

## SHORT REPORT: A FOCUS OF *LEISHMANIA MEXICANA* NEAR TUCSON, ARIZONA

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**Abstract.** Twenty-eight white-throated woodrats (*Neotoma albigula*) collected in Pima County, Arizona were screened for *Leishmania* using culture and the polymerase chain reaction (PCR). Two rodents were culture positive. Isoenzyme analysis determined the isolates to be *Leishmania mexicana*. The two culture-positive and four additional rodents were determined to be *Leishmania*-positive by the PCR. These isolates extend the geographic and ecologic range of enzootic leishmaniasis in the United States and represent a new host record.

The leishmaniasis are vector-borne, parasitic diseases caused by protozoans in the genus *Leishmania*. *Leishmania mexicana*, which causes cutaneous leishmaniasis, infects humans and a variety of vertebrate hosts in North, Central and South America and occurs in a wide variety of ecologic settings.<sup>1</sup> Twenty-nine autochthonous cases of human cutaneous leishmaniasis are known from the United States, all in southern or central Texas.<sup>2</sup> Enzootic foci have been identified only in the semi-arid brush country of southern Texas, in a southern plains woodrat (*Neotoma micropus*)–sand fly (*Lutzomyia anthophora*) cycle.<sup>3–5</sup>

We hypothesized that enzootic foci of *L. mexicana* could exist in the United States wherever sand flies occur in close association with rodents or other potential hosts of *Leishmania*.<sup>2</sup> Mead and Cupp<sup>6</sup> found *Lu. anthophora* associated with white-throated woodrats (*Neotoma albigula*) and rock squirrels (*Spermophilus variegatus*) at the Buenos Aires National Wildlife Refuge (BANWR) in Pima County, Arizona, motivating us to collect and screen rodents from that area for *L. mexicana*. We report the results of those screenings.

Live trapping with Sherman traps (13 × 13 × 37.5 cm; H. B. Sherman Traps, Inc., Tallahassee, FL) was conducted from September 27–October 1, 1998. Seventy-three trap nights were conducted in the southern part of the BANWR along Arivaca Creek, about 70 km southwest of Tucson. This intermittent stream is lined with netleaf hackberry (*Celtis laevigata*), algerita (*Mahonia trifoliata*), fremont cottonwood (*Populus fremontii*), and Arizona walnut (*Juglans major*). Numerous large, fallen trees provided excellent den sites for rodents. Traps were placed near burrow openings, tree cavities, and brush piles. Twenty-seven trap nights were conducted at a dump site near the El Cazador camp area at the northern end of the refuge. Woodrat nests were common in the piles of discarded lumber, equipment, and cars in the dump. The vegetation in this area was desert grassland and mesquite savanna.

Captured rodents were transferred from the trap to a cloth bag, weighed with a precision spring scale (Avinet Inc., Dryden, NY), and then placed in a hardware cloth cone for restraint during handling.<sup>7</sup> The sex and species of each individual was determined, and a biopsy punch was taken from each ear using a sterile, disposable, 2-mm biopsy punch (Sklar Instruments, West Chester, PA). Rodents were then released at the site of their capture.

In the laboratory, each tissue biopsy was cut in two. Half of the biopsy from each ear was placed in a flat-sided Nunc tube (Nalge Nunc International, Roskilde, Denmark) containing M199 medium (Gibco-BRL, Gaithersburg, MD) sup-

plemented with 20% (v/v) heat-inactivated fetal bovine serum (Summit Biotechnology, Fort Collins, CO), 10 mM adenine (Sigma Chemical Co., St. Louis, MO), 0.25% (v/v) bovine hemin (Sigma), 50 mM HEPES buffer (Sigma), 50 IU/ml of penicillin, and 50 µg/ml of streptomycin. Cultures were maintained at room temperature and were examined several times a week for four weeks. Positive cultures were passed in supplemented M199 medium until sufficient promastigotes were available for isoenzyme analysis. Isoenzyme analysis of isolates was performed as described by Kreutzer and others<sup>8</sup> and consisted of preliminary and confirming electrophoretic tests for the enzymes glucose phosphate isomerase, mannose phosphate isomerase, phosphogluconate dehydrogenase, and leucyl proline peptidase. Specific identification was made by comparison of isolate profiles with those of World Health Organization (WHO) reference strains.

The remaining halves of the biopsies were prepared and screened using the method described by Rodgers and others.<sup>9</sup> The DNA was extracted by incubating the tissue in a lysing buffer consisting of 10 mM Tris-HCl (Fisher Biotech, Fair Lawn, NJ) and 10 mM EDTA (LabChem Inc., Pittsburgh, PA) for 30 min at 95°C. Two microliters of the lysed supernatant were used as DNA template in a 25-µl polymerase chain reaction (PCR) mixture containing primers 13A and 13B, which amplify a 120-basepair conserved region of the *Leishmania* kinetoplast DNA minicircle. The PCRs were allowed to proceed through 30 cycles of amplification, with each cycle consisting of denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min, and chain extension at 72°C for 90 sec. Ten microliters of each amplified sample were analyzed alongside a DNA ladder (Gensura Laboratories, San Diego, CA) on a 2% ultra pure DNA-grade agarose gel (Bio-Rad Laboratories, Hercules, CA) prepared with 0.5 mg/ml of ethidium bromide. Samples positive for *Leishmania* were identified by the presence of a band at the 120-basepair region of the gel.

Eighteen *N. albigula* were collected along Arivaca Creek; 10 were collected at the El Cazador refuse area. No *S. variegatus* were captured at either location. Six days after capture, promastigotes (WHO reference code MNEO/US/98/Pima1) were observed in a tube containing biopsies from a 215-g female *N. albigula* collected on October 1 at Arivaca Creek. Eight days after capture, promastigotes (MNEO/US/98/Pima2) were observed in a sample taken from a 175-g female also collected on October 1 at Arivaca Creek. Isoenzyme analysis determined the isolates to be *L. mexicana*. Subcultures of the two isolates were deposited with the Wal-

ter Reed Army Institute of Research *Leishmania* reference cryobank in Washington, DC. No isolates were made from the 10 white-throated woodrats collected at the El Cazador dump site.

The PCR detected *Leishmania* in the two culture-positive animals and identified four additional positive animals among woodrats collected at Arivaca Creek: females weighing 203 g and 255 g captured on September 28; a 165-g individual the sex of which was not recorded captured on September 29; and a male weighing 240 g captured on October 1. The PCR did not detect any positive animals among the 10 woodrats captured at the El Cazador refuse area.

Prevalence of infection at Arivaca Creek was 33% (6 of 18), well above the 20% set by WHO<sup>1</sup> as the level necessary to implicate *N. albigula* as a likely reservoir of *Leishmania*. The failure to find *Leishmania*-positive woodrats at the El Cazador camp is not surprising. Surveys in southern Texas determined that only one site in four sampled was a focus of *L. mexicana*.<sup>5</sup>

The detection of *L. mexicana* in *N. albigula* in southern Arizona is significant for three reasons. First, this is a westward extension of more than 1,100 km in the known range of enzootic leishmaniasis in the United States. All previously recognized foci were located in southern and central Texas, the most westerly of which was near Uvalde, Texas.<sup>2,5</sup> Second, it documents a focus in a new ecologic setting. All previous rodent isolates in the United States have been from the Tamaulipan biotic province; the Arizona focus is in the Sonoran province,<sup>10</sup> albeit in a riparian setting. Third, the detection of *L. mexicana* in *N. albigula* is a new host record for the parasite.

This focus is not far from the greater Tucson area, with a population of approximately 730,000 people. There are probably additional foci in the region and human contact with them is likely, especially where suburban areas intrude into native habitat or where landscaping consists of native vegetation. However, the public health significance of enzootic leishmaniasis in that area is unclear. In Texas, some foci, including some inside the city limits of San Antonio, have existed for years in close proximity to humans,<sup>5</sup> yet human cases of leishmaniasis are rare.<sup>2</sup> The factor determining whether zoonotic transmission of *Leishmania* is possible may be what species of *Lutzomyia* occur in an area. At the BANWR, *Lu. anthophora*<sup>6</sup> and three other species, *Lu. californica*, *Lu. apache*, and an undescribed *Lutzomyia* sp., have been collected (McHugh CP, unpublished data). Based on what is known of its host preferences, vector competence, and association with foci in Texas,<sup>4</sup> *Lu. anthophora* is the most likely vector at the Arizona focus. However, *Lu. anthophora* is a non-anthropophilic feeder, and the presence of a human-feeding species such as *Lu. diabolica* may be necessary to act as a bridge between the enzootic cycle and humans.<sup>2</sup> To date, no anthropophilic sand flies have been reported from southern Arizona.

Although all autochthonous human cases and all known foci in rodent populations are located in the southwestern United States, there is no apparent reason why transmission could not occur in other areas of the country. Canine cases

of leishmaniasis have been reported from Texas,<sup>11</sup> Oklahoma,<sup>12</sup> and Ohio,<sup>13</sup> outside the ranges of *N. micropus* and *N. albigula*.<sup>14</sup> These cases, the origins of which have not been satisfactorily explained, suggest that *Leishmania* may, in fact, be more widely distributed than currently believed. Much additional screening of potential vertebrate hosts and sampling of associated sand flies will be necessary to define the distribution and dynamics of *Leishmania* in the United States.

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