

LEISHMANIA MEXICANA COMPLEX: HUMAN INFECTIONS IN THE REPUBLIC OF PANAMA

ANA MARIA DE VASQUEZ, ROLANDO E. SAENZ,
 JOHN L. PETERSEN, HOWARD A. CHRISTENSEN, AND
 CARL M. JOHNSON

Gorgas Memorial Laboratory, Panama

Abstract. Parasites of the genus *Leishmania* responsible for human cutaneous leishmaniasis in the New World form 2 major taxonomic divisions: the *Leishmania braziliensis* and the *L. mexicana* complexes. We report the isolation and characterization of the *L. mexicana* complex among humans in the Republic of Panama. Characterization was based on parasite morphology, pathogenesis in infected golden hamsters, cellulose acetate isoenzyme electrophoretic mobilities, and membrane-specific monoclonal antibodies using the radioimmune binding assay technique.

Human cutaneous leishmaniasis was first reported in the Republic of Panama in 1910 by Darling.¹ Research on the ecology of the disease was initiated in 1947 at Gorgas Memorial Laboratory (GML) where 5 sand fly species²⁻⁶ and 8 sylvatic hosts⁷⁻⁹ were incriminated in the epidemiology of the parasite *Leishmania panamensis*, a member of the *L. braziliensis* complex which, until recently, was thought to be the only agent responsible for human cutaneous leishmaniasis in Panama.

The isolation of *L. aristidesi* from 3 species of rodents and a single marsupial, *Marmosa robinsoni*, in Sasaki, San Blas Territory in 1968 documented the presence of a member of the *L. mexicana* complex.¹⁰ To date, this parasite has never been isolated from humans. We report 2 cases of human cutaneous leishmaniasis due to *L. mexicana* complex in Panama.

MATERIALS AND METHODS

During 1985-1986, 839 cases of cutaneous leishmaniasis were seen at the GML clinic. Diagnostic procedures included the Montenegro skin test, Giemsa-stained smears, and culture of tissue sample. The methodology for skin tissue cultures has been described.¹¹

Three hundred isolates from the 839 patients seen, mainly from the provinces of Panama, Colon, and Darien, have been screened by isoenzyme analysis using cellulose acetate electrophoresis (CAE). Preparation of extracts and conditions for antigens used in the technique have been described.¹² Nine enzymes systems were used in the CAE testing: ALAT E.C.2.6.1.2. (alanine amino transferase), ASAT E.C.2.6.1.1. (as-

partate amino-transferase), PGM E.C.2.7.5.1. (phosphoglucumutase), PGI E.C.5.3.1.9. (phosphoglucoseisomerase), ME E.C.1.1.1.40. (malic enzyme), MDH E.C.1.1.1.37 (malic dehydrogenase), G-6-PDH E.C.1.1.1.49. (glucose 6-phosphate dehydrogenase), 6-PGDH E.C. 1.1.1.44. (6-phosphogluconic dehydrogenase), and MPI E.C.5.3.1.8. (mannose phosphate isomerase). World Health Organization (WHO) strains for *L. pifanoi* (MHOM/VE/57/LL1), *L. venezuelensis* (MHOM/VE/74/PM-H3), *L. garnhami* (MHOM/VE/73/JAP 78), *L. mexicana* (MHOM/BZ/82/BEL21), *L. amazonensis* (MHOM/BR/73/M2269), and *L. aristidesi* (MORY/PA/68/GML3) were used as *L. mexicana* complex reference markers.

Only 3 of the isolates, which were recovered from 2 patients, differed in their isoenzyme patterns from those of *L. panamensis*. These isolates were also characterized by radioimmune binding assay (RIA) at Yale University, New Haven, CT, using monoclonal antibodies (Mabs) against *L. mexicana* (L11, M379) and *L. amazonensis* (WR 303, H6, LV72).^{13, 14} Promastigotes isolated from both patients were characterized by parasite morphology and pathogenesis in infected golden hamsters (*Mesocricetus auratus*).

RESULTS

During investigations on leishmaniasis in eastern Panama in April 1985, a 41-year-old Caucasian entomologist spent many hours with the field crew in human-bait collections of phlebotomines. In June of that year, a papule was observed on his lower right leg which later developed into a 17 × 21 mm dry, scaly lesion (Case

No. 1). Giemsa-stained skin scraping of the lesion showed numerous amastigotes larger than those observed in most cases seen at the GML clinic. The Montenegro skin test was positive with an induration 9 mm in diameter. Sodium stibogluconate (Pentostam) was prescribed but the patient received treatment for only 2 days. The treatment was discontinued, at the patient's insistence, to allow study of the natural course of the infection. The lesion healed spontaneously 11 months after the infection was first observed. No relapse has been observed after 3 years.

Between December 1985 and April 1986, 8 isolates were cultured in Senekjic's medium and the original isolate was cloned. Nine of the clones and 7 of the other isolates showed identical CAE electrophoretic mobilities for the 9 enzymes systems tested. These mobilities were indistinguishable from those of the WHO reference strains of *L. amazonensis* (MHOM/BR/73/M2269) and *L. garnhami* (MHOM/VE76/JAP78). The original isolate reacted against *L. amazonensis* (WR, 303, H6, LV72) Mabs by radioimmune binding assay.

Case No. 2, a 17-year-old mestizo involved in hunting and agriculture in the northern and eastern part of the country, noted skin lesions 2 months prior to visiting the GML clinic in October 1986. He was exposed to sand fly bites during hunting in the sylvatic areas of Alcalde Diaz in Panama Province and during agricultural activities in Darien Province. The patient had 2 ulcerative lesions on his right forearm (17 × 19 mm and 17 × 23 mm). There was no evidence of local lymphatic dissemination. The Montenegro skin test was positive with an induration of 12 mm. He was treated with oral ketoconazole 600 mg daily for 28 days. At the end of treatment, November 1986, the ulcers were 16 × 13 mm and 20 × 20 mm. An evaluation in December 1986 showed complete healing of both ulcers. In January 1987 he developed a reactivation with the appearance of a new papular lesion surrounding the area of the scars. He was treated im with sodium stibogluconate (Pentostam), 850 mg every other day for 20 days. He did not return until August 1987, by which time both lesions had healed. No reactivation has been observed for 2 years. Two isolates were made, 1 in October and 1 in November 1986. Isoenzyme analysis on these isolates revealed profiles that did not co-migrate with any of the known species within the *L. mexicana* complex used as markers by WHO. At least

3 enzymes, MPI, PGI, and ME, separated them from *L. amazonensis* and *L. mexicana* (Fig. 1). Eight clones each were made of the 2 original isolates by streaking a loop of diluted, 8-day-old culture onto plated solid blood agar media, which was then sealed and incubated at 22°C for 8 days in a humidified chamber. Individual colonies were placed in culture tubes by means of a bacteriological needle. This was followed by massive growth. Colonies were tested by CAE. All clones from each original isolate showed identical isoenzyme profiles, but the clones from each isolate differed from those of the other, as well as from the original isolates.

Clones from the October isolate co-migrated with the WHO reference strain for *L. amazonensis* (MHOM/BR/73/M2269), while clones from the November isolate co-migrate with the WHO reference strain for *L. mexicana* (MHOM/BZ/82/BEL21). Characterization of clones using Mabs against *L. mexicana* (L11, M379) and *L. amazonensis* (WR 303, H6, LV 72) corroborated the CAE results.

Isolates from both patients inoculated in noses of golden hamsters (*M. auratus*) produced the large tumors characteristic of *L. mexicana* infection at the site of inoculation within 45 days. Stained smears from the tumors showed abundant intracellular amastigotes. Metastatic tumors in the paws developed within 7 months.

DISCUSSION

The Republic of Panama forms the geographical link between Central and South America, sharing their fauna and zoonoses. The region is undergoing marked ecological changes. Settlers from arid regions of the central provinces, where cutaneous leishmaniasis is no longer endemic, have been moving into forested areas seeking free land for agriculture and cattle raising. Studies by GML staff in 18 communities along the Inter-American Highway in eastern Panama showed a leishmaniasis prevalence rate of 4–78% (A. M. de Vasquez, personal communication).

L. panamensis, which has been reported from most Central American countries as well as from Colombia and Ecuador, was considered to be the only causative agent of human leishmaniasis in Panama.¹⁵ In 1977, a group of U.S. Army personnel stationed at Fort Sherman near the Caribbean entrance to the Panama Canal, acquired leishmaniasis after conducting jungle exercises

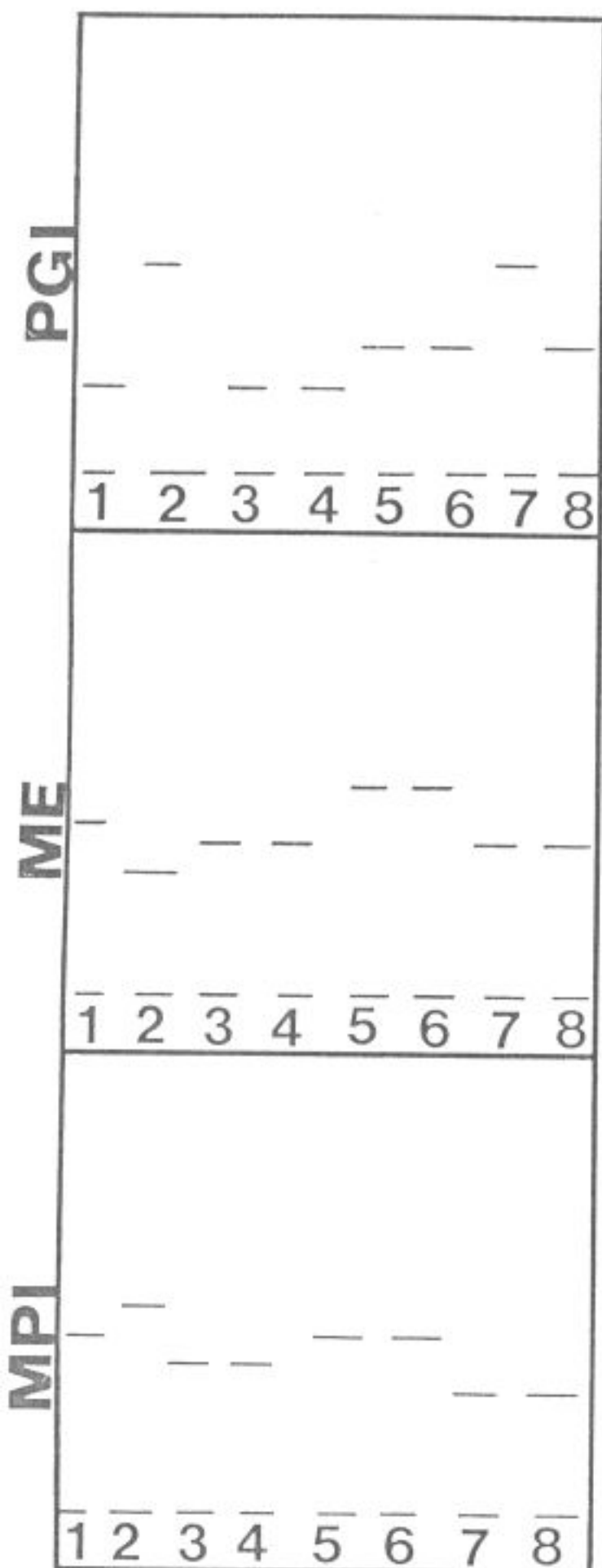


FIGURE 1. Enzyme profiles that separated original isolates. Location of antigens are as follows: 1, *L. pifanoi* (MHOM/VE/57/LL1); 2, *L. venezuelensis* (MHOM/VE/74/PM-H3); 3, October isolate; 4, November isolate; 5, *L. amazonensis* (MHOM/BR/73/M2269); 6, *L. garnhami* (MHOM/VE/76/JAP78); 7, *L. mexicana* (MHOM/BZ/82/BEL21); 8, *L. aristidesi* (MORY/PA/68/GML3).

in the area.¹⁶ Several isolates from these soldiers were characterized in our laboratory by CAE as *L. amazonensis* (data not shown).

L. amazonensis has been reported from Bolivia, Brazil, Peru, Venezuela,¹⁴ Ecuador,¹⁷ and French Guiana¹⁸ and probably occurs in the Amazon Region in Colombia.¹⁹ In Brazil, 30% of the patients found to be infected with *L. amazonensis* developed diffuse cutaneous leishmaniasis, while the remainder developed lesions that healed spontaneously.²⁰ *L. mexicana* has been reported from Mexico, Guatemala, and Belize.²¹

Case No. 2 probably represents a dual infection by *L. amazonensis* and *L. mexicana*, both of the *L. mexicana* complex. It is not known whether the samples were collected from the same ulcer. Characterization of the parasites was not possible until cloning. Isoenzyme profiles on original, uncloned isolates could not be interpreted. Similar results have been obtained by other investigators when co-cultivating *L. amazonensis* and *L. mexicana*.²²

The rarity of human cutaneous leishmaniasis due to *L. amazonensis* and *L. mexicana* in Panama may be partially attributed to the rodentophilic feeding habits of *Lutzomyia olmeca bicolor*, the implicated vector of *L. aristidesi*,²³ and potential vector of the *L. mexicana* complex in this country.

Acknowledgments: The authors wish to thank Robert Tesh and Gabriel Grimaldi at Yale University for the characterization of isolates by serodeme analysis. We also thank David Evans, London School of Hygiene and Tropical Medicine, for providing reference strains and corroborating isoenzyme electrophoresis and C. Gonzales and E. London for their technical assistance.

Authors' address: Gorgas Memorial Laboratory, P.O. Box 6991, Panama 5, Republic of Panama.

Reprint requests: Ana Maria de Vasquez, Gorgas Memorial Laboratory, P.O. Box 6991, Panama 5, Republic of Panama.

REFERENCES

1. Darling ST, 1910. Autochthonous oriental sore in Panama. *Trans Soc Trop Med Hyg* 4: 60-63.
2. Johnson PT, McConnell E, Hertig M, 1962. Natural and experimental infections of leptomonal flagellates in Panamanian Phlebotomus sandflies. *J Parasitol* 48: 158.
3. McConnell E, 1963. Leptomonalads of wild-caught Panamanian Phlebotomus: culture and animal inoculation. *Exp Parasitol* 14: 123-128.
4. Schneider CR, Hertig M, 1966. Immunodiffusion

- reactions of Panamanian Leishmania. *Exp Parasitol* 18: 25-34. file m66; (ui) 66110006
5. Christensen HA, Herrer A, Telford SR Jr, 1969. *Leishmania braziliensis* s. lat., isolated from *Lutzomyia panamensis* in Panama. *J Parasitol* 55: 1090-1091.
 6. Christensen HA, Herrer A, 1973. Attractiveness of sentinel animals to vectors of leishmaniasis in Panama. *Am J Trop Med Hyg* 22: 578-584. file m72; (ui) 73249949
 7. Thatcher VE, Eisenmann C, Hertig M, 1965. A natural infection of Leishmania in the kinkajou, *Potos flavus*, in Panama. *J Parasitol* 51: 1022-1023. file m66; (ui) 66050689
 8. Herrer A, Telford SR Jr, 1969. *Leishmania braziliensis* isolated from sloths in Panama. *Science* 164: 1419-1420. file m66; (ui) 69202702
 9. Herrer A, Christensen HA, Beumer RJ, 1973. Reservoir hosts of cutaneous leishmaniasis among Panamanian forest mammals. *Am J Trop Med Hyg* 22: 585-591. file m72; (ui) 73249950
 10. Herrer A, Telford SR Jr, Christensen HA, 1971. Enzootic cutaneous leishmaniasis in Eastern Panama. I. Investigation of the infection among forest mammals. *Ann Trop Med Parasitol* 65: 349-358. file m66; (ui) 72007903
 11. Herrer A, Thatcher VE, Johnson CM, 1966. Natural infections of Leishmania and trypanosomes demonstrated by skin culture. *J Parasitol* 52: 954-957. file m66; (ui) 67049487
 12. Kreutzer RD, Christensen HA, 1980. Characterization of Leishmania spp. by isozyme electrophoresis. *Am J Trop Med Hyg* 29: 199-208. file m80; (ui) 80172968
 13. Pratt DM, Bennett E, Grimaldi G, Jaffe CL, 1985. Subspecies- and species-specific antigens of *Leishmania mexicana* characterized by monoclonal antibodies. *J Immunol* 134: 1935-1940. file m83; (ui) 85106290
 14. Grimaldi G Jr, David JR, McMahon-Pratt D, 1987. Identification and distribution of New World Leishmania species characterized by serodeme analysis using monoclonal antibodies. [Correction appears in *Am J Trop Med Hyg* 37: 414]. *Am J Trop Med Hyg* 36: 270-287. file m86; (ui) 87154108
 15. Petersen J, Johnson CM, de Vasquez AM, Saenz R, 1987. [Cutaneous leishmaniasis caused by *Leishmania mexicana amazonensis* in Panama]. *Rev Med Panama* 12: 158-164. file m86; (ui) 88098391
 16. Takafugi ET, Hendricks LD, Daubek JL, McNeil KM, Scagliola HM, Diggs CL, 1980. Cutaneous leishmaniasis associated with jungle training. *Am J Trop Med Hyg* 29: 516-520.
 17. Mimori T, Grimaldi G Jr, Kreutzer RD, Gomez EA, McMahon-Pratt D, Tesh RB, Hashiguchi Y, 1989. Identification, using isoenzyme electrophoresis and monoclonal antibodies, of *Leishmania* isolated from humans and wild animals of Ecuador. *Am J Trop Med Hyg* 40: 154-158. file med; (ui) 89148559
 18. Dedet JP, Pajot FX, Desjeux P, Goyot P, Chippaux JP, Geoffroy B, 1985. Natural hosts of *Leishmania mexicana amazonensis* Lainson and Shaw, 1972 (Kinetoplastida: Trypanosomatidae) in French Guiana. *Trans R Soc Trop Med Hyg* 79: 302-305. file m83; (ui) 85301387
 19. Shaw JJ, Lainson R, 1987. Ecology and epidemiology: new world. Peter W, Killick-Kendrick R, eds. *The leishmaniasis in biology and medicine*, vol. 1. London: Academic Press, 291-363. (ui) 8805854
 20. Lainson R, 1983. The American leishmaniasis: some observations on their ecology and epidemiology. *Trans R Soc Trop Med Hyg* 77: 569-596. file m83; (ui) 84098932
 21. Lainson R, Shaw JJ, 1987. Evolution, classification and geographical distribution. Peters W, Killick-Kendrick R, eds. *The leishmaniasis in biology and medicine*, vol. 1. London: Academic Press, 1-128. (ui) 8805854
 22. Pacheco RS, Grimaldi Junior G, Morel CM, 1987. Inhibition of growth of *Leishmania mexicana mexicana* by *Leishmania mexicana amazonensis* during "in vitro" co-cultivation. *Mem Inst Oswaldo Cruz* 82: 537-542. file m86; (ui) 89200863
 23. Christensen HA, Herrer A, Telford SR Jr, 1972. Enzootic cutaneous leishmaniasis in eastern Panama. II. Entomological investigations. *Ann Trop Med Parasitol* 66: 55-66. file m72; (ui) 72167792