

SUSCEPTIBILITY OF *AOTUS TRIVIRGATUS* TO *LEISHMANIA BRAZILIENSIS* AND *L. MEXICANA*

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Abstract. Two *Aotus trivirgatus* (owl monkeys) were infected experimentally with *Leishmania braziliensis* and two with *L. mexicana* strains of Panamanian origin in a pilot study to determine the susceptibility and the course of infection of cutaneous leishmaniasis in this primate species. Montenegro skin tests performed on all animals prior to parasite inoculation were negative. A standardized inoculum of promastigotes was injected intradermally on the nose of each monkey. All of the animals developed infections which lasted from 3.5 to 8.5 months. Depigmentation developed at the site of the inoculation in all of the subjects. The severity of the resulting lesions was greater in the animals infected with *L. braziliensis*. Positive skin tests developed in three *A. trivirgatus* at days 62, 76, and 139 postinoculation, respectively. An explanation for the negative skin test in the fourth animal is discussed.

In view of the availability of owl monkeys, *Aotus trivirgatus*, in Panama for research purposes, and the substantial inventory of these animals following antimalarial drug evaluation trials at Gorgas Memorial Laboratory (GML), a pilot study was conducted to determine the susceptibility of these animals to leishmaniae and to study the nature of infections in this primate species. The importance of *A. trivirgatus* as a primate model for studies on human malarias is well known. However, the usefulness of this species as a potential model to study cutaneous leishmaniasis was questionable since only one of 64 owl monkeys examined at GML for natural leishmanial infections was positive for *Leishmania braziliensis*.¹

MATERIALS AND METHODS

Experimental animals

Four *A. trivirgatus*, previously used in malaria studies, were selected for experimental inoculation; all were intact animals (not splenectomized or otherwise immunologically altered). Each animal was skin tested on a shaved portion of the lateral aspect of the thigh with 0.1 ml of the GML Tejada strain Montenegro antigen (1×10^6 promastigotes) prior to inoculation with *Leishmania*.

The development of an induration of 5 mm or greater at the site of injection after 48 hours was regarded as a positive skin test.

Leishmania strains

Two indigenous *Leishmania* species were used: (a) *L. braziliensis* LC 71 isolated from a human case of cutaneous leishmaniasis from Cerro Azul, Panama Province, and (b) *L. mexicana*, 1746, isolated from a rice rat, *Oryzomys capito*, from Sardi, San Blas, Panama.²⁻³

Two animals each were inoculated intradermally on the nose with 10×10^6 promastigotes of *L. braziliensis* and *L. mexicana*, respectively, harvested from Senekjic's medium⁴ (1st and 3rd in vitro subcultures, respectively). The animals were examined approximately every 2 weeks for signs of infection. Finely drawn glass needle aspirates of nasal tissue were taken at the time of each examination and cultured for growth of promastigotes. Montenegro skin tests were performed on days 0, 62, 76, and 139 post-inoculation.

All animals were killed at day 237 post-inoculation, and tissue biopsies were cultured from the nose, ears, feet, liver, spleen, and bone marrow.

RESULTS

All experimental animals were Montenegro skin test negative prior to inoculation with *Leishmania*. Promastigotes were isolated in culture from needle aspirates of the nose in three of the four animals at 20 days post-inoculation (Table 1). The cultures from the fourth animals were contami-

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TABLE 1

Results of skin tests and cultures following experimental inoculation of *Aotus trivirgatus* with *Leishmania* strains

| Animal no. | Species and strain | Montenegro skin test on post-inoculation day (diameter of induration*) | | | | Skin culture result on postinoculation day | | | | | | | | | | |
|------------|------------------------------|--|-------|--------|--------|--|----|----|----|----|----|-----|-----|-----|-----|-----|
| | | 0 | 62 | 76 | 139 | 20 | 35 | 48 | 62 | 76 | 90 | 104 | 125 | 139 | 168 | 207 |
| NM 8478 | <i>L. braziliensis</i> LC 71 | - | - | + (16) | + (7) | + | + | + | + | + | + | - | + | + | + | + |
| NM 8479 | <i>L. braziliensis</i> LC 71 | - | - | - | + (16) | + | + | + | C† | + | + | + | - | - | - | - |
| NM 8943 | <i>L. mexicana</i> 1746 | - | + (5) | NT‡ | + (16) | C | + | + | + | + | + | + | + | - | + | - |
| NM 8944 | <i>L. mexicana</i> 1746 | - | - | - | - | + | + | + | + | + | + | + | - | - | - | - |

* In millimeters.

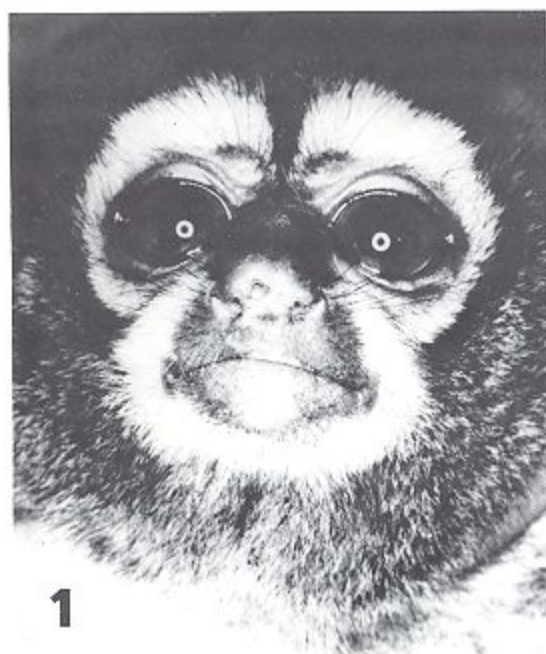
† C, cultures contaminated with fungus.

‡ NT, not tested.

nated with fungus. All animals were positive for *Leishmania* at day 35 post-inoculation and remained positive through day 104. The *Aotus* inoculated with *L. braziliensis*, NM 8478 (Figs. 1 and 2) and NM 8479, remained infected through 8.5 and 3.5 months, respectively. The animals inoculated with *L. mexicana*, NM 8943 and NM 8944, remained infected through 6 and 3.5 months, respectively. *L. braziliensis*-infected animals became skin test positive at day 76 and day 139. Only one of the *L. mexicana*-infected *Aotus* showed a positive skin test (NM 8943 at day 62). The other remained negative through day 139

when last skin tested. The positive skin tests were characterized by raised, slightly erythemic, indurated areas 5–16 mm in diameter (Table 1).

All animals manifested depigmentation and profuse bleeding on aspiration during their infections. Both monkeys infected with *L. braziliensis* also showed edema, erythema, encrustation and ulceration of the nose. Although NM 8943, infected with *L. mexicana*, also showed edema, erythema and encrustation, the severity of these manifestations was not as great as that of both *L. braziliensis*-infected animals. NM 8944, although positive by culture for *L. mexicana* through 104



FIGURES 1 and 2. Frontal and profile views of *Aotus trivirgatus* NM 8478 2 months after intradermal inoculation of *Leishmania braziliensis* on the nose.

days, appeared normal throughout the period except for slight depigmentation and excessive bleeding of the nose on aspiration. The infection of *L. braziliensis* in NM 8478 became cryptic at day 168, and although promastigotes were isolated on day 168 and day 237 the gross appearance of the nose was completely normal.

Culture from the ears, feet, liver, spleen, and bone marrow on day 237 were negative in all animals.

DISCUSSION AND CONCLUSION

This pilot study established that *A. trivirgatus* is susceptible to both *L. braziliensis* and *L. mexicana*.

Montenegro skin testing prior to experimental infection is important to such studies to insure the use of nonimmune animals. The test was negative in all four animals prior to inoculation, and became positive in both *L. braziliensis*-inoculated monkeys and one of two *L. mexicana*-inoculated monkeys after infection was established. The negative skin test in NM 8944 after *L. mexicana* was proven extant by positive culture may be due, in part, to the fact that this animal appeared to develop the least severe infection, which may have resulted in insufficient antigenic stimulation to activate those components of the immune system responsible for the Montenegro reaction.

Cebus apella (weeping capuchin) and *Macaca mulatta* (rhesus) have been used successfully as models in cross-immunity experiments involving *L. braziliensis* and *L. mexicana*.^{5,6}

The infections in all *Aotus* used in the present study persisted for a minimum of 3.5 months, which is a sufficient period for testing the efficacy of antileishmanial drugs in prophylactic or treatment trials.

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