

## EPIDEMIOLOGICAL AND NOSOLOGICAL ASPECTS OF *LEISHMANIA NAIFFI* LAINSON & SHAW, 1989

R. D. NAIFF; R. A. FREITAS; M. F. NAIFF; J. R. ARIAS;  
T. V. BARRETT; H. MOMEN\* & G. GRIMALDI JR. \*\*

Instituto Nacional de Pesquisas da Amazônia, Coordenação de Pesquisas  
em Ciências da Saúde, Caixa Postal 478, 69083 Manaus, AM, Brasil

\*Instituto Oswaldo Cruz, Departamento de Bioquímica e Biologia Molecular

\*\* Departamento de Imunologia, Caixa Postal 926, 20001 Rio de Janeiro, RJ, Brasil

*Leishmania naiffi* was isolated from 10 out of 64 armadillos (*Dasyus novemcinctus*) examined in Amazonas, Pará and Rondônia States in the Brazilian Amazon Region. The isolates were obtained in culture from samples of liver (3), spleen (3), lymph nodes (2), skin (1) and blood (1) from the infected animals. Heavy infections with the same parasite were detected for the first time in *Psychodopygus squamiventris*, a common man-biting phlebotomine, in Amazonas and Pará. A new case of cutaneous leishmaniasis caused by *L. naiffi* is described from the Manaus area, making a total of three known cases of human infection by this parasite.

Key words: cutaneous leishmaniasis - *Psychodopygus squamiventris* -  
*Leishmania naiffi* - *Dasyus novemcinctus* - Amazônia

Lainson et al. (1979, 1981, 1982) isolated a new peripylarian *Leishmania* from armadillos (*Dasyus novemcinctus*) in Pará State, Brazil. The same parasite was later reported from the phlebotomines *Psychodopygus ayrozai* and *P. paraensis* in Amazonas and Rondônia States (Arias et al., 1985). This parasite was formally described as *L. naiffi* by Lainson & Shaw (1989) who had already placed it in the subgenus *Viannia* Lainson & Shaw, 1987 (q.v.). Reports of human cutaneous leishmaniasis caused by *L. naiffi* (Menezes et al., 1988, 1989; Naiff et al., 1989) were based in part on unpublished results of molecular characterization (Grimaldi et al., 1991).

The purpose of the present communication is to complement our formal identification of the material (Grimaldi et al., 1991) with information on the isolates of *L. naiffi* characterized in that study, to report further isolations, and to discuss the epidemiological implications of these findings.

This study received financial support from the research agreements ELETRONORTE/INPA, PETROBRAS/INPA, FUNDAÇÃO BANCO DO BRASIL/INPA, and from PADCT-FINEP, CNPq, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, National Institutes of Health, and Rockefeller Foundation Biotechnology Career Fellowship Program.

Received 29 March 1991.

Accepted 24 May 1991.

## MATERIALS AND METHODS

Collection sites and probable origins of human infections, all in the Brazilian Amazon Region, were as follows: Pará State (PA), Repartimento, near Tucuruí lake, study area described previously (Naiff et al., 1986, 1988); Amazonas (AM) and Rondônia (RO) States, BR-319 and BR-364 highways, described previously (Arias et al., 1985); Balbina (AM), 01°55'S 59°28'W, near site of hydroelectric dam on the Uatumã river, high ground (180m above sea level) tall terra-firme primary forest on red-yellow sandy clay, behind residential area; Reserva Ducke (AM), terra-firme forest 26 km northeast of Manaus, described previously (Penny & Arias, 1982); Puraquequara (AM), military training area in forest near Reserva Ducke; Cachoeira Porteira (PA), terra-firme primary forest near the left bank of the Trombetas river, just above the confluence with the Mapuera river.

Phlebotomines were taken on human bait. Dissection, and procedures for isolation of *Leishmania*, were as described previously (Arias et al., 1985). Isolates from armadillos (Table) and human cases MHOM/BR/86/IM-2773 and MHOM/BR/86/IM-2736 were made directly into blood agar culture medium using rabbit blood with Difco Blood

TABLE  
 Details of *Leishmania naiffi* isolated from *Dasypus novemcinctus*

Strain Code	Origin	Tissue	Parasite identified by	No. of animals positive/ No. examined
MDAS/BR/82/IM-671	AM: BR-319 Km 40	skin (ear)	M	1/20
MDAS/BR/82/IM-1267	RO: BR-364 Km 119	liver	M	
MDAS/BR/82/IM-1311	RO: BR-364 Km 113	spleen	M	4/10
MDAS/BR/82/IM-1335	RO: BR-364 Km 119	liver	M	
MDAS/BR/83/IM-1538	RO: Ji-Paraná	blood	M	
MDAS/BR/87/IM-3280	PA: Repartimento	spleen	M,S,Z	
MDAS/BR/87/IM-3281	PA: Repartimento	spleen	M,S,Z	
MDAS/BR/87/IM-3292	PA: Repartimento	lymph nodes	M,S,Z	5/34
MDAS/BR/87/IM-3307	PA: Repartimento	liver	M,S,Z	
MDAS/BR/87/IM-3320	PA: Repartimento	lymph nodes	M,S	

M = morphology, and behaviour in hamsters and culture.

Z = zymodeme analysis by isoenzyme electrophoresis.

S = serodeme analysis with monoclonal antibodies.

Agar Base B45, and condensation fluid as overlay (Walton et al., 1977).

Armadillos were tracked with dogs until they went to ground, and captured after excavation of the burrow, or in emergence traps (*jequi*) made of a closed cylinder of strong wire mesh with a one-way hinged door placed in the entrance of the occupied burrow. The animals were placed in cotton sacks and transported on mules to the field laboratory (Repartimento), or by motor vehicles. Immediately before examination they were anaesthetized and killed with parenteral barbiturate solution (200-400 mg Thionembatal). After external asepsia with soap and water, iodated alcohol and 70% ethanol, samples of skin, liver, spleen, blood, and subcutaneous lymph nodes were inoculated separately into 2-4 tubes of the culture medium. Overlay from positive cultures was inoculated intradermally on the nose of hamsters (*Mesocricetus auratus*).

Leishmanin skin tests on humans were carried out using an antigen prepared in our laboratory

from *L. amazonensis* promastigotes (INPA antigen), and another prepared from a mixture of *L. braziliensis*, *L. guyanensis*, *L. amazonensis*, and *L. mexicana*, (Belo Horizonte antigen), as previously described (Naiff et al., 1988).

Procedures used for zymodeme analysis by enzyme electrophoresis, and serodeme analysis by radioimmune assay with monoclonal antibodies, have been described or cited elsewhere (Grimaldi et al., 1991).

## RESULTS

### RECENT ISOLATIONS OF *L. NAIFFI* FROM PHLEBOTOMINES

Strain code ISQU/BR/85/IM-2264 from *P. squamiventris* at Cachoeira Porteira (Grimaldi et al., 1991) — Flagellate infection in the insect heavy. Hamster inoculated with culture presented a slight swelling of the nose with a minute nodule when necropsied after 4 months; 5/5 cultures from

the nose positive, 2/2 cultures from the spleen and 2/2 cultures from the liver all negative.

Strain code ISQU/BR/86/IM-2833 from *P. squamiventris* at Balbina (Grimaldi et al., 1991) — Midgut without trace of a bloodmeal, ovaries in the earliest stage of egg development, flagellate infection very heavy, with parasites observed throughout the digestive tract including the hind triangle and mouthparts and also in the crop, salivary glands, and malpighian tubules. Hamster inoculated with culture showed no external sign of infection, but culture of skin from the nose was positive when the animal was necropsied after 18 months.

#### ISOLATION OF *L. NAIFFI* FROM HUMANS

Strain code MHOM/BR/87/AXEL, from cutaneous leishmaniasis, Trombetas river (PA) (Grimaldi et al., 1991) — (See Menezes et al., 1988; 1989).

Strain code MHOM/BR/86/IM-2773 (Grimaldi et al., 1991) — Male, 31 years old, professional soldier from Campinas (São Paulo). Presented with a small (10 mm diameter), painful, ulcerated lesion, without raised margins, surrounded by an erythematous area, on the index finger of the left hand. The lesion had been noted six months previously, shortly after the patient had taken part in exercises in the forest at Puraquequara (AM). No previous history of leishmaniasis. Leishmanin skin test results with INPA antigen 3.5 x 3.5 mm, with Belo Horizonte antigen 8.0 x 8.0 mm. Smears of exudate obtained by scarification of the edge of the lesion contained various small amastigotes. Luxuriant growth of small promastigotes in culture of biopsy material. Hamsters inoculated with biopsy material or culture showed no external sign of infection within 8 months; inoculation sites with rare small amastigotes at necropsy, cultures of hamster material positive. The patient's lesion healed completely after a single intravenous injection of 480 mg pentamidine methanesulphonate (Major Ageu Silva, Hospital Geral de Manaus, personal communication).

Strain code MHOM/BR/86/IM-2736 (new record): male, 34 years old, research scientist from Rio Grande do Sul (Brazil) working at Reserva Ducke (AM). Presented with an ulcerated lesion approximately 10 mm in diameter on the abdomen, that had first been noted one month previously. No

previous history of leishmaniasis. Leishmanin skin test results with INPA antigen 4.0 x 5.0 mm, with Belo Horizonte antigen 8.5 x 8.5 mm. The patient took 30 injections of glucantime, and later reported that the lesion had healed after taking the 25th ampoule. This parasite was indistinguishable from the *L. naiffi* reference strain (MDAS/BR/78/M-5169) based on (1) their reactivity patterns using specific monoclonal antibodies (serodeme analysis) and (2) their electrophoretic patterns of 11 enzymic loci (zymodeme analysis).

#### ISOLATION OF *L. NAIFFI* FROM ARMADILLOS

*Leishmania naiffi* was isolated from 10 *D. novemcinctus*, out of a total of 64 *D. novemcinctus* and one *D. kappleri* examined in areas where armadillos infected with this parasite were found (Table).

#### DISCUSSION

We have described in some detail the methodology used for collecting armadillos for parasitological studies, because these animals are not readily captured in conventional live-traps, and shot specimens are often unsatisfactory because of bacterial contamination. *L. naiffi* can be isolated directly by hamster inoculation, but in our experience many of the animals inoculated with armadillo tissues develop fatal infections with *Histoplasma capsulatum* or *Paracoccidioides brasiliensis*. Hamster inoculation can however be useful for isolating *L. naiffi* from mixed culture of *L. naiffi* with *Trypanosoma cruzi*. The fact that various tissues contained *L. naiffi* in the infected armadillos, whereas in all our samples the parasite was only isolated from a single site in individual animals, suggests that use of an increased number of tubes of culture medium would improve detection rates. We know of no previous reports of the isolation of *L. naiffi* from either blood, lymph nodes or the skin of armadillos.

Our observation of flagellates in the salivary glands of *P. squamiventris* is included here for the sake of completeness. We recognize that an objective demonstration of the phenomenon remains desirable, especially since leishmanial infections in phlebotomine salivary glands have not previously been reported.

The vertical distribution above the forest floor of *P. squamiventris* and of several other species of *Psychodopygus* in light-trap samples, with a strong bias to lower levels (Barrett & Freitas, unpublished data), is consistent with the ground-dwelling habits of armadillos. *P. squamiventris* is strongly attracted to humans at ground level; in the Balbina area this species made up 56% of all phlebotomines taken on human bait, and on the Mapuera river (PA), out of 3139 female phlebotomines taken in 90 min by a single collector sitting in darkness, 2182 (70%) belonged to this species, and 859 (27%) to other species of *Psychodopygus* (Barrett & Freitas, unpublished data).

Also of interest from this study was the confirmation that not all leishmania isolates from *P. squamiventris* belong to *L. naiffi*, Cf. ISQU/BR/86/IM-2832 (Grimaldi et al., 1991).

If, as we suggest here, *L. naiffi* is a common parasite of armadillos, transmitted by common man-biting phlebotomines, then some explanation for the low number of reported cases of human infection is required. We have not dissected sufficient numbers of *P. squamiventris* to give meaningful estimates of the frequency of infection in these phlebotomines, but infection rates in recognized or suspected vectors of other leishmanias are often low (Arias et al., 1987). It is noteworthy that all human infections by *L. naiffi* were detected in patient who not only were visitors to the area of probable contagion, but also of a social class and/or profession with ready access to specialized diagnosis and treatment of what were in most cases relatively benign lesions. We do not know of any case of *L. naiffi* infection among residents of forest areas or professionals engaged in collecting phlebotomines, although many of these have a history of *L. guyanensis* infection. Acquired resistance to *L. naiffi* may play a part in the distribution of human cases, as may successful treatment with household remedies or spontaneous cure without recourse to specific diagnosis and treatment (Cf. Naiff et al., 1988), or again many human infections may be clinically imperceptible, as in hamsters. Resolution of this question will probably have to await the availability of species-specific immunodiagnostic reagents for epidemiological field studies.

#### ACKNOWLEDGEMENTS

To Dr Wilson Mayrink, Universidade Federal de Minas Gerais, for the Belo Horizonte leishmanin. To Major Ageu Silva, M. D., of the Hospital

Geral de Manaus, for his interest in referring patients with atypical cutaneous leishmaniasis to our laboratory for specific diagnosis. To two anonymous referees, for suggesting improvements of the manuscript.

#### REFERENCES

- ARIAS, J. R.; FREITAS, R. A.; NAIFF, R. D. & BARRETT, T. V., 1987. Observations on the parasite *Leishmania mexicana amazonensis* and its natural infection of the sand fly *Lutzomyia olmeca nociva*. *Bull. Pan Am. Hlth. Org.*, **21**: 48-54.
- ARIAS, J. R.; MILES, M. A.; NAIFF, R. D.; POVOA, M. M.; FREITAS, R. A.; BIANCARDI, C. B. & CASTELLON, E. G., 1985. Flagellate infections of Brazilian sand flies (Diptera: Psychodidae): Isolation in vitro and biochemical identification of *Endotrypanum* and *Leishmania*. *Am. J. Trop. Med. Hyg.*, **34**: 1098-1108.
- GRIMALDI Jr., G.; MOMEN, H.; NAIFF, R. D.; McMAHON-PRATT, D. & BARRETT, T. V., 1991. Characterization and classification of leishmanial parasites from humans, wild mammals, and sand flies in the Amazon Region of Brazil. *Am. J. Trop. Med. Hyg.*, **44**: 645-661.
- LAINSON, R. & SHAW, J. J., 1987. Evolution, classification and geographical distribution, p. 1-120. In W. Peters & R. Killick-Kendrick (eds.). *The Leishmaniasis in Biology and Medicine*, Vol. 1. London, Academic Press.
- LAINSON, R. & SHAW, J. J., 1989. *Leishmania (Viannia) naiffi* sp. n., a parasite of the armadillo, *Dasypus novemcinctus* (L.) in Amazonian Brazil. *Ann. Parasitol. Hum. Comp.*, **64**: 3-9.
- LAINSON, R.; SHAW, J. J.; MILES, M. A. & PÓVOA, M., 1982. Leishmaniasis in Brazil: XVII. Enzymic characterization of a *Leishmania* from the armadillo, *Dasypus novemcinctus* (Edentata), from Pará State. *Trans. R. Soc. Trop. Med. Hyg.*, **76**: 810-811.
- LAINSON, R.; SHAW, J. J.; READY, P. D.; MILES, M. A. & PÓVOA, M., 1981. Leishmaniasis in Brazil: XVI. Isolation and identification of *Leishmania* species from sandflies, wild mammals and man in north Pará State, with particular reference to *L. braziliensis guyanensis* causative agent of "pian-bois". *Trans. R. Soc. Trop. Med. Hyg.*, **75**: 530-536.
- LAINSON, R.; SHAW, J. J.; WARD, R. D.; READY, P. D. & NAIFF, R. D., 1979. Leishmaniasis in Brazil: XIII. Isolation of *Leishmania* from armadillos (*Dasypus novemcinctus*), and observations on the epidemiology of cutaneous leishmaniasis in north Pará State. *Trans. R. Soc. Trop. Med. Hyg.*, **73**: 239-242.
- MENEZES, J. A.; BRAZIL, R. P.; MOMEN, H. & GRIMALDI, G., 1988. Atypical human leishmaniasis: Isolation of a parasite from the *L. braziliensis* complex — a new species? *Mem. Inst. Oswaldo Cruz*, Suppl. I, **83**: 39.

- MENEZES, J. A.; BRAZIL, R. P.; MOMEN, H. & GRIMALDI, G., 1989. Atypical human leishmaniasis due to a parasite of the *Leishmania braziliensis* complex in north Brazil. *Trans. R. Soc. Trop. Med. Hyg.*, 83: 429.
- NAIFF, R. D.; BARRETT, T. V.; ARIAS, J. R. & NAIFF, M. F., 1988. Encuesta epidemiologica de histoplasmosis, paracoccidioidomicosis y leishmaniasis mediante pruebas cutaneas. *Bol. Of. Sanit. Panam.*, 104: 35-50.
- NAIFF, R. D.; FERREIRA, L. C. L.; BARRETT, T. V.; NAIFF, M. F. & ARIAS, J. R., 1986. Paracoccidioidomicose enzoótica em tatus (*Dasypus novemcinctus*) no Estado do Pará. *Rev. Inst. Med. Trop. São Paulo*, 28: 19-27.
- NAIFF, R. D.; FREITAS, R. A.; NAIFF, M. F.; ARIAS, J. R.; BARRETT, T. V.; GRIMALDI, G. & MOMEN, H., 1989. Aspectos epidemiológicos de uma *Leishmania* de tatus (*Dasypus novemcinctus*). *XI Congresso Sociedade Brasileira de Parasitologia*. Resumo 24. Rio de Janeiro.
- PENNY, N. D. & ARIAS, J. R., 1982. *Insects of an Amazon Forest*. New York, Colombia University Press, 269 p.
- WALTON, B. C.; SHAW, J. J. & LAINSON, R., 1977. Observations on the *in vitro* cultivation of *Leishmania braziliensis*. *J. Parasitol.*, 63: 1118-1119.