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THE PRINCIPAL RESERVOIR HOST OF CUTANEOUS LEISHMANIASIS IN THE URBAN AREAS OF MANAUS, CENTRAL AMAZON OF BRAZIL

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*Twenty one opossums (*Didelphis marsupialis) *from disturbed primary forest in the vicinity of Manaus, and seven from an isolated island of secondary vegetation within the city of Manaus, were examined for the presence of* Leishmania. *Of the opossums from the primary forests, 13 (61.9%) were found to be positive for* Leishmania braziliensis guyanensis. *One additional opossum was found to be positive for* Le. mexicana amazonensis.

A simple and economical trapping technique for opossums is presented here, and a control method in special cases is suggested.

Recently Arias et al (1981) showed that the opossum *Didelphis marsupialis* was one of the wild animals in the Central Amazon region of Brazil that was to be considered an important natural reservoir of *Leishmania braziliensis guyanensis* in regions where man has entered and much disturbed the jungle. At the same time, Lainson et al (1981b) had isolated the same parasite from other sylvatic animals and had discussed the importance of edentates (sloths and anteaters) as probable natural reservoir hosts. Other work (Lainson et al, 1981a) confirmed the two-toed sloth *Choleopus didactylus* as the principal sylvatic natural reservoir. In a separate work analyzing sandfly bloodmeals, Christensen et al (in press) have found that the principal vector of *Le. b. guyanensis* in the Manaus region (*Lutzomyia umbratilis,* Arias & Freitas 1977, 1978) feeds primarily on animals in the family Bradypodidae, supporting the findings of Lainson et al (1981a, 1981b).

We plan to show here that *Didelphis marsupialis* is a principal natural reservoir host in special circumstances, possibly substituting the two-toed sloth, and propose a cycle of transmission for *Le. b. guyanensis* that encompasses two principal and distinct reservoir hosts.

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MATERIAL AND METHODS

Collection Sites

The three areas where we focused our collections were: a residential area of Manaus called "Parque das Laranjeiras" where much of the primary forest was left standing, the "Reserva Ducke" Forest Reserve as described in Arias et al (1981), and the INPA campus. The latter is a 10 acre patch of secondary growth with the laboratory buildings situated to one side. The campus is completely surrounded by residential areas with little or no vegetation (INPA campus thus represents an island of secondary vegetation within the city of Manaus).

Human cases of cutaneous leishmaniasis have frequently been reported from the first two areas and the sandly vector species has readily been captured there. Neither of these factors is true for the INPA campus.

Capturing of Opossums

We designed traps that would contribute to an economical and practical control of cutaneous leishmaniasis in selected areas where opossums have been found to be the natural reservoir hosts. The "traps" consisted of empty 55 gal. (200 liter) oil drums (Fig. 1) which had the tops cut out and the bottoms perforated to allow water to run out and avoid creating breeding sites for mosquitoes or drowning of captured animals. These oil drums were baited with generous quantities of leftover food scraps and aromatic tropical fruit (pineapples, bananas, cupuaçu, etc.) as well as other aromatic opossum "attractants" such as some palm oils and peanut butter. After the traps were baited, a board or large branch was placed as a ramp going up the side of the oil drum. The principle in use here is the following: the opossum goes up the ramp, jumps into the oil drum to get the food, and then is not able to climb up the steep smooth sides of the drum to get out again. We found the traps very effective for opossums; even though other animals have been taken in these areas, only opossums were taken in the oil drum traps.

Isolation of Stocks from Opossums

The methodology followed here was the same as previously used in Arias et al (1981). One of the most important factors we noted was that all of the visceral tissue triturates produced *Leishmania* in the hamsters, and that all of these opossums were positive for *Trypanosoma cruzi.* While studying the *Trypanosoma* stocks we noted what appeared to be *Leishmania* parasites mixed with the *Trypanosoma* parasites. Parasites from the cultures injected into some of the hamsters showed that we were dealing with mixed parasitemias and, if let run its course, culture would end up losing the *Leishmania* parasites to the more dominant *Trypanosoma* parasites. Pure *Leishmania* cultures were later obtained by re-isolating the parasites from the infected hamsters.

Isolates were identified biologically, on the basis of behaviour in hamsters and in culture. All of the isolates were further identified by Dr. M.A. Miles and Ms. M. Povoa of the Seção de Parasitologia, Instituto Evandro Chagas, Belém, Pará, Brazil by enzyme electrophoresis, using 5 enzymes (ASAT, ALAT, PGM, GPI and G6PD) (Miles et al, 1980) with modifications as given by Miles et al (1981).

RESULTS

In Table I we can see that 71.4% (5 of 7) opossums collected in the Ducke Forest Reserve were found positive for *Le. b. guyanensis*, which is comparable with the 20% (3 of 15) found by Arias et al (1981) in the same location. In the elite residential area of

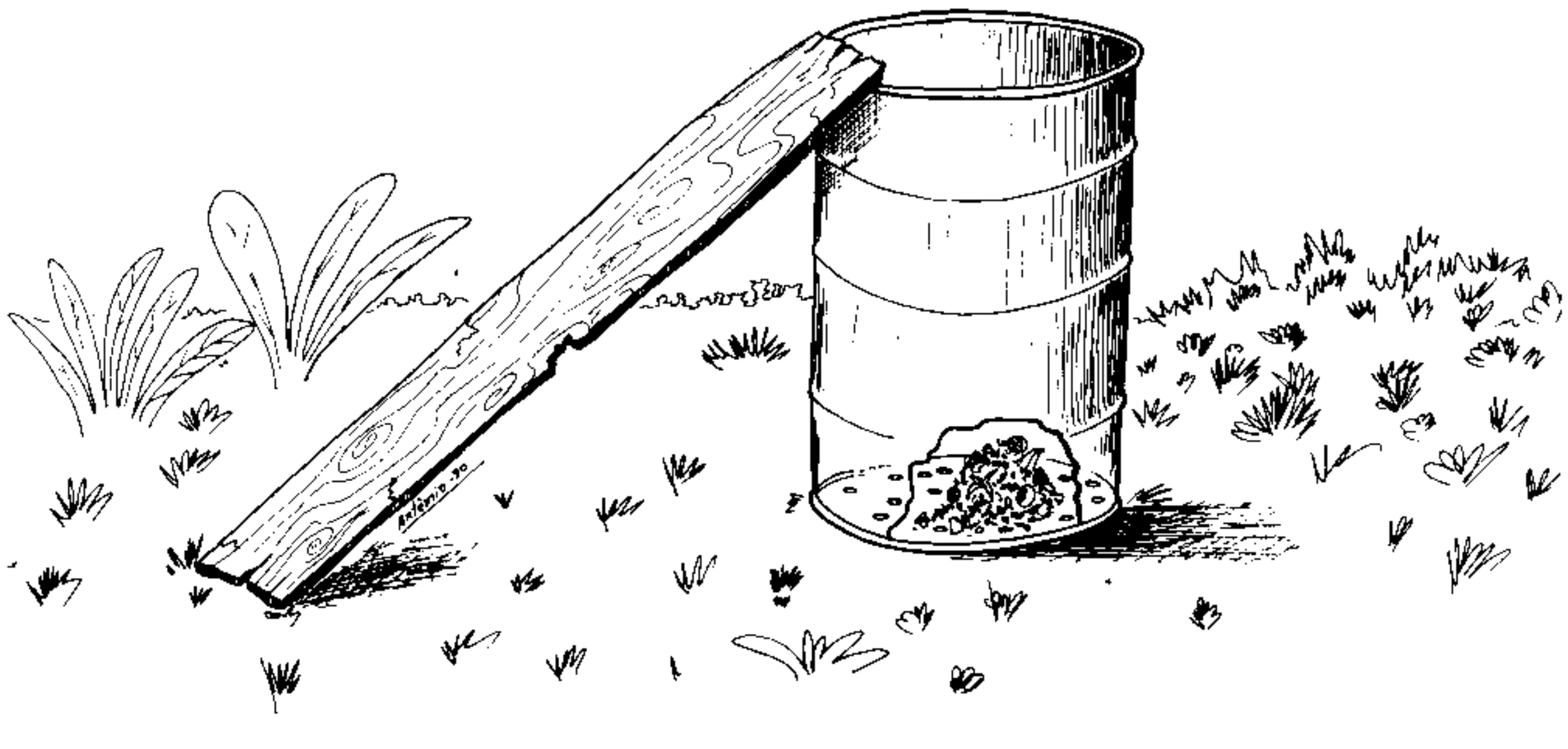

Fig. 1 – 55 gal. (200 liter) oil drum trap for opossums.

TABLE I
Source of *Leishmania braziliensis guyanensis* isolated from the common opossum *Didelphis marsupialis* in the Central Amazon Region of Brazil.

Locality	*Total Examined*	*No Positive*	*Nº Positive*
Ducke Reserve	7	5	71.4
Parque das Laranjeiras	14	8	57.1
Subtotal*	21	13	61.9
INPA Campus	7	0	0
Total	28	13	46.4

*Total of opossums from endemic areas of disturbed forest.

Parque das Laranjeiras, where several residents have contracted the disease and from where we have isolated stocks of *Le. b. guyanensis* from *Lu. umbratilis*, 57.1% (8 of 14) of the opossums were found positive for this *Leishmania*. *Leishmania* isolates were all from the viscera of the opossums, none from the skin triturates. We also isolated *Trypanosoma cruzi* from the viscera of all the positive opossums.

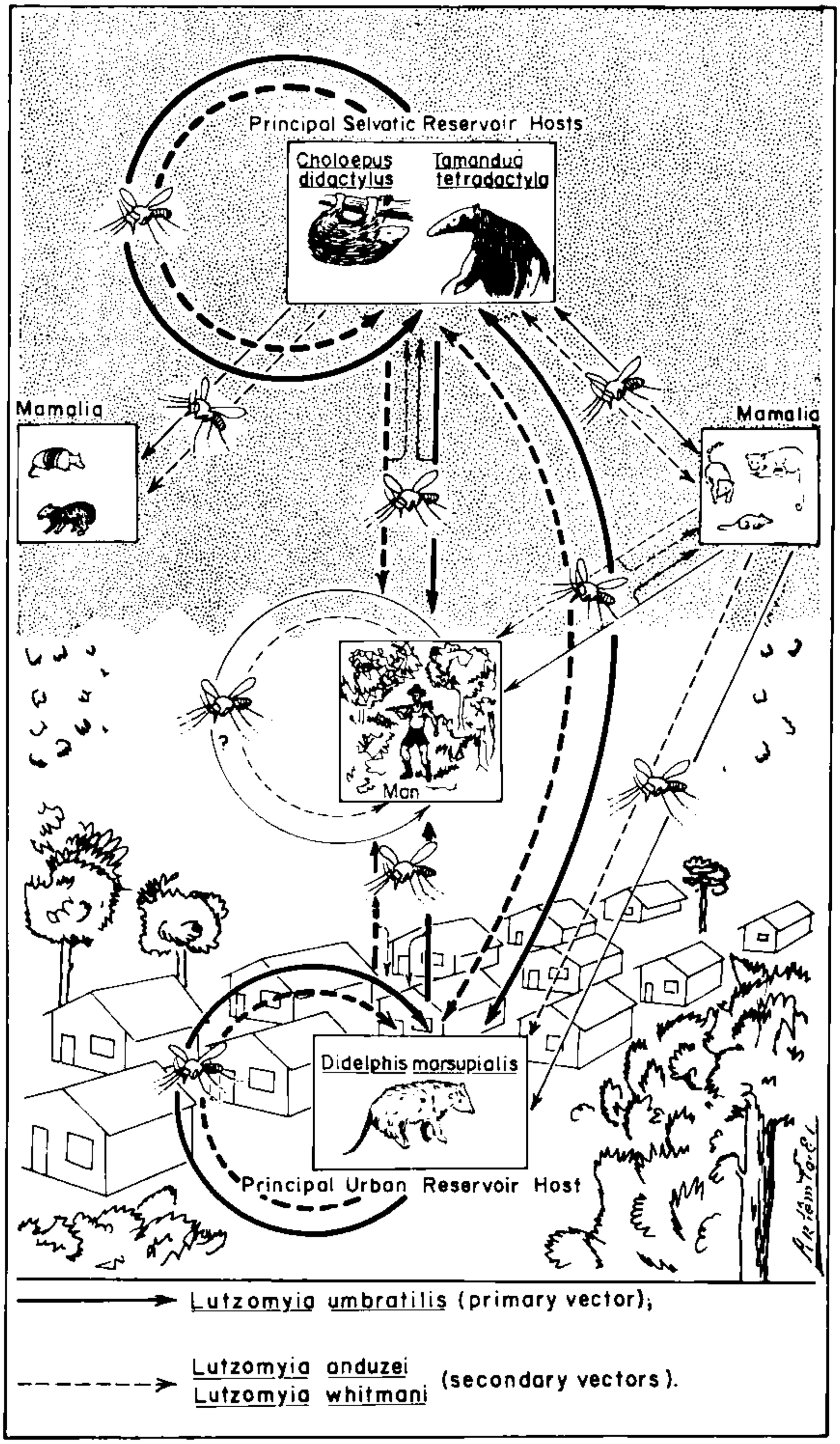

Fig. 2 – Transmission cycle for *Leishmania braziliensis guyanensis*.

In contrast, none of the seven opossums captured on the Institute grounds were found to be infected with *Leishmania;* 5 of these were positive for *Trypanosoma.* Of the 28 opossums captured, only one was infected with *Leishmania mexicana amazonensis*; this one individual, which we believe had a mixed *Le. b. guyanensis* and *Le. m. amazonensis* infection, was one collected from Parque das Laranjeiras and is considered as only being *Le. m. amazonensis* since we were not able to isolate the *guyanensis* parasite. (Both in culture media and in the hamster, the *Le. m. amazonensis* stock will be more competitive and mask or hide the *Le. b. guyanensis* stock).

DISCUSSION

The importance of the common opossum, *Didelphis marsupialis,* as a principal reservoir host of *Leishmania braziliensis guyanensis* in disturbed areas of tall forest was suggested by Arias et al (1981), and its importance is further emphasized here by the fact that 61.9% of 21 individuals that were captured over a period of 4 months were found positive for this parasite. These figures do not include the seven negative animals captured on the INPA campus grounds. These opossums from INPA are presumed to belong to a population restricted to the Institute grounds and, if this is the case, they should be expected to be negative since the principal vector of *Le. b. guyanensis* in the Manaus area, *Lutzomyia umbratilis* (Arias & Freitas, 1978), has not been collected at INPA in the past 7 years. We believe that these factors, together with other available information, suggests a more complex transmission cycle for *Le. b. guyanensis* than originally implied.

Considering the recent findings on *Leishmania braziliensis guyanensis,* we propose a cycle for the probable mode of transmission of "pian bois" on the Guyana Shield. Fig. 2 shows the transmission pathways, with always a sandfly being involved. The solid lines represent the transmission pathways of the principal vector and the dotted lines those of the secondary vectors. The thickness of each line suggests the degree of possibility of transmission; the thicker the line, the more the possibility of transmission. *Choloepus didactylus* of the family Bradypodidae is the primary natural reservoir host in the forest with *Tamandua tetradactyla* being another important natural reservoir host (Lainson et al, 1981b). There is probably a natural cycle of *Lu. umbratilis* and a secondary cycle with *Lu. anduzei* or *Lu. whitmani,* with the principal natural reservoir host in the "terra firme" forest. Mammals (left side) that become infected can be dead-end tangents while others (right side) may play minor roles in the propagation of the disease. Man plays the role of an important dead end, as he penetrates the forest, primarily when disrupting the natural zoonotic harmony of "pian bois". He may also provide a minor source of infection for the vector species. Man to man transmission via sandflies is possible, but not very probable.

As man enters and disrupts the forest, establishing residences (as occurs in certain localities of Manaus), a new transmission cycle is established. Many of the houses are constructed in close proximity of natural standing forests (Fig. 3). The construction of these houses, as well as the preliminary roads (Fig. 4) disrupt the natural habitat of many wild animals and attracts the immigration of others. Of the animals which enter this new "forest" system, *Didelphis marsupialis* is one of the most common. This scavenger roams through the garbage seeking scraps of food. The principal natural reservoir host moves to a more continuous forest nearby. The opossum probably supports a zoonosis with vector sandflies, and there probably exists a direct principal route of transmission between sloths and opossums as the latter goes back into the semidisturbed or undisturbed forest. The opossum to man path of transmission becomes more viable. Infected opossums infect the natural sandfly vector population, establishing different transmission pathways.

Arias et al (1981) initially only reported 3 isolates of *Le. b. guyanensis* from *D. marsupialis:* together with these 13 isolates the total number of isolates is 16 from a total of 36 opossums.

Fig. 3 – House constructed in close proximity of forest in Parque das Laranjeiras where *Leishmania* has been isolated from man, sandflies and opossums.

Of the 29 *D. marsupialis* examined here, we did not expect of find *Leishmania* in the 7 opossums collected in the secondary forest situation of the INPA campus, considering the fact that the sandfly population here is very low. If we don't include these in the final tabulation, we find that the incidence of infection in the opossums is 44.4% (16 of 36) in modified "terra firme" forests. The presence of the parasite only in the viscera suggests similar host-parasite relationships as do *Le. b. guyanensis* and *C. didactylus* in

Fig. 4 – Preliminary road which cuts the forest throughout the Parque das Laranjeiras residential area.

Pará state (Lainson et al, 1981). On the other hand, Herrer and Christensen (1980) isolated *Le b. panamensis* from 2.1% of *C. hoffmani* blood samples, 11.3% from skin culture samples,and 12.0% from viscera samples. The infective incidence of *C. didactylus* and *D. marsupialis* with *Le. b. suyanensis* is much higher than that of *C. hoffmani* with *Le. b. panamensis.*

Since the trapping method is economical, readily available, and effective for opossums, we feel that in specific areas, such as the residential area of Parque das Laranjeiras, the setting of these traps and the drastic reduction in population of the opossums will greatly reduce the incidence of the disease in recently colonized areas in a region where *Le. b. guyanensis* is endemic.

RESUMO

Vinte e uma mucuras (*Didelphis marsupialis*), procedentes de uma floresta primária explorada, e sete de uma vegetação secundária em uma ilha isolada, nas cercanias de Manaus, foram examinadas para detectar a presença de *Leishmania.*

Das mucuras da floresta primária, 13 (61.9%) foram detectadas positivas para *Leishmania braziliensis guyanensis.* Somente uma mucura foi dada como positiva para *Le. mexicana amazonensis.*

Apresentamos aqui uma técnica simples e econômica para captura de mucuras e é sugerido um método de controle, em casos especiais.

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REFERENCES

ÁRIAS, J.R. & FREITAS, R.A. de, 1977. On the vectors of Cutaneous Leishmaniasis in the Central Amazon of Brazil. I. Preliminary Findings. *Acta Amazonica, 7*(2) :293-294.

ÁRIAS, J.R. & FREITAS, R.A. de, 1978. Sobre os vetores da Leishmaniose cutânea na Amazônia Central do Brasil. 2. Incidência de flagelados em flebótomos selváticos, *Acta Amazonica, 8* :387-396.

ÁRIAS, J.R.; NAIFF, R.D.; MILES, M.A. & SOUZA, A.A., 1981. The opossum, *Didelphis marsupialis* (Marsupialia: Didelphidae), as a reservoir host of *Leishmania braziliensis guyanensis* in the Amazon Basin of Brazil. *Trans. Roy. Soc. Trop. Med. Hyg.* (in press).

CHRISTENSEN, H.A.; ÁRIAS, J.R.; VASQUEZ, A.M. & FREITAS, R.A. Hosts of sandly vectors of *Leishmania braziliensis guyanensis* in the Central Amazon of Brazil. (in press).

HERRER, A. & CHRISTENSEN, H.A., 1980. *Leishmania braziliensis* in the Panamanian two-toed sloth, *Choloepus hoffmanni. Am. Jour. Trop. Med. Hyg. 29*(6) :1196-1200.

LAINSON, R.; SHAW, J.J. & POVOA, M., 1981a. The importance of Edentates Sloths and anteaters as primary reservoirs of *Leishmania braziliensis guyanensis,* causative agent of "Pian-bois" in Northern Brazil. *Trans. Roy. Soc. Trop. Med. Hyg.* (in press).

LAINSON, R.; SHAW, J.J.; READY, P.D.; MILES, M.A. & POVOA, M., 1981b. *Leishmania braziliensis guyanensis,* causative agent of "pian-bois". *Trans. Roy. Soc. Trop. Med. Hyg.* (in press).

MILES, M.A.; LAINSON, R.; SHAW, J.J. & POVOA, M., 1981. Leishmaniasis in Brazil: XV. Biochemical distinction of *Leishmania mexicana amazonensis, Leishmania braziliensis braziliensis* and *Leishmania braziliensis guyanensis* – etiological agents of cutaneous Leishmaniasis in the Amazon Basin of Brazil. *Trans. Roy Soc. Trop. Med. Hyg.* (in press).

MILES, M.A.; POVOA, M.; SOUZA, A.A. de; LAINSON, R. & SHAW, J.J., 1980. Some methods of the enzymatic characterization of Latin American *Leishmania,* with particular reference to *Leishmania mexicana amazonensis* and subspecies of *Leishmania hertigi. Trans. Roy. Soc. Trop. Med. Hyg., 74* :243-252.