis demonstrated that the two isolates (MTB-581/583 and MTB-574) were indistinguishable from each other and differed from standard isolates of *L. m. mexicana*, *L. m. amazonensis*, *L. m. aristedesi*, *L. m. pifanoi*, *L. m. garnhami* and *L. m. ssp.* (Goias - W. Barbosa) when the following enzymes were used: G6PD, MDH,

Table 1 – Reactivity of promastigotes of isolates of *Leishmania* from the rodent *Proechimys iheringi denigratus* from Três Braços, Bahia, Brazil to monoclonal antibodies by indirect immunofluorescence.

Clone Code (*)	Specificity	Isolates of <i>Leishmania</i>				
		MPRO/BR/83/ MTB-574	MPRO/BR/83/ MTB-581/3	MPRO/BR/83/ MTB-584/5	MHOM/BR/83/ LTB-363(**)	MHOM/BR/75/ L-3(***)
VI 4B9D10	<i>L. braziliensis</i>	-	-	-	++	-
XIII 3F4F6	<i>L. b. braziliensis</i>	-	-	-	++	-
XIII 3E6B11	<i>L. b. braziliensis</i>	-	-	-	+++	-
XIII 2A5A10	<i>L. b. braziliensis</i>	-	-	-	++	-
IX 1F9D8	<i>L. m. amazonensis</i>	+++	++	+++	-	+++
LXVII 1D7B8	<i>L. m. mexicana</i>	+++	++	+++	-	+++
LXVIII 4E12D8	<i>L. m. mexicana</i>	-	-	-	-	-

(\*) McMahon Pratt and David<sup>12</sup>

(\*\*) Control: human isolate of *L. b. braziliensis*

(\*\*\*) Control: human isolate of *L. m. amazonensis* (Josefa)

MPI, ASAT, 6PGDH, PGM, GPI, ALAT, and PEP. These isolates seem to be a subspecies of *L. mexicana* very closely related to *L. m. amazonensis*, from which they differ by decreased electrophoretic mobility of GPI, PEP and ALAT. Also, these two isolates differed from *L. m. aristedesi* by decreased mobility of MPI and greater mobility of ASAT.

## DISCUSSION

Species of the genus *Proechimys* have been previously found infected with parasites of the *L. mexicana* complex. *Proechimys guyannensis* (Geoffroy) is considered to be the primary reservoir of *L. m. amazonensis* in the state of Pará, Brazil<sup>10</sup>, and *Proechimys semispinosus* (Torres), in Panama, was found to be infected with a *Leishmania*<sup>9</sup> which was later designated as *L. m. aristedesi*<sup>11</sup>. Recently Dedet et al<sup>7</sup> examined a new rodent species, *Proechimys cuvieri* Petter, from which a parasite was isolated from normal skin and identified by isoenzymes as *L. m. amazonensis*. Nine enzymes were used, and five of them (MDH, MPI, PGM, 6PGDH and GPI) coincided with those used in this study. While the GPI enzyme did not differentiate the *P. cuvieri* isolate from *L. m. amazonensis*, this same enzyme along with ALAT and PEP were able to separate the *Leishmania* isolated from the *P. i. denigratus* from *L. m. amazonensis*. On the other hand, Christensen et al<sup>6</sup> were able to distinguish electrophoretically a Panamanian strain of *L. m. aristedesi* from *L. m. amazonensis*. The former has a more rapid anodic migration for ALAT and a slower anodic mobility for malic enzyme (ME). In our laboratory we did not observe any differences between electrophoretic mobilities of two strains (MTB-574 and MTB-763/766)<sup>(\*)</sup> isolated

from *P. i. denigratus* and two strains of *L. m. amazonensis* for malic enzyme (ME) (Figure 2) using the cellulose-acetate plate technique. The examination of a larger number of enzymes will be necessary for a better characterization of this parasite.

These isolates apparently represent the third subspecies of the *L. mexicana* complex isolated in the Três Braços region. It differs in isoenzyme characteristics and in reactivity with monoclonal antibodies from LTB-16 (*L. m. amazonensis*) and LTB-55 (*L. mexicana* ssp.), both of human origin, reported earlier<sup>4,5</sup>. Biologically (growth in culture, inoculation in hamsters and development in the digestive tube of *Lu. longipalpis*), the isolates from the rodents are

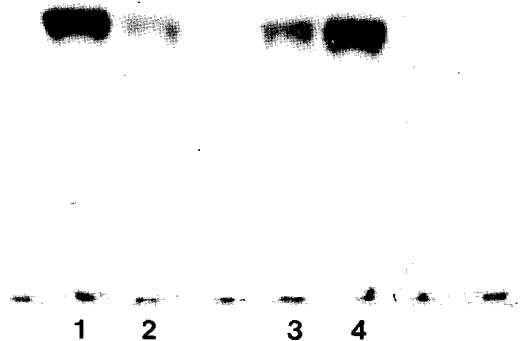


Figure 2. Cellulose-acetate plate electrophoresis showing the mobilities of the malic enzyme (ME): (1) *L. m. amazonensis*, 136241, (2) *L. mexicana* ssp., MPRO/BR/83/MTB-584/585, (3) *L. mexicana* ssp., MPRO/BR/83/MTB-763/766, and (4) *L. m. amazonensis*, MHOM/BR/75/L-3 Josefa. The *L. m. amazonensis* strains are used as standard references in our laboratory.

(\*) One strain, MPRO/BR/84/MTB-763/766, was isolated after this manuscript was submitted. It is mentioned here to substantiate our original findings.

indistinguishable from LTB-16, but differ from LTB-55 which has characteristics of both *L. m. amazonensis* and *L. b. braziliensis*<sup>5</sup>.

This is the first record of the isolation of a parasite of the genus *Leishmania* in a rodent captured in the state of Bahia. As far as we can determine, this is also the first time that a natural infection of *L. mexicana* has been reported in *Proechimys iheringi denigratus*.

## RESUMO

Três isolados de *Leishmania* foram obtidos de cinco entre 27 exemplares do roedor *Proechimys iheringi denigratus*, capturados na região de Três Braços, na mata atlântica do Estado da Bahia, Brasil. O isolamento desse parasito foi feito através de inoculação de triturado de pele, baço e fígado em patas de hamsters. Em pelo menos um dos casos, (MTB-574), o parasito foi isolado da pele. Metástase foi observada nos hamsters inoculados, os parasitos cresceram abundantemente em meios artificiais de cultura e um padrão suprapapilar típico foi obtido em *Lutzomyia longipalpis*, indicando que o parasito pertence ao complexo *L. mexicana*. Todos os isolados reagiram positivamente com anticorpos monoclonais de *L. m. mexicana* e *L. m. amazonensis*. A análise isoenzimática diferenciou o parasito de isolados padrões de *L. m. mexicana*, *L. m. amazonensis*, *L. m. aristadesi*, *L. m. pifanoi*, *L. m. garnhami* e *L. m. ssp (Goiás-W. Barbosa)*. O parasito parece ser uma subespécie de *L. mexicana* muito próxima à *L. m. amazonensis*, da qual difere pela menor mobilidade eletroforética de GPI, PEP e ALAT.

Este é o primeiro registro do isolamento de um parasito do gênero *Leishmania* em um roedor capturado no Estado da Bahia.

Palavras chaves: *Leishmania mexicana* ssp. Reservatório natural. Estudo de campo. Bahia, Brasil.

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