# LEISHMANIASIS CONTROL STRATEGIES

A CRITICAL EVALUATION OF IDRC-SUPPORTED RESEARCH

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# Leishmaniasis control strategies

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# Leishmaniasis control strategies: A critical evaluation of IDRC-supported research

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# Observations on the Ecology of Visceral Leishmaniasis in Jacobina, State of Bahia, Brazil (1982 to 1986)

**I.A. Sherlock[1] & J.C. Miranda[1]**

## Introduction

This is a report on the project entitled "Ecology of visceral leishmaniasis in Jacobina, Bahia", which received financial support from the International Development Research Centre, Canada (IDRC) with the agreement of the "Fundaçåo Oswaldo Cruz" and " Conselho Nacional de Desenvolvimento Científico e Tecnológico of Brazil". The project was carried over the years 1982 to 1986, with some interruptions due to administrative and bureaucratic problems.

The specific aims of the project were:

a) to compare the importance of sylvatic and domestic reservoir hosts in the transmission of visceral leishmaniasis in Jacobina, Bahia;

b) to obtain information on the behaviour and vectorial capacity of vectors in Jacobina;

c) to observe the importance of the human reservoir in the cycle of the transmission of visceral leishmaniasis in Jacobina.

## I. The Study Area

Jacobina is one of the oldest known and most important foci of visceral leishmaniasis in the American continent. It is a city located in the county of Jacobina in the interior of the State of Bahia, Brazil at 11°11'08" S latitude and 40°31' W Gr longitude, 465 m above sea level, in the zone of which is climatically prone to drought. The physiography of the area is composed of mountains and elevated plains. The Itapicuru river runs though the mountains and receives several tributaries, among them the "rio do Ouro", that passes through the city of Jacobina. The mean annual rainfall is 550 mm and the mean annual temperature is 24°C, ranging from the mininum 24°C to the maximum 31°C (12). About three decades ago the area of Jacobina was forested. Presently, primary vegetation of tall trees is rare due to indiscriminate deforestation. In those forests of the past lived several species of sylvatic animals, including mammals which are now restricted to a few specimens and belong to a small number of species.

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The soil is rich in minerals such as gold, saltpetre, silica, copper, amianthus, iron, manganese and uranium (12). Nowadays, these are exploited by multinational companies benefitting from the cheap manual labor of the inhabitants of northeast, who are attracted to the area looking for work. This is the principal reason, together with the drastic conditions of the climate, for the intense migration that exists in Jacobina. This fact has a deep influence on the dispersion and distribution of the visceral leishmaniasis (9,27).

Granitic and calcareous rocks are very abundant, often forming caves that are common in the area of the geographical distribution of the Lutzomyia longipalpis, the principal vector of the American visceral leishmaniasis (9,27).

In 1984, the estimated population of the municipality of Jacobina was 50,000 inhabitants and 83% of them lived in the rural area. Ethnically the population was composed principally of mestizos. Badaro (5) provides a bibliographic review of the clinical and epidemiological aspects of visceral leishmaniasis in Jacobina.

According to the first case registration by Sherlock (27) in 1959, the incidence of visceral leishmaniasis does not show any difference in relation to the sex or race of the patients. However, there were pronounced differences depending on age. Children from 0 to 5 years old were attacked more than adults. This fact has been corraborated with findings in other endemic foci in the northwest of Brazil.

The per capita income of inhabitants of the peripheral sites of Jacobina is very low. As the city grows, the poor population is being pushed far from the periphery once again.

The most common type of dwelling is one that is built with stones or bricks. These buildings can be considered of good standard when compared with those in other endemic areas, where they are predominately made of mud bricks or sticks and mud. Probably, for this reason, Jacobina is pratically free of the proliferation of the insect vectors of Chagas' disease. Consequently, there are not any autocthonous human cases of Chagas' disease in Jacobina. It is, however, necessary to emphasize that type of housing does not have any importance in the epidemiology of visceral leishmaniasis (27, 29, 35).

The city of Jacobina was arbitrarily divided into 7 sub-areas, with different incidence rates of human cases, based on the work of Teixeira (40). The sub-area "Grotinha" was selected for our observations because human cases of visceral leishmaniasis have been occurring there in the last three years. Our 1982 census in "Grotinha" showed 323 dwellings where 1,128 inhabitants lived.

It is important to emphasize that the population of Jacobina is constantly increasing with individuals that emigrate from other areas, including those endemic areas of visceral leishmaniasis. Some individuals also emigrate from Jacobina to other states, principally to São Paulo. Thus, from time to time, the specialized bibliography mentions human cases of visceral leishmaniasis in São Paulo.

The name "Grotinha" means little valley. There is a disorderly row of houses along a tortuous way between two mountains. At the end of this valley, there is still some vegetation, composed of shrubs and small trees of secondary formation. The consequences of years of uncontrolled deforestation, which remains very intensive even now, is that, continuous erosion exists in the hills around the city. Siliceous and calcareous stones roll from the hills and drop near houses. These stones are useful in building houses. They also represent an most important economic source for many of the inhabitants, because they break the stones and sell them to other areas. Both adults and children do this work until late, outside houses, increasing their contact with the peridomestic vector.

During the four years of our observations on visceral leishmaniasis, there was active transmission of the disease, although in the first two years, few human cases were detected. In the two subsequent years an epidemic was verified when more than 50 cases were registered. According to Badaro (4) the annual global incidence (1980 to 1984) was 4.3/1000 children, but in Grotinha the incidence was 13.7/1000 children.

Analysis of risk factors for the development of the disease, according to Badaro (4), revealed that the age 0 to 5 years as the principal factor, followed by bad nutritional status. Thus, 75% of the malnourished children, when infected developed severe symptoms. The serological rate for positive antibodies, predominates in children above 5 years of age. Another important observation of Badaro was the confirmation of seropositive children with visceromegalia but without the manifest symptomatology of the disease. There exists a greater number of asymptomatic seropositive children than those with typical clinical disease.

The general epidemiological conditions of visceral leishmaniasis in Grotinha are shown in the Table I. As one can see from the table the area is characterized by a large number of infected dogs, a high rate of people with visceral leishmaniasis, and a high rate of sandfly infested houses.

**TABLE I**
**NUMBER OF HOUSES WITH PATIENTS EXAMINED, SICK DOGS AND SANDFLIES IN GROTINHA, A SUBURB OF THE CITY OF JACOBINA, BAHIA (1982 to 1984), ENDEMIC AREA FOR VISCERAL LEISHMANIASIS**

| Houses | Number | Percentage |
|---|---|---|
| Total Inhabitants | 1137 | 100 |
| Number of houses | 323 | 100 |
| Houses surveyed for sandflies | 299 | 92 |
| Houses infested with sandflies | 63 | 21 |
| Houses with dogs examined | 62 | 19 |
| Houses with infected dogs | 7 | 11 |
| Houses with Kalazar patients | 26 | 8 |

## II. Etiologic Agent

*Methods* - The specific leishmanias identification was made by zymodeme analysis based on its characteristic reactivity with species specific monoclonal antibodies using either an indirect radioimmune binding assay or immunofluorescence and immunoperoxidase technique (30). The behaviour of the leishmanias was observed in cultures, in the digestive tract of L. longipalpis and in the challenge of hamsters by intraperitoneal and intracutaneous inoculation of leishmanias. Histopathologic, immunological, biochemical and molecular biology techniques are described by Sadigursky (23) and Grimaldi (30) who were responsible for the realization of such techniques for this work.

For the diagnosis of the infected hosts and the isolation of the parasites, besides IFT we have employed other techniques such as culture of leishmanias in several media (NNN, LIT, RPMI, BHI), Giemsa stained smears of organs, inoculation of macerates of organs into hamsters and posterior confirmation of the hamster infection six months later end histopathologic study of organs. Xenodiagnosis were made with laboratory bred L. longipalpis. Human cases were diagnosed by Dr. Badaro's clinical staff in Jacobina, by means of the clinical symptomatology and isolation of leishmanias from bone marrows and spleens.

*Results* - The following species of trypanosomatids were found in Jacobina: Leishmania chagasi (8) infecting man, dog and opossum; Leishmania amazonensis Lainson & Shaw, 1972 infecting man and opossum; Leishmania guyanensis Vianna, 1911 (sub sp.) infecting opossum; bodies like leishmania amastigotes, that could not be specifically identified, were seen in the aguti Dasyprocta aguti and in the sylvatic rodents Cercomys cunicularius and Oryzomys eliurus, and in a domestic cat. Trypanosomatids other than leishmania were also found: Trypanosoma cruzi in the opossum Didelphis albiventris, and an epimastigote not specifically idenfified and not isolated, that we think to be Blastocrithidia, in three smears from the ear of a dog from Grotinha.

Leishmania chagasi was the causative organism of visceral leishmaniasis in man. But we have to emphasize that recently Barral et al. (6) have also been isolating only Leishmania amazonensis from the bone marrow of patients with the typical symptomatology of visceral leishmaniasis in Jacobina (5, 6). So, besides L. chagasi, this other species is also part of the ecology of american visceral leishmaniasis, at least in Jacobina. We tend to admit that the viscerotropic Leishmania amazonensis is a rather new species of leishmania pertaining to Jacobina.

The species of leishmania were maintained in hamsters and in several culture media, as mentioned before. L. chagasi was reisolated 89 times from the experimentally infected hamsters. The best culture medias were LIT and NNN. L. guyanensis (like) was reisolated 13 times and its best culture medium was BHI. L. amazonensis was reisolated 71 times and its best culture medium was NNN.

Direct isolation of L. chagasi from sylvatic hosts was not feasible. Prior inoculation of hamsters with macerates of spleens of the suspected animals is necessary to confirm and isolate the infection of the sylvatic host six months later. It seems that there is a specific dominance of the leishmania species that eliminate the weaker species from the culture media as follows: L. amazonensis domines, L. chagasi domines, L. guyanensis.

It was also possible to isolate L. chagasi through the xenodiagnosis with L. longipalpis from man, dog, oppossum and hamster (Table V-VIII).

The behaviour of some species of leishmanias in the tract of L. longipalpis will be dealt with in detail in the section of this paper on infection of the vector.

**III. Vector**

*Methods* - Sandflies were caught in the morning, afternoon and at night inside houses, in chicken pens, pig pens, corrals, natural caves, tree trunks, etc., by means of direct localization with hand lights and suction with Castro's collector apparatus, and with a Falcao electric trap (34).

During 24 consecutive months the light traps were set at 6 p.m. and collected at 6 a.m. the following day, in a cave located near houses and where cocks were mantained as live bait. Inside the houses from 7 to 8 p.m., sandfilies were collected off human baits. The collections were made for two days of every month during the years 1982 to 1984. Some of the collected sandflies were dissected and examined for flagellates and some of the engorged females were mantained alive in the laboratory for oviposition and maintaining of a laboratory colony of L. longipalpis. All the 49,048 sandflies collected were specifically identified.

Samples of several materials, suspected as possible breeding places of sandfilies, were observed according to techniques described before (26). The methods for mantaining sandiflies in the laboratory, for xenodiagnosis, feeding and infecting sandflies through membranes, were based on Sherlock (37) and Ward, Lainson & Shaw (43).

*Results* - Of the total 49,048 collected sandflies, 45,419 were L. longipalpis (Lutz & Neiva, 1912) (92%). Other collected species were Lutzomyia lenti Mangabeira, 1942, Lutzomyia evandroi (Costa Lima & Antunes, 1936), Lutzomyia trinidadensis (Newstead, 1922) and Lutzomyia oswaldoi (Mangabeira, 1942) (Table II).

**TABLE II**
**SANDFLIES COLLECTED AROUND HOUSES AND CAVES IN JACOBINA, BAHIA (1982 - 1986)**

| SPECIES | MALE | FEMALE | TOTAL |
|---|---|---|---|
| L. longipalpis | 29,365 | 16,054 | 45,419(92%) |
| L. lenti | 926 | 439 | 1,365 |
| L. evandroi | 1,101 | 652 | 1,753 |
| L. trinidadensis | 16 | 16 | 32 |
| L. oswaldoi | 2 | 5 | 7 |
| Lutzomyia sp | 254 | 218 | 472 |
| TOTAL | 31,664 | 17,384 | 49,048 |

In spite of the difficulties of obtaining successful transmission of L. chagasi by the bite of L. longipalpis this species is considered the principal vector of the parasite in the american continent (1,9,27). The basis for this is: coincidence of the geographical distribution of human and canine visceral leishmaniasis with one of these species; its natural and experimental infection with L. chagasi; its pronounced antropophilic habits, feeding frequently on man.

The formal proof of the transmission of L. chagasi by L. longipalpis, in spite of our persistent attempts was not acquired (35). In contrast, it was possible to transmit L. amazonensis by means of L. longipalpis bite (Table III and IV).

**TABLE III**
**ATTEMPTS TO TRANSMIT LEISHMANIAS TO HAMSTERS THROUGH BITES AND MACERATES OF L. LONGIPALPIS**

| TYPE OF INOCULATION | INFECTING LEISHMANIAS | HAMSTERS No. Used | No. Examined | No. of Positives |
|---|---|---|---|---|
| Bites of Sandflies | L. chagasi | 22 | 19 | 0 |
| | L. amazonensis | 9 | 9 | 6 |
| Macerates of infected sandflies | L. chagasi | 19 | 16 | 0 |
| | L. amazonensis | 2 | 2 | 1 |

**TABLE IV**
**ATTEMPTS OF TRANSMISSION OF LEISHMANIA CHAGASI (FROM JACOBINA) TO HAMSTERS, BY THE BITE OF LUTZOMYIA LONGIPALPIS, OR INOCULATION OF MACERATES OF THESE INFECTED SANDFLIES, WITH NEGATIVE RESULTS**

| TYPES OF INOCULATION HAMSTERS | No OF EXAMINE\NEGATIVES |
|---|---|
| Bites of the suspected sandflies collected in Jacobina. | 69 |
| Macerates of naturally infected sandflies from Jacobina. | 6 |
| Bites of experimentally sandflies in amastigotes infected organs through membranes. | 5 |
| Macerates experimentally infected sandflies in infected organs mascerated, through membranes. | 6 |
| Bites on infected sadflies in dogs from Jacobina. | 9 |
| Macerates of infected sandflies in hamsters. | 12 |
| TOTAL | 107 |

In Jacobina we found only the type of L. longipalpis with two spots (on the 3th and 4th tergites), but in a locality near Jacobina, just one spot L. longipalpis (on 3th segment) was the only species found. In this other locality, visceral leishmaniasis ocurrs sporadically. Ward et al. (44) suggested that L. longipalpis with two spots is probably the vector of visceral leishmaniasis in endemic areas, while L. longipalpis with just one spot is responsible for sporadic transmission. The two types of L. longipalpis have different pheromone composition in their spots, according to those authors (43). Once more, the population of L. longipalpis with two spots in Jacobina, is composed of two distinct types of individuals that can be distinguished by the naked eye: one big, dark and hairy and the other small, clear and hairless. The differences are possibly due to the ages of the individuals. L. longipalpis was found naturally infected with promastigotes, epimastigotes and tripomastigotes of non-specifically identified trypanosomatids in

Jacobina. It was also found naturally infected with ascocistes and microfilaria still not identified specifically.

There is a seasonal fluctuation in the density of L. longipalpis. This vector is more abundant during the colder and drier months and also in the hotter and rainier periods. The seasonal fluctuation correlates with the forthcoming of new human patients and with the increasing peridomestic frequency of the opossum Didelphis albiventris. The hourly activity of L. longipalpis begins at dusk and reaches its highest peak from 9 to 11 p.m. After 11 p.m. the number of sandflies decreases until the sandflies totally disappear at 6 a.m.

Two other species of sandflies are very much antropophilic, usually biting man, but preferring to feed on dogs; these species are L. lenti and L. evandroi. The first species was found naturally infected with promastigotes. Since they are usually found together with L. longipalpis in several focus of visceral leishmaniasis, there is some suspicion of their involvement in the transmission chain of american kalazar (25).

The natural breeding places of sandflies were not found in Jacobina. In the laboratory L. longipalpis is easily bred (37). During the present project some observations were done on its biology in the laboratory. We emphasized the development of a second batch of eggs from which adults were obtained. Engorged females can survive for more than 14 days. Species of Acari still not specifically identified and two species of fungi: Paecilomyces heliothis (Charles) and Cunninghamella elegans Lender (1907), destroy the laboratory colonies of L. longipalpis.

Lutzomyia longipalpis was easily infected with the following species of leishmanias: L. chagasi (human strain from Jacobina, Bahia and Imperatriz Maranhao; dog strain from Jacobina and Conde, Bahia; opossum strain from Jacobina, Bahia); L. amazonensis (opossum strain from Jacobina). It was not infected with human strain of L. braziliensis (Table X). L. longipalpis was also infected with L. donovani from India. Several species of animals served as blood source for the infection of L. longipalpis with L. chagasi such as man, dog, opossum and hamster. Experimentally infected mice infected well with L. longipalpis and with L. amazonensis. The behaviour of the promastigotes in the gut of L. longipalpis was that of characteristic suprapylarian position with L. chagasi and L. amazonensis, but this last species sometimes showed peripylarian position.

It was possible also to infect L. longipalpis with T. cruzi feeding on infected guinea pig. However, this trypanosome did not develope well in the gut of the sandfly (32).

Looking for the possibility of other existing vectors, we examined 136 specimens of Rhipcephallus sanguineus, 26 specimens of Ctenocephalides sp and 36 specimens of Stomoxys calcitrans collected feeding on five infected dogs in Jacobina. They were not found to be infected with leishmania.

**TABLE V**
**XENODIAGNOSIS WITH L. LONGIPALPIS IN EXPERIMENTALLY INFECTED HAMESTERS WITH DIFFERENT STRAINS OF L. CHAGASI**

| TYPES OF LEISHMANIA | No. XENOS REALIZED | +ve XENOS | No. SANDFLIES EXAMINED | +ve SANDFLIES |
|---|---|---|---|---|
| L. donovani (India) | 05 | 01 | 82 | 10 |
| L. chagasi (Dog) (Jac.\BA) | 05 | 01 | 85 | 08 |
| L. chagasi (Man) (Imp.\MA) | 03 | 01 | 92 | 01 |
| L. chagasi (Man) Risea (Jac.\BA) | 01 | 0 | 20 | 0 |
| L. brasiliensis (man\Bahia) | 01 | 0 | 28 | 0 |
| TOTAL | 15 | 03 | 307 | 29 |

**IV. Reservoirs**

**Domestic reservoirs**

*Methods* - In Grotinha, only dogs and cats were examined because other domestic mammals like pigs, donkeys, horses, cows and goats were very rare. It was a pity because equines have been found to be infected with leishmanias in some foci of cutaneous leishmaniasis in Brazil (14).

In our 1982 survey we found 245 dwellings and 83 houses where there were domestic animals. Among these, 78 were dogs and 62 cats. The rest were represented by a few goats, 3 donkeys, 9 pigs and some chickens. Only dogs and cats were submitted to biopsies of ears for smears and the obtainment of drops of blood on filter papers for immunofluorescent testing (IFT) with L. chagasi antigen. The dogs were gagged, the ears cleaned and the point cut, in accordance with alrealy described techniques (1,9,27). We proceeded in the same manner with the cats but it was necessary to contain them in canvas sacks.

All positive animals were brought to the laboratory in Salvador for a more detailed examination. This included the realization of xenodiagnosis with triatomines bugs and sandflies and submission to necropsy for histopathologic examination and culture of leishmanias from several organs, and inoculation into hamsters.

*Results* - **Cats** - Fifty three cats were examined with smears and IFT. In the skin of one cat, we found just one typical amastigote. This cat could not be re-examined and for this reason the specific identification of the amastigote was not made. All IFT were negative for leishmania.

As in other foci of Brazil it is possible that cats do not have any importance as a reservoir of L. chagasi. Cats almost never attract L. longipalpis and until now only one cat has been found naturally infected with a non specified leishmania. In an important focus of visceral leishmaniasis in the northeast of Brazil, Deane (9) examined 142 cats and all were negative for leishmania (Table VI).

**TABLE VI**
**CATS EXAMINED FOR LEISHMANIASIS IN JACOBINA, BAHIA, THROUGH SEROLOGICAL TEST AND STAINED EAR SMEAR EXAMINATION**

| TECHNIQUES | N0. EXAMINED | POSITIVES | %POSITIVES |
|---|---|---|---|
| Serology (IFT) | 53 | 0 | 0 |
| Ears smears | 53 | 1* | 1.9 |

* Only one typical amastigote was found in an ear smear from a healthy cat; it was not possible to re-isolate and definitely identify the flagelate.

**Dogs** - Two sero-parasitologic surveys were made with dogs in Grotinha. In 1982, 23.9% of the dogs were seropositive while at the same time a small number of human cases were ocurring. In 1984 the seropositivity rate was 47.5% among dogs, and at the same time a human epidemic ocurred (Table VII).

**TABLE VII**
**DOGS POSITIVE FOR LEISHMANIASIS IN JACOBINA, BAHIA IN THE YEARS 1982 AND 1984, THROUGH SEROLOGICAL TESTS AND STAINED SMEARS.**

| | DOGS EXAMINED* | | | | | |
|---|---|---|---|---|---|---|
| YEAR | SEROLOGYCAL TEST (IFT) | | | SMEARS | | |
| | EXAMINED | POSITIVE | % | EXAMINED | POSITIVE | % |
| 1982 | 46 | 11 | 23.9 | 46 | 01 | 2.0 |
| 1984 | 40 | 19 | 47.5 | 42 | 05 | 12 |

* Those positive in smears were killed soon after the results.

Confirming anterior observations in Jacobina (27,28,29) and in other foci of Brazil (1-9), dogs also represent a better source for the infection of L. longipalpis than man does. In our experiments 78% of dogs infected 29% of the sandflies (Table VIII). Man infected only 15% of the sandflies in 33% of the attempts.

In edemic areas, because of their higher prevalence of infection and their infectivity for the vector, dogs are the most important domestic reservoir during epidemics, in spite of their being victims of the pathogenic action of the L. chagasi (1,9,28).

**Man** - The results obtained regarding the capacity of man to attract and infect the vector L. longipalpis were similar to those obtained by Deane (9) with patients of other foci. In our results 15% of the sandflies were infected by the six patients with visceral leishmaniasis who were submitted to xenodiagnosis with L. longipalpis in 2 of 6 attempts (33%) (Table VIII).

**TABLE VIII**
**COMPARATIVE RESULTS OF THE CAPACITY OF LUTZOMYIA LONGIPALPIS TO INFECT MAN, DOG AND OPOSSUM INFECTED WITH LEISHMANIA CHAGASI FROM JACOBINA-BAHIA**

| Host Type | Number | XENOS Realized | Positive | % | Examined | SANDFLIES Infected | % |
|---|---|---|---|---|---|---|---|
| Man | 2 | 6 | 2 | 33 | 201 | 32 | 15 |
| Dog | 8 | 20 | 13 | 78 | 368 | 107 | 29 |
| Opossum | 1 | 8 | 2 | 25 | 193 | 27 | 14 |

In the endemic area, the role of man as a reservoir is small, since man's capacity to infect the vector is poor. During epidemics, because of the increase in new cases and the higher density of L. longipalpis, the importance of the role of man as a reservoir, also increases due the existence of more opportunities for the infection of the vector. This fact was suggested by the greater occurrence of new cases after the peak of the density of L. longipalpis during its seasonal fluctuation.

**Domestic rodents** - The domestic rodents: Rattus rattus, Rattus novergicus and Mus musculus were not studied in significant numbers in Jacobina, in spite of the former findings of the L. infamtum infection in Rattus rattus from the Mediterranean (10-22). Also, years ago we speculated about their possible involvment in the chain of transmission of visceral leishmaniasis in Jacobina (29). Only 20 specimens of Rattus rattus were examined and none were found to be infected with leishmanias. The same happened with 42 specimens of Mus musculus.

**Sylvatic reservoirs**

*Methods* - Over four years, for a week every month, observations were made in Jacobina on sylvatic reservoirs. Forty wire traps for small animals and three for big ones, were set in strategic places to catch mammals, in the remaining forests of Grotinha. They were set in the morning and evening and collected the following day and the baits were changed. Fruit, cheese, corn, peanuts, etc, were used as bait. Pineapple was the best bait to catch opossums and pumpkin seeds were the best to catch wild rodents.

Besides the collected specimens of mammals, we acquired from local hunters specimens of foxes, agutis and armadillos caught around the city of Jacobina, that were studied here.

The species captured in Jacobina are very common in the State of Bahia. Thus, specific identification was made by the senior author, based on comparison of the taxidermized laboratory samples. These were previously identified by experts of the National Museum of Brazil. The specific identification of foxes as Lycalopex vetulus was made because they looked identical to those studied by Deane in the State of Ceará. In 1954 the senior author had the opportunity to work with Deane during the investigation of the visceral leishmaniasis in that focus (9).

The species Cerdocyon thous (= Dusycion thous?) studied by Lainson (13) in the State of Pará, Brazil, look bigger and darker than the specimens we have studied here.

All animals were submitted to xenodiagnosis with triatomines bugs and L. longipalpis and were examined by means of: fragment cultures of their organs in several media (NNN, LIT, RPMI, BHI); inoculation into hamsters of macerates of their spleens; stained smears of their spleen, liver and skin; hystopathologic examination of fragments of several organs and sometimes IFT of their sera.

*Results* - **The sylvatic fauna** - In Grotinha 3,353 traps were set during the years 1982 to 1984 in which only 166 (5%) caught 178 mammals specimens. The results do not mean, that the traps were inefficient but rather they showed that there was a very poor fauna with few species and specimens exists in the periphery of Jacobina. Only 11 mammal species were obtained, included two species of domestic rodents (Table IX).

**TABLE IX**
**MAMMALS CAPTURED AROUND THE CITY OF JACOBINA, BAHIA (1982-1986), FOR RESEARCH ON LEISHMANIAS**

| SPECIES OF MAMMALS | N° CAPTURED | % TOTAL |
|---|---|---|
| Dasyprot. aguti | 8 | 3.8 |
| Didelphis albiventris | 94 | 44.1 |
| Cabassous unicinctus | 6 | 2.8 |
| Cavia porcellus | 7 | 3.2 |
| Lycalopex vetulus | 14 | 6.6 |
| Cercomys cunicularius inermis | 11 | 5.2 |
| Mus musculus | 42 | 19.7 |
| Rattus rattus alexandrinus | 20 | 9.4 |
| Chiroptera | 1 | 0.5 |
| Oryzomis subflavus | 1 | 0.5 |
| Oryzomis eliurus | 9 | 4.2 |

The predominant species of mammal was the marsupial opossum Didephis albiventris Lund, 1841, that reached 44% of the total. Following this were rodents with a frequency of 39%. However, sylvatic rodents were less frequent than domestic ones. Only 14 specimens of foxes were obtained with much difficulty even though attractive financial offers were made to buy foxes.

**Infected animals** - A total of 213 specimens were studied. Among them 193 underwent all types of examination mentioned above. Besides the specimens infected with leishmanias that will be referred to later, bodies like leishmania promastigotes were seen in the smears of liver and spleens of four specimens of Dasyprocta aguti and in smears of spleen of a Cercomys cunicularius and a Oryzomys eliurus. Unfortunately they could not be specifically identified.

Among 94 specimens of D. albiventris that were studied we obtained results from 84, of which four were infected with three leishmania species and three with Trypanosoma cruzi (Table X). The species of leishmanias and the number of oposums were: two with Leishmania chagasi, one with Leishmania amazonensis and one with Leishmania guyanensis, sub-species.

**TABLE X**
**WILD AND COMMENSAL ANIMALS OBTAINED FROM 1982 TO 1986 IN JACOBINA, BAHIA FOUND NATURALLY INFECTED WITH TRYPANOSOMATIDS**

| MAMMAL SPECIES | Examined | INFECTED WITH L.chagasi | L.amazonensis | L.braziliensis sp | Bodie amastigotes | T.cruzi |
|---|---|---|---|---|---|---|
| D. albiventris | 84 | 2 | 1 | 1 | - | 3 |
| Das. aguti | 7 | - | - | - | 4 | - |
| C. porcellus | 6 | - | - | - | - | - |
| C. cunicularius | 12 | - | - | - | 1 | - |
| O. eliurus | 8 | - | - | - | 1 | - |
| O. subflavus | 1 | - | - | - | - | - |
| R. rattus | 19 | - | - | - | - | - |
| M. musculus | 35 | - | - | - | - | - |
| Chiroptera | 1 | - | - | - | - | - |
| C. unicinctus | 6 | - | - | - | - | - |
| L. vetulus | 14 | - | - | - | - | - |
| TOTAL | 193 | 2 | 1 | 1 | 6 | 3 |

Human and canine infection with T. cruzi, that are not authocthonous in Jacobina, are rare, problably because triatomine bugs do not exist in the city.

Although D. alviventris is found infected with L. amazonensis and L. braziliensis, cutaneous leishmaniasis is not usually found in the city. However, we have to emphasize that L. amazonensis has been found in several patients with visceral leishmaniasis by Barral et al. (6), as mentioned before. Lutzomyia flaviscutellata, the principal vector of L. amazonensis was not registered in Jacobina. But other species belonging to its same sub-genus Nyssomyia, the Lutzomyia whitmani that is a vector of L. braziliensis, were registered in Jacobina (9,27) and can probably also transmit L. amazonensis.

Didelphis albiventris is the first non canid mammal shown to be naturally infected with L. chagasi (30-33-36) and was also challenged in the laboratory with the same leishmania sp. The histopathologic examination of the specimens naturally infected showed proliferation of macrophages cells but did not reveal parasites or typical evolutive lesions of visceral leishmaniasis. Also, the hystopathologic examination of the experimentally infected specimen, with the same opossum strain of L. chagasi, only revealed amastigotes in the spleen and liver and grannulome formation as in the involutive forms of visceral leishmaniasis (23,36).

D. albiventris was captured throughout the year but during the colder months June, July and August the number of specimens collected was higher. This possible variation in the oposum population correlates with the higher density of L. longipalpis and the forthcoming of new human cases of visceral leishmaniasis in Jacobina (33). It is in the colder and rainy months that the opossum frequents human dwellings more often, looking for food. Besides leftovers from human meals, the opossum also eat chickens, eggs and fruit (30).

D. albiventris is the most frequent wild mammal to be found infected with L. chagasi around houses in Jacobina. In two houses close to one where two infected opossums were captured, human and canine cases of visceral leishmaniasis were encoutered. Lutzomyia longipalpis feeds readily on D. albiventris as we verified in traps with opossums used as bait in Jacobina, and in the laboratory where the challenged animals infected the sandfly (30).

D. albiventris is an american mammal and does not strongly suffer from infection with L. chagasi. It seems to indicate the existence of an evolved parasite host association that permits this marsupial to be one of the primary natural reservoirs of the parasite of american visceral leishmaniasis.

However, in spite of some evidence, one can not yet eliminate the hypothesis that oposum in its approximation to man, has been infected with leishmanias by the same vector that is infecting man, dogs and other reservoirs.

**Foxes** - Lycalopex vetulus can be seen, during the night, crossing roads and are sometimes killed by cars. It is not easy to catch foxes and for this reason on 14 specimens bought from local hunters were examined. All the specimens were apparently healthy and were not positive for leishmanias. It is impossible to make conclusions about the role of these canids in the epidemiology of visceral leishmaniasis in Jacobina. More studies must be carried out on the foxes.

**V. Epidemic Periodicity of American Visceral Leishmaniasis**

Based on (i) observations in Jacobina, where the senior author has been working since 1959 (27,28,29,31), (ii) a bibliographic review of the registration of human cases of visceral leishmaniasis in Brazil (2,3,7,8,15,18,19,21,39,40,42) and (iii) on data of Ministery of Health in Brazil (38), it was possible to conclude that visceral leishmaniasis has been occurring endemically with cyclic decenal epidemics. Every epidemic lasts for three years, after which the number of cases begins to decline until they reach the endemic condition. Generally, the peaks of the epidemics have taken place in years ending with the number three and years before and after the principal year, as explained as follows. Beginning from 1913, when Migone (15) described the first human case on the american continent, the highest number of cases were reported in the scientific literature, principally in the years 1923, 1933, 1943, 1953, 1963, 1973 and 1983 (Table XII). The next epidemic is expected to occur in the years 1992, 1993 and 1994 (31).

It is not yet possible to know the real causes of the periodic epidemic cycle. It seems possible that there exists some correlation between the immunological and nutritional status of individuals between 0-5 years of age that are exposed to the disease. We know that malnourished children from the ages 0-5 years old are more frequently suffer visceral leishmaniasis in Brazil (4,5). The serological positive rates of infection predominates in children above 5 years old as noted by Badaro (4,5). Climatic factors and ecological unbalances probably also have a connection to the epidemics. Droughts that provoke a successive chain of interrelative factors such as farming devastation, scarcity of food and decrease of the nutritional and immunological condition of the population challenged. In drought periods the sylvatic reservoir fauna encroach on the peridomestic environment looking for food or shelter which facilitates the transmission of the disease. The periodic occurence of visceral leishmaniais is very important to direct more correct and economic control measures at the right time.

**TABLE XI**
**CASES OF VISCERAL LEISHMANIASIS REPORTED TO SUCAM FROM 1976 TO 1983 (FROM THE REPORT OF THE FIRST MEETING ABOUT RESEARCH ON LEISHMANIASIS, S.PAULO, 1984**

| YEAR | NUMBER OF CASES | % TOTAL |
|---|---|---|
| 1976 | 95 | 3.1 |
| 1977 | 57 | 1.8 |
| 1978 | 86 | 2.8 |
| 1979 | 91 | 3.0 |
| 1980 | 165 | 5.3 |
| 1981 | 359 | 11.6 |
| 1982 | 1,120 | 36.3 |
| 1983 | 1,124 | 36.3 |
| TOTAL | 3,097 | 100% |

**TABLE XII**
**CASES OF VISCERAL LEISHMANIASE REPORTED IN BRAZIL BY SEVERAL AUTHORS (1913-1986)**

1913 - MIGONE, L.E. - Bull.Soc.Pathol.Exot.,6:118-120
1923 - TAVARES, A.- In Parahym O, 1961 - Endemias Bras. Impr. Univ. Recife
1934 - PENNA, H.A. - Brasil Medico Rio de Janeiro, 11:1-5
1942 - TAVARES, A.F.R & FONTES, J. - Lab.&Clinica RJ,21:61
1942 - ALBUQUERQUE, A.F.R. et al. - O Hospital RJ,21:61-69
1942 - PONDE,R.,MANGUEIRA,Fo.,O.& JANSEN,G. - Mem. Inst. Oswaldo Cruz, 37:333-7
1942 - BARROS, O.M & ROSENFELD,G. - Rev. Clinica S.Paulo, 15:97-102
1943 - VERSIANI, O. - Rev.Hosp.Clin.S.Paulo, 9:13-50
1944 - ALMEIDA, E. - Brasil Medico, 59:83
1953 - ARAGãO, T.C. - 30 Reunião Saúde Pública Ceará,9\1953
1954 - PESSOA, S.B. - Revista Paulista Medicina, 44:141-142
1954 - DEANE, L.M. & DEANE, M.P. - O Hospital, RJ,45:697-791
1954 - COUTINHO, J.O. & LIRA, A. - Rev.Brasil.Med.,11:89-97
1954\56- PRATA, A. - Tese Faculdade Med.-Univ.Fed.Ba,1957
1954 - VERONESI, R. et al. - Rev.Hospital das Clinicas, S.Paulo, 9:13-50
1955 - PESSOA, S.B. et al. - XII Cong.Soc.Bras.Hig.,Belem, 1954
1955 - CARVALHO, S.B. & PESSOA, S.B. - An.Fac.Med.Paraiba, 1955
1955 - ALENCAR, J.E. de. - XII Cong.Soc.Bra.Hig,PR,1955
1955 - ALENCAR, J.E. de et al. - XII Cong.Bras.Hig., Pará
1955 - FIGUEREDO, J. - XII Reun.Dermat.-Sifilógrafos Bras.BA
1955 - VERONESI, R. et alli - Rev.Hosp.Clinicas,SP,9:13-50
1956 - DEANE, L.M. - Tese - Serv.Nac.Educ.Sanitária,RJ,1956
1964 - SHERLOCK,IA - Rev.Brasil.Malar.D.Trop.,16:157-160
1966 - BARBOSA, W. - Rev.Goiania Medicina, 12:3-26
1954\77 TEIXEIRA, R. - Tese Fac.Med.-Univ.Fed.Bhia, 1980
1973 - SUCAM, M.S. - I Reunião Nac.Pesq.Leishmaniose,SP,1984
1985 - MARZOCCHI, M.C.A. et al. - Cadernos Saùde Pub.,RJ,1:5-7
1980 - BADARO, R.J. - Tese. Fac.Med.-Univ.Fed.Bahia, 1985.
1982\86 SHERLOCK, I.A. et al. - Dados inéditos ref. Bahia

## VI. Conclusions

Several papers, principally those of Deane (9) have established the most important geographical and climatic characteristics of the ecology of visceral leishmaniasis in Brazil, its principal vector, and dogs and foxes as domestic and sylvatic hosts, respectively. For the first time, we have recently found the marsupial opossum Didelphis albiventris, naturally infected with Leishmania chagasi, which was confirmed in foci in Colombia (30,42). However, several aspects of the ecology of visceral leishmaniasis still remain unknown making the understanding and resolution of the problem for control difficult.

Several aspects are still not clear. First Chagas et al. (8), and more recently other authors, principally Lainson (13), defend the authocthonicity of Leishmania chagasi to be the ethiologic agent of leishmaniasis in America continent. According to this author, if L. infantum from Europe had been introduced in the american continent, human leishmaniasis could not have dispersed so quickly througout South America, because in the actual areas of distribution of the disease, the means of dispersion were very deficient. So, those authors speculate that dogs, the presumable primitive hosts of viscerotropic leishmanias, were already well established in America, before the arrival of the white man. Thus, there were no reasons for these animals not to have their own leishmaniasis (13).

Truly, one does not know if the american dogs had their own leishmanias in the pre-colombian period. In the same way, at least in Brazil, one does not have any evidence that the natives suffered from visceral leishmaniasis (27). On the contrary, it is certain that in the pre-colombian period,the american indians suffered from cutaneous leishmaniasis (20).

However, as an example of the introduction of Leishmania infantum in the America, Lainson (13) has pointed to the recent establishment of a small foci of canine leishmaniasis in Oklahoma, USA, involving, at least, four dogs outside of the local area. Canine leishmaniasis was also reported seven times in dogs that came from Greece. This evidence suggested that imported dogs were probably the source of origin for the Oklahoma focus. Since the only species of sandfly (Lutzomyia diabolica) is rare in Oklahoma, one can speculate that congenital transmission could have occurred. Later, in Oklahoma it was proven that leishmaniasis was transmitted among dogs via ticks (16).

Most of the leishmanias produce mild or asymptomatic infection in natural hosts, maintaining the balanced relationship between parasite and host. Commonly dogs and foxes have a fulminating infection, thus they are not well adapted to serve as a reservoir for the parasite. On the contrary, like man, they seem to be recent hosts that suffer from the disease. This fact, according to Lainson (13), has been used as an argument for the support of the hypothesis that visceral leishmaniasis was caused by the imported L. infantum. From an epidemiological point of view, regardless of whether or not dogs suffer from visceral leishmaniasis, they are the principal domestic reservoirs. However, another primary wild reservoir must also exist in order to explain some aspects apparent in the natural history of the disease.

The four first specimens of foxes found naturally infected in America by Deane (9) were sick, but the three other foxes found naturally infected by Lainson and Shaw (13) were apparenthy healthy. For this reason, those authors think that if a more significant number of infected foxes without the symptoms of the disease were found, one could explain that american visceral leishmaniasis could have originated from old american sylvatic cycles involving foxes and their commensals (13).

In Jacobina the examination of only 14 specimens of foxes with negative results, did not permit any conclusions (Table X). It is suggested that more specimens should be examined (35).

The domestic rodents Rattus rattus and Rattus norvegicus have been recently found infected with L. infatum in Mediterranean areas (10, 22). Alencar (1) found amastigotes in R. rattus in the State of Ceará, Brazil. Lainson and Shaw (13) believe that the parasite could be L. chagasi. The finding of infected rodents with leishmanias in Central America, is believed by Lainson (13) also to be L. chagasi.

Some years ago, the senior author of the present paper, made the hypothesis that the domestic rodents in Jacobina could be the hosts of L. chagasi (29). Unfortunelely, like the foxes, the number of rodents examined was very small and thus, no conclusions can be drawn.

According to Lainson (13) serologic studies and isoenzymes electrophoresis do not have definite value in distinguishing L. infatum and L. chagasi. It was possible for him to find differences among the proteic profiles of several leishmanias, which was confirmed by means of polyclonal and monoclonal antibodies with surface antigens of three species. However, it was observed that some close relation existed among L. chagasi from Brazil (13), L. infatum from the Mediterranean and L. donovani from India. Nevertheless Momem, Grimaldi Jr. and Deane (17) did not find differences in the proteic profile using 11 enzymes in 100 isolates of L. chagasi from Brazil (17). They concluded that the profiles of L. chagasi was identical to those profiles of L. infantum standardized by WHO. Thus, they proposed the designation of L. infatum also for the species that cause american visceral leishmaniasis (17). Later, Grimaldi et al. concluded the species L. infantum for Mediterranean visceral leishmaniasis and L. chagasi for american visceral leishmaniasis (11) and this is now the general consenses of several authors.

Studies of Ward et al. (44) confirmed Mangabeira's observations about the morphological differences in the vector L. longipalpis. They detected different compositions of pheromones in accordance with the morphological type, suggesting that one type is more antropophilic than the other. This fact could explain the existence of areas with sporadic disease and other areas with epidemic disease. Transmission is made by the different morphological types respectively. Despite the morphological variations, the sandfly that is designated as L. longipalpis is surely the principal vector of visceral leishmania in the epidemic, endemic, and sporadic foci (1,9,34).

Relative to inherent factors of the vector, we emphasize some recent observations published in specialized literature. Enzymes, like trypsine, inhibit the sandfly infection with some species of leishmanias (24). Such enzymes are self produced in the digestive tract of the sandfly and their production stimulates the presence of certain species of leishmanias (24). This corresponds to a natural protective mechanism of the sandfly against infection, probably prejudicial for itself. Some investigators discovered that the inhibition of the action of the tripsin enzyme is made by the same substances that inhibit soybean tripsin. When it is ingested by a sandfly an infection with leishmania easy (24). We know that sandflies feed on vegetation both in nature and in the laboratory. In the laboratory, sandflies feed on afiudiulutein produced by aphids, which contains several aminoacids and sugars, important in the metabolism of the trypanosomatids. In the laboratory, sugars facilitate the infection of sandflies and increase the number of promastigotes (24).

The type of host seems to be important for improving the infection of the sandfly. Bird blood diminishes the capacity of infection in the sandfly, probably because it contains enyzmes like tripsine.

Recently Titus and Ribeiro (9) discovered an acentuated increase for the infectivity of Leishmania major when inoculated together with salivar glands of L. longipalpis in Balb\C mice.

In spite of the recent findings mentioned above, we were unable to make a sucessful experimental transmission of L. chagasi by means of the bite of L. longipalpis (Table IV). We do not know if the above mentioned factors are interfering in the results of our experiments.

At the present time it is possible to control visceral leishmaniasis at a temporary level through the treatment of human cases, elimination of sick dogs and using insecticides to control vectors. However, it is not possible to obtain permanent control of the disease, since as soon as expensive prophylactic measures are interrupted, the disease continues its natural course. Basic questions still remain without answers such as: Does a natural primary reservoir exist? What are the real roles of man, dogs, foxes, opossums and rats as reservoirs of visceral leishmaniasis? Where are the natural breeding places of the vectors? etc.

**Implications for Control**

From the point of view of the control of american visceral leishmaniasis the contributions and conclusions of our project are as follows:

a) We still do not have sufficient knowledge to include fox and opossum reservoirs in control strategies. It appears that dogs are a more important domestic reservoir than man.

b) Control strategies must include three principle measures: treatment of human cases, elimination of infected dogs, and the use of insecticides to control vectors. The active use of these measures can result in temporary control of the disease.

c) However, no one measure in isolation is sufficient to produce an acceptable level of control. As has been emphasized before, treatment of human cases together with the killing of sick dogs is not enough to prevent the occurrence of new human cases in Jacobina.

d) Routine spraying with insecticides every six months when the density of sandflies high, is necessary to contol the vector.

e) Health and sanitation education is necessary for communities to avoid the migration of infected humans and dogs from endemic to indene areas.

f) Understanding of the cyclic decennial periodicity in the natural histroy of american visceral leishmaniasis is important to maximize the effectiveness and economy of control measures.

g) Further multidisciplinary studies are necessary to understand how permenant and cost-effective control of visceral leishmaniasis can be achieved.

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