

Lutzomyia longipalpis and the eco-epidemiology of American visceral leishmaniasis, with particular reference to Brazil - A Review

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An historical review is given of American visceral leishmaniasis (AVL), with particular reference to the eco-epidemiology of the disease in Brazil. Following the first records of AVL in this country, in 1934, the sandfly Lutzomyia longipalpis (Lutz and Neiva, 1912) was incriminated as the principal vector. It is now generally accepted, however, that there exist a number of cryptic species under the name of Lu. longipalpis s.l. and that variations in the quantity of the vasodilatory peptide maxadilan in the saliva of flies from different populations of Lu. longipalpis s.l., may account for the variable clinical manifestations of AVL seen in different geographic regions. Distribution of AVL has been shown to extend throughout most of South and Central America, with the domestic dog serving as the principal reservoir of infection for man. However, while one hypothesis suggests that the causative parasite is Leishmania infantum, imported from Europe with the Portuguese and Spanish colonists, the demonstration of a high rate of benign, inapparent infection in foxes in Amazonian Brazil raised an opposing suggestion that the parasite is indigenous to the Americas. Recent reports of similar infections in native marsupials, and possibly rodents, tend to support this view, particularly as Lu. longipalpis is primordially a silvatic sandfly. Although effective control measures in foci of the disease will diminish the number of canine and human infections, the presence of such an enzootic in a variety of native animals will render the total eradication of AVL unlikely.

Key words: *Lutzomyia longipalpis* - sandflies - American visceral leishmaniasis - eco-epidemiology - Brazil

Early history: studies in the states of Sergipe, Pará, and Ceará

Following the first description of the sandfly *Lutzomyia longipalpis* Lutz and Neiva, 1912, in an indeterminate locality in Brazil, interest in this insect remained largely entomological until the mid-1930s. In 1934, however, Henrique Penna used the viscerotome to examine liver samples from persons who were suspected to have died from yellow fever in various rural localities in Brazil, and found that 41 of these deaths were, in fact, due to visceral leishmaniasis. His results suggested the major foci of the disease to be in the northeastern states, particularly in Ceará, and Carlos Chagas, at that time Director of the Instituto Oswaldo Cruz in Rio de Janeiro, sent his son Evandro Chagas to investigate the epidemiology. His first study was made in Sergipe where, in addition to giving the first clinical description of a living case of American visceral leishmaniasis (AVL) in Brazil, he made the important observation that the most frequent blood-sucking insect in and around the patient's house was the phlebotomine sandfly *Lu. longipalpis* (Chagas 1936).

Evandro Chagas was appointed head of a Commission set up in 1936 to continue his studies and, in view of the higher prevalence of AVL in the Northeast, it was there that he wished to work. Perversely, the only state governor

who offered the necessary financial and logistic back-up was Da Gama Malcher of Pará in the North of Brazil, where the number of recorded cases was low, and a huge old colonial-style mansion was made available for conversion into the Commission's laboratories, which received the imposing name of "The Institute of Experimental Pathology for the North" (IPEN).

Working in the rural areas of Abaetetuba and Moju, where cases of AVL were recorded by Penna, the Commission uncovered more cases of the disease in both humans and dogs (Chagas et al. 1938). Once more *Lu. longipalpis* was shown to be the principal man-biting insect in and around the houses of the infected persons, and this sandfly became the major suspect as the vector. It was concluded that the disease was essentially rural and only occurred in the close vicinity of forest or copses. For this reason it was suggested that the origin of the causative parasite, named as *Leishmania chagasi* by Cunha and Chagas (1937), was in some wild animal. The Commission's hope of confirming the role of *Lu. longipalpis* as the vector and indicating the wild animal reservoir were dashed in 1940, however, when the tragic death of their leader Evandro Chagas in a mid-air plane collision put an abrupt end to their epidemiological studies. Although the IPEN was renamed "Instituto Evandro Chagas" in his honour, his little band of dedicated workers never recovered fully from the loss of their brilliant and colourful leader, and research on the epidemiology of visceral leishmaniasis in Brazil went into steady decline.

A rude awakening to the real importance of AVL in Brazil did not occur until 1953, when over 100 inhabitants of the small town of Sobral, Ceará, died in a severe out-

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break which jolted the health authorities into activity. Another epidemiological enquiry was organized, involving three prominent figures in Brazilian tropical medicine – JE Alencar, and the married couple LM Deane and MP Deane who had formed part of the Evandro Chagas team in Pará. In Ceará they made two vitally important findings: heavy flagellate infections of what they considered to be promastigotes of *L. (L.) chagasi* in wild-caught specimens of *Lu. longipalpis* (Deane & Deane 1954a), and the natural infection of foxes with that parasite (Deane & Deane 1954b). The foxes were identified as *Lycalopex vetulus*, but evidence exists that they were more likely to have been *Cerdocyon thous* (Courtenay et al. 1996). Infections in *Lu. longipalpis* were readily obtained when these sandflies were experimentally fed on an infected fox (Deane & Deane 1954c).

By 1955, Alencar and the Deanes had recorded nearly 1000 new cases of human AVL in Ceará and neighbouring northeastern states. They noted that these occurred in the humid, wooded foothill valleys (boqueiros), and not in the dry lowland plains (sertões) or on the exposed slopes of the hills where the arid conditions and strong winds were unfavourable for *Lu. longipalpis*. Dogs suffered as badly as man from the infection: that they were the major reservoir of the human disease was clearly indicated by the high rate of canine infection and the ease with which *Lu. longipalpis* could be infected when fed on infected dogs. On the other hand, it was found that man was a somewhat poor source of the parasite for *Lu. longipalpis* when these were fed on infected patients (Deane 1956).

Distribution of AVL

Human visceral leishmaniasis was soon shown to have a very wide distribution throughout Latin America, extending from Mexico in the north to Argentina in the south.

Up to 1984 it was estimated, however, that over 90% of the recorded cases in the New World were from Brazil, and of a total of 8959 cases registered in this country 7882 were from the Northeast and 992 from the Southeast (Deane & Grimaldi 1985). Considering inadequacy of diagnosis and a general reluctance in permitting autopsies in the more remote rural communities, these figures are likely to have been considerably higher. To date, the distribution of AVL in Brazil includes the states of Alagoas, Bahia, Ceará, Distrito Federal, Espírito Santo, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará, Paraíba, Pernambuco, Piauí, Rio de Janeiro, Rio Grande do Norte, Roraima, Sergipe, São Paulo, and Tocantins.

Taxonomic position and origin of the aetiologic agent of AVL

The name *Leishmania chagasi* Cunha and Chagas, 1937 long remained in use in spite of considerable debate regarding the origin of the parasite and its taxonomy. Thus, Lainson and Shaw (1987, 1998) gave their reasons for considering the parasite as indigenous to the Americas, with an origin in native wild animals, particularly foxes, whereas Killick-Kendrick (1985) and Rioux et al. (1990) favoured the view that it was in fact *Leishmania infantum* which had been imported into Latin America during the

Portuguese and Spanish colonization of that continent. Similarities in the enzyme profiles of stocks of *L. chagasi* and *L. infantum* led Lainson et al. (1981) to suggest that taxonomic separation of the two parasites “would best be at subspecific level”, but following their studies on the genomic diversity of members of the *Leishmania donovani* complex, Mauricio et al. (1999) considered that there were no grounds for any separation. More recently Lainson and Rangel (2003) and Lainson and Shaw (2005) have used the subspecific names of *L. infantum infantum* and *L. infantum chagasi* in consideration of previous claims that distinct differences exist between the two organisms based on the kDNA fragment patterns using the restriction endonuclease digestion technique (Jackson et al. 1982, 1984); comparison of radioiodinated surface proteins of their promastigotes, and monoclonal antibodies generated against promastigote surfaces (Santoro et al. 1986); and comparative radiorespirometry studies (Decker-Jackson & Tang 1982). Recently, Martinez et al. (2003) claimed to differentiate *L. infantum* and *L. chagasi* by the Random Amplification Polymorphic DNA technique (RAPD) and a single 10-mers long primer.

The origin of AVL thus remains debatable. To account for the immense geographic distribution of the disease following the introduction of *L. infantum* and adaption of the parasite to *Lu. longipalpis*, one must postulate either that this took place at very many points on the Latin American continent, or that there was a rapid spread of the parasite throughout South and Central America. Such a spread from a few isolated points of introduction by way of *Lu. longipalpis* seems unlikely, due to the limited flight range of phlebotomines in general (Alexander 1987), the fairly static nature of populations of this insect and the improbability of infected adult sandflies being unintentionally transported by man. Again, although spread by way of infected dogs might take place in a given country, it remains unlikely that this could account for the presence of the parasite in almost the whole of the Latin American continent in such a short time.

Lu. longipalpis: the major vector of AVL

The coincidental distribution of *Lu. longipalpis* and AVL throughout most of Central and South America greatly strengthened the Deanes' conviction that this was the major vector of the disease. Strangely enough, however, although *Lu. longipalpis* is perhaps the most easily colonized of all sandflies in the laboratory, repeated attempts to experimentally transmit the parasite by the bite of this insect failed. Appropriately enough it was in the Instituto Evandro Chagas, where so much of the early history of AVL began, that the chain of evidence incriminating this sandfly was finally completed when five separate transmissions to hamsters were obtained by the bites of experimentally infected laboratory-bred *Lu. longipalpis* (Lainson et al. 1977a). The same laboratory (Lainson et al. 1984, 1985) studied a serious outbreak of AVL in the outskirts of Santarém, Pará, where they found this sandfly to be the only species consistently present in and around houses with human and canine infections. Large numbers were captured in the back-yard of one house and fed on clean hamsters, four of which subsequently developed

fulminating infections. Dissections of the sandflies used in this experiment indicated an infection-rate of 7%, and 16 isolates were identified as *L. (L.) chagasi* on enzyme profiles and by monoclonal antibodies. This transmission by the bites of naturally infected *Lu. longipalpis* provided the most conclusive proof possible of the role of this sandfly as a major vector of AVL.

The ecology of *Lu. longipalpis*: a sylvatic origin

Most early studies on AVL in Brazil were made in the sparsely forested northeastern states, or in other parts of the country that have suffered considerable deforestation and, as a result, there developed the tendency to think of the disease only as one which involves the dog and *Lu. longipalpis* in a domestic environment. Observations in the Amazon region of Brazil (Chagas et al. 1938, Lainson et al. 1986, Ryan et al. 1986c), however, indicated that *Lu. longipalpis* is primordialily a sylvatic species and that it can still be captured in remote primary forest that is far from human habitation. In Northern Brazil this is particularly evident along the length of newly opened roads that pass through forested areas. Primitive houses, inevi-

tably with dogs, chicken houses and other animal shelters, are rapidly thrown up along their length, in very close proximity to the forest edge. *Lu. longipalpis* females have catholic feeding habits and quickly invade such habitations: thus, in an epidemiological investigation of cases of AVL along the forest-fringed Igarapé Miri-Tucuruí highway this sandfly was found in the chicken houses of numerous widely separated houses only 18 months after the road had been opened (Lainson, Shaw, Silveira & Souza, unpublished observations). Finally, even more conclusive evidence came from studies in the municipality of Salvaterra, Island of Marajó, Pará, in a focus of AVL (Lainson et al. 1990). Using CDC light-traps variously placed over caged chicken, a fox, and sawdust impregnated with the urine and faeces of a fox, attempts were made to capture *Lu. longipalpis* in a pocket of residual primary forest, the back-yard of a house some 500 m distant, and neighbouring open savanna. During the dry season, 80 trapping-nights in the forest produced a total of 47 of these sandflies, consisting of 22 males and 25 females: none were caught following 14 captures in the savanna, and 2 captures in the back-yard of the house



Figs 1-3: stages in the development of foci of leishmaniasis in Pará, Amazonian Brazil. Fig. 1: primary forest in the Serra dos Carajás, where the sandfly vector *Lutzomyia longipalpis* forms part of the phlebotomine fauna. Figs 2, 3: the margins of new roads cut through the forest (Fig. 2) are soon occupied by rustic houses (Fig. 3), with a subsequent infestation of chicken houses and other animal shelters by *Lu. longipalpis* coming from the adjacent forest.

provided only one male and four females. During the wet season the results were much more impressive: 32 trapping-nights in the forest provided 1161 (463 males and 698 females); 26 captures in the savanna gave a total of 4 (one male and 3 females); and 24 captures in the backyard of the house produced a total of 1274 (572 males and 702 females). From this and other studies it was clear that the natural savanna is an unattractive breeding-site for *Lu. longipalpis*. On the other hand the large numbers of this sandfly caught in the patch of forest, and the marked association of males and females during both the dry and wet seasons, strongly suggested this to be an important breeding-site. Galati et al. (2003) recently reported the capture of *Lu. longipalpis* in the forest environment in the state of Mato Grosso do Sul, Brazil. It remains to be determined, however, if the peridomestic accumulation of this sandfly is entirely due to their migration from the sylvatic habitat or, at least in part, to the establishment of a secondary peridomestic breeding-site.

The discovery of the latter would be a significant step forward in the control of AVL, but to date all available evidence suggests that the immature stages of *Lu. longipalpis* are thinly dispersed, and not concentrated in any particular microhabitat (Deane 1956). In Salvaterra, on the Island of Marajó, the results of an examination of soil removed within and around a small, heavily infested chicken house suggested that the sandflies were not breeding in that microhabitat, but had migrated to the chicken house from elsewhere (Dye & Quinnell, pers. commun.). It has been shown, in the laboratory, that the male produces a pheromone which attracts the female from a substantial distance (Morton & Ward 1989), leading these authors to suggest that the attraction of host odour and male pheromone worked together at the same time and synergistically. On the other hand, following their observations on the progressive infestation of newly con-

structed chicken houses by *Lu. longipalpis*, Dye et al. (1991) and Quinnell and Dye (1994a) were led to the conclusion that the females, accompanied by some males, are at first attracted by host odour and latterly by the pheromone. It was noted, however, that whereas the males tended to remain longer in the chicken houses, most of the females did not rest there during the day.

That *Lu. longipalpis* females feed readily on the domestic chicken suggests that wild birds are likely to be among their sylvatic hosts. This sandfly's concentration in chicken houses is of considerable epidemiological importance, because it is not customary to spray these with insecticides during antimalarial campaigns – which still remain the principal, indirect control of AVL.

Following experimental studies on the peridomestic distribution of *Lu. longipalpis* in Salvaterra, Island of Marajó, Quinnell and Dye (1994a,b) concluded that this sandfly tends to congregate at sites outdoors, including animal sheds, where leks can most easily form on abundant, stationary (sleeping) hosts. The flies are much less frequently encountered within houses and, as most dogs sleep outdoors, this probably accounts for a much higher infection-rate of AVL in dogs than in man. It was also suggested that human exposure to the bites of *Lu. longipalpis* was greatest in poorly constructed houses with abundant holes in the walls and the roof.

The *Lu. longipalpis* complex

Mangabeira (1969) first drew attention to small morphological differences between male examples of *Lu. longipalpis* from Ceará, Northeast Brazil, and others from Pará, North Brazil, and Lainson and Shaw (1979) suggested that the presence of "...a *Lu. longipalpis* complex of very similar sandflies...may account for certain anomalous situations" and that "a taxonomic revision is needed of...*Lutzomyia longipalpis*"



Fig. 4: final stage in the formation of a focus of Amazonian visceral leishmaniasis. Disorganised growth of a shanty-town, high density of the sandfly vector and an abundance of dogs. Outskirts of Santarém, Pará, North Brazil.

Ward et al. (1983) confirmed Mangabeira's finding; namely, that the male flies from Pará had a pair of white spots on the 4th abdominal tergite, whereas those from Ceará had two pairs of spots on the 3rd and 4th tergites. Furthermore, they showed that the two forms were sexually isolated, suggesting that they represented two cryptic species. It was also suggested that this might account for epidemiological differences in AVL in the two geographic areas (Ward et al. 1985).

By use of the electron-microscope, Lane et al. (1985) showed that the tergal spots were in fact the site of pheromonal glands, and further studies (Ward et al. 1988, Hamilton et al. 1996) went on to show that the different populations of *Lu. longipalpis* produced different pheromones and that the female flies could differentiate the correct one. Correct mating depended on different "songs" produced by the vibrating wings of the males.

More evidence of the existence of a species complex of *Lu. longipalpis* s.l. was offered by Crampton et al. (1989), who prepared a DNA probe which was specific for a Bolivian population of this sandfly, and Lanzaro et al. (1993) who compared examples from Costa Rica, Colombia, and Brazil by enzyme electrophoresis and cross-breeding experiments. They concluded that these populations were of three distinct species, but refrained from using any new specific names. Mutebi et al. (2002) added support to this conclusion by demonstrating genetic differentiation of these populations and this was also demonstrated in populations of the sandfly in Venezuela (Arrivillaga et al. 2000). Further evidence for the presence of cryptic species within a *Lu. longipalpis* complex has been provided by numerous other workers (Dujardin et al. 1997, Lampo et al. 1999, Uribe, 1999, Yin et al. 1999, Arrivillaga & Feliciangeli 2001, Soto et al. 2001, Arrivillaga et al. 2002, 2003).

The existence of such cryptic species in Brazil was disputed by Mukhopadhyay et al. (1998), Mutebi et al. (1999), Azevedo et al. (2000), and Arrivillaga et al. (2002, 2003) who considered that there is only a single species in that country, based on a study of several widely separated populations for genetic variability in biochemical characters. They felt that the reasons for any epidemiological variations in AVL should be sought elsewhere. In favour of this view, a recent study of *Lu. longipalpis* populations from six locations in a transect across eastern Brazil by mitochondrial cytochrome b gene sequence analysis suggested that sequence divergence also did not adequately indicate cryptic species (Hodgkinson et al. 2003). On the other hand, comparison of the courtship "songs" has detected differences of song pattern between Brazilian populations of *Lu. longipalpis*, and these were consistent with the level of molecular divergence, found at the *cacophony* locus, among the different populations (Bottecchia et al. 2004, Souza et al. 2004). These groups of workers suggest that their findings, together with other evidence, does suggest the existence of a cryptic species-complex under the name of *Lu. longipalpis* in Brazil, with as many as four sibling species (Souza et al. 2004). Among the additional evidence, for example, populations of this sandfly from Jacobina (Bahia), Lapinha (Minas Gerais), and Natal (Rio Grande do

Norte) had been differentiated on genetic grounds (Bauzer et al. 2002), and Maingon et al. (2003) produced genetic evidence of the existence of two sibling species of *Lu. longipalpis*, that produce distinct male sex pheromones in Sobral, Ceará, Northeast Brazil. Finally, Watts et al. (2005) investigated the phylogeographic pattern of variation at microsatellite loci among 11 populations from Brazil and Venezuela, related to their male pheromone. They concluded that "Temporal genetic differentiation was mostly not significant at the same site. Spatial genetic differentiation was, however, strong, although there was only a weak relationship between genetic differentiation and the geographic distance separating the samples.... Geographic separation explained a much greater.... percentage of the genetic differences among populations when samples with the same pheromone type were analyzed separately." A cluster analysis showed 5 groups: *Lu. cruzi* (Brazil) and *Lu. pseudolongipalpis* (Venezuela) as separate species, two Venezuelan and Brazilian groups, and a very distinct cluster of Brazilian cembrene populations.

Most authors have cautiously refrained from giving names to examples of "cryptic species", and it has rightly been asked if these different populations might not simply indicate the *initiation* of a speciation process rather than the existence of valid species (Bottecchia et al. 2004). It has also been questioned as to whether *Lu. longipalpis* "...is a highly polymorphic and geographically variable species, but not a species complex" (Bauzer et al. 2002).

Arrivillaga and Feliciangeli (2001), however, gave the name of *Lutzomyia pseudolongipalpis* to a sandfly in Venezuela. The adults are apparently morphologically indistinguishable from those of *Lu. longipalpis*, but the larvae are morphologically distinct. In addition, the adult fly's biting activity was shown to be continuous throughout the night, unlike that of two populations of Venezuelan *Lu. longipalpis* which was found to be at its greatest before 23.00 h and to steadily decrease from that time onwards (Feliciangeli et al. 2004). Arrivillaga et al. (2003) made phylogenetic analyses of thirty-one populations of *Lu. longipalpis* s.l. originating throughout this species' geographic range, using seven isozyme loci and genes in the mitochondrial genome. The analyses revealed four distinct clades which, it was considered, supported the existence of four species. These had distinct geographic ranges, defined as (1) Brazil (*Lu. longipalpis sensu stricto*); (2) Laran (Northwestern Venezuela populations); (3) cis-Andean-Colombia; and (4) *trans*-Andean-Central American populations. The Brazilian clade was represented by 11 populations sampled throughout this country, including the areas in which *Lu. longipalpis* was originally described; the sandfly of the Laran clade = *Lu. pseudolongipalpis* from Northwest Venezuela; the cis-Andean clade consisted of Colombian populations in Bucaramanga, Palo Gordo, Neiva, Durania, and a population from Pacaraima, North Brazil (a mountainous area in Roraima, on the borders of Venezuela and Guyana); the *trans*-Andean clade included 11 populations from various parts of Central America. The authors have proposed to prepare descriptions and new specific names for the sandflies of the latter two clades.

Differing opinion will doubtless continue concerning the criteria needed before a considered “cryptic species” can be given specific rank, but intrinsic reproductive isolation, as demonstrated by cross-breeding experiments, must surely be high on the list. In this connection, the work on the sandfly’s mating “song” is particularly interesting, as the vocalization of the males of a number of insects appears to be the most important barrier isolating the different species (Imms 1964, Perdeck 1957).

The existence of a complex of cryptic species, under the name of *Lu. longipalpis* s.l., helps considerably in explaining why very different clinical manifestations of AVL exist in Latin America, especially when this is considered in the light of studies on the nature of the saliva of *Lu. longipalpis* s.l. from widely separated geographical areas.

The influence of the saliva of *Lu. longipalpis* s.l. on infection of man with *L. infantum chagasi*

Although infection with *L. i. chagasi* is predominately associated with a visceral disease, the same parasite has been shown to produce only non-ulcerative cutaneous lesions in Costa Rica (Zeledón et al. 1989), while in Honduras it may cause both visceral and cutaneous leishmaniasis in the same focus (Ponce et al. 1991). The saliva of *Lu. longipalpis* contains a potent vasodilatory peptide, ‘maxadilán’ (Lerner et al. 1991). In experiments investigating the possible influence of the sandfly’s saliva on the course of human infection with *L. i. chagasi*, Warburg et al. (1994) fed *Lu. longipalpis* s.l. of Brazilian, Colombian and Costa Rican origin on the arms of volunteers. They found that the measurements of the resulting erythemas at the sites of the bites correlated well with the levels of maxadilán in the sandflies from the three geographical areas. Saliva from the Brazilian colony was the most potent, while that from the Colombian flies was less so. Saliva from the Costa Rican specimens had very little maxadilán, a very low vasodilatory activity and produced negligible erythema: when mixed with promastigotes of *Leishmania major* and inoculated into the foot-pads of mice it strongly enhanced proliferation of cutaneous lesions. On the other hand, similar inoculations of mixtures of promastigotes and saliva from Colombian and Brazilian *Lu. longipalpis* exacerbated the development of cutaneous lesions to a lesser degree. It was suggested that some of the variability in the clinical presentations of *L. i. chagasi* infections may be due to the different composition of the saliva of the sandfly, presumably accounting for the manifestation of *L. i. chagasi* infection in man as either a visceral or a cutaneous disease. The significance of these findings regarding the nature of infections in wild or domestic reservoir hosts in foci of human cutaneous and/or visceral leishmaniasis due to this parasite remains to be studied. A cutaneous lesion due to *L. i. chagasi* has been reported in a patient from the state of Rio de Janeiro, Brazil (Oliveira et al. 1986). Unlike those described in Costa Rica and Honduras, however, the lesion was ulcerative, and cutaneous manifestations of infection with this parasite in Brazil would appear to be a rarity.

Other possible vectors of *L. i. chagasi* in Brazil and neighbouring countries

The assumption that *Lu. longipalpis* s.l. was the sole sandfly vector of *L. i. chagasi* throughout the whole geographical range of AVL was to persist for over 50 years. Suspicions were raised, however, that other species of sandflies might be involved in Venezuela when cases of the disease were recorded in the apparent absence of this sandfly.

Thus, Potenza and Anduze (1942) were unable to find *Lu. longipalpis* in two districts of the state of Bolívar, where two cases of infantile visceral leishmaniasis had been diagnosed, and Pifano and Romero (1964) suggested that *Lu. evansi* (Nuñez-Tovar) might be an alternative vector in a focus of AVL in the Turmiquire hills, state of Sucre, Venezuela, where *Lu. longipalpis* was seemingly absent. A further 26 years were to elapse, however, before this suspicion was substantiated, when Travi et al. (1990) showed that 87% of the sandflies captured in a focus of AVL in the Córdoba Department of Colombia were *Lu. evansi* and that one of these flies was infected with *L. i. chagasi*, as identified by isolation of the parasite and its characterization by isoenzyme electrophoresis. In further studies in north Colombia, promastigotes were found in nine more specimens of *Lu. evansi* and the parasite again identified as *L. i. chagasi* on two occasions (Travi et al. 1996). This, the presence of *Lu. evansi* in peridomestic and intradomestic habitats throughout the year, and the apparent absence of *Lu. longipalpis*, led to the conclusion that *Lu. evansi* is the principal vector of AVL in that region of Colombia, although elsewhere the vector has been shown to be *Lu. longipalpis* (Ferro et al. 1995). Recently, in Carabobo state, Venezuela, Aguilar et al. (1998) recorded the presence of promastigotes in a single specimen of *Lu. evansi* captured in an area endemic for AVL, and among 1757 sandflies caught in and around houses 72.9% were *Lu. evansi* and only 1.3% *Lu. longipalpis*. Finally, Feliciangeli et al. (1999) used k-DNA restriction analysis to show high homologies between the culture forms of the parasite from *Lu. evansi* and a standard stock of *L. i. chagasi*. These findings regarding *Lu. evansi* raise two major questions: firstly, whether or not it may be an alternative vector of AVL in other parts of this sandfly’s geographical range, and secondly if there exist other alternative vectors. In addition to Colombia and Venezuela, *Lu. evansi* has been recorded in Costa Rica, Honduras, Nicaragua, El Salvador, Guatemala (Young & Duncan 1994), and Mexico (Ibáñez-Bernal et al. 2004). Evidence suggests that adaptation of *L. i. chagasi* to *Lu. evansi* is a relatively recent event which is still in progress. Thus, Montoya-Lerma et al. (2003) made a study of the infection-rates and development of *L. i. chagasi* in *Lu. longipalpis* and *Lu. evansi* in natural and experimental conditions. Experimental infection-rates and the cycle of *L. i. chagasi* in the two flies have shown that parasite colonization, differentiation, attachment to the gut epithelium and migration to the fore-gut were all more frequent and uniform in *Lu. longipalpis* than they were in *Lu. evansi*.

As far as we can ascertain, *Lu. evansi* has not been found in Brazil, but speculations have been made regarding the possible role of a variety of other sandfly species in the transmission of *L. i. chagasi*. Oliveira et al. (1959) failed to find *Lu. longipalpis* in a village in Minas Gerais where there was a high incidence of AVL, and suspicion fell principally on *Lu. intermedia* and *Lu. whitmani*. Coelho et al. (1965) were also unable to capture *Lu. longipalpis* in a focus of the disease in southwest Goiás, where the most common sandflies were *Lu. intermedia*, *Lu. whitmani*, *Lu. shannoni*, and *Lu. (Psychodopygus) davisi*. Ryan et al. (1984) recorded heavy promastigote infections in *Lu. antunesi* captured in a focus of AVL on the Island of Marajó, Pará. Although the organism remained unidentified, its suprapylarian development in the sandfly raised the question as to whether or not it was *L. i. chagasi*. The same authors (unpublished observations) found heavy infestations of *Lu. furcata* in pigsties in an area near Belém, Pará, where isolations of *L. i. chagasi* had been made from foxes but where *Lu. longipalpis* could not be found. *Lu. furcata* is not anthropophilic but attacks a variety of wild and domestic animals. It could possibly represent, therefore, an alternative vector among such reservoir hosts of *L. i. chagasi* as dogs and foxes: experimentally, it has been shown to be capable of transmitting another species of *Leishmania*, *L. (L.) amazonensis* (Ryan et al. 1986a).

The female of the sandfly *Lu. cruzi* is considered to be morphologically indistinguishable from that of *Lu. longipalpis* (Martins et al. 1984), and the two species can only be reliably separated by small differences when comparing the males. To add to the confusion, a distribution overlap makes it difficult to incriminate either species as the vector of AVL in areas where the two are found together. Santos et al. (1998) dissected a large number of sandflies captured in CDC light-traps around houses in a focus of AVL in Corumbá and Ladário, Mato Grosso do Sul, and found promastigotes in 14 female specimens, all with the morphology of *Lu. longipalpis/Lu. cruzi*. The parasite was identified as *L. i. chagasi* by monoclonal antibodies and, in virtue of the apparent absence of males of *Lu. longipalpis* in their captures, these authors concluded that all the infected flies were *Lu. cruzi* and that "...*L. cruzi* is the vector of *Leishmania chagasi* in the area of Mato Grosso do Sul, Brazil". Although the evidence for this supposition is strong, it is not yet conclusive, and in a more recent publication Santos et al. (2003) have, in fact, confirmed the presence of *Lu. longipalpis* in the Corumbá area of study. Until infected females can be conclusively identified as *Lu. cruzi* – by way of biochemical methods (Ryan et al. 1986b), by DNA probes (Ready et al. 1991), or after the production of adults by raising them from the eggs of infected flies (Ryan et al. 1987), the role of *Lu. cruzi* as a vector of *L. i. chagasi* must remain doubtful. Little information exists on the distribution of *Lu. cruzi*. Young and Duncan (1994) suggest that in Brazil this sandfly is restricted to the state of Mato Grosso do Sul. Santos et al. (1998) suggest that the epidemiology of AVL in the area of Bolivia bordering Mato Grosso do Sul "...certainly should be the same..." : namely, that *Lu. cruzi* also occurs in Bolivia. The limited

distribution of both *Lu. cruzi* and *Lu. evansi* compared with that of *Lu. longipalpis* leaves no doubt regarding the overwhelming importance of the latter as the principal sandfly host of *L. i. chagasi*.

Among other possible "alternative" vectors, *Lu. intermedia* and *Lu. whitmani* must be included. *Lu. intermedia*, highly suspected as a vector of *L. (V.) braziliensis* in southeast Brazil, shares a similar habitat to that of *Lu. longipalpis*, is highly anthropophilic and also known to feed on dogs: in addition, it has been experimentally infected with *L. i. chagasi* (Chagas 1940, Paraense & Chagas 1940). On the other hand, *Lu. intermedia* has not been recorded further north than Paraíba, in Alagoa Grande and Areia, and part of Pernambuco in Lagoa dos Gatos, Nazaré, Quipapá, Timbaúba and Vitória de Santo Antão (Martins et al. 1978, Young & Duncan 1994). Consequently, it cannot be involved as a secondary vector of AVL in the highly endemic areas in Ceará and Piauí, or in the states of Maranhão and Pará. *Lu. whitmani* sensu stricto is a confirmed vector of *L. (V.) braziliensis* in Northeastern Brazil (Rangel & Lainson 2003) and, as mentioned above, has been suspected as a vector of AVL in Minas Gerais and Goiás. It is highly anthropophilic and frequently found, together with *Lu. longipalpis*, in chicken houses and human dwelling places. Regarding transmission in the sylvatic habitat in north Brazil, *Lu. whitmani* sensu lato might function as a vector among foxes, but its rarity near houses and its non-anthropophilic habits militates against it being a vector of *L. i. chagasi* to man.

In the Amazon region, suspicion must fall on *Lu. flaviscutellata* as a conceivable alternative vector of *L. i. chagasi*. It is better known as the major sylvatic vector of *L. (L.) amazonensis* among a variety of rodents and marsupials, but this parasite has been isolated from a fox in Pará (Lainson & Shaw 1987), indicating that this sandfly does include foxes among its hosts, and these animals are natural hosts of *L. i. chagasi*. *Lu. flaviscutellata* is occasionally found invading the peridomestic habitat in areas where isolated cases of Amazonian AVL have been diagnosed (Lainson et al. 1994). It is not greatly attracted to man, however, so its role as a secondary vector, if indeed it exists, would be of minor importance. Souza et al. (2003) were unable to find *Lu. longipalpis* in 6 of 18 foci of AVL in the municipality of Rio de Janeiro and suggested the participation of other species of sandflies such as *Lu. migonei* and *Lu. firmatoi*. When considering the apparent absence of *Lu. longipalpis* in such foci of AVL, however, it must be remembered that with the change of rainy to dry season the population density of this sandfly may fall to such an extent that no examples can be found until advent of the next wet season.

Amazonian AVL: indigenous or introduced?

While there remains little doubt that peridomestic/intradomestic infestations by *Lu. longipalpis* and/or *Lu. evansi* originate(d) from sylvatic populations of these sandflies, the origin of *L. i. chagasi* has remained controversial, particularly in the more remote forested areas.

It has been argued that in Brazil the parasite was introduced into the Amazon region by way of infected dogs

accompanying immigrants coming from major foci of AVL in the northeastern states, such as Ceará and Piauí. At the time of Penna's discovery of human visceral leishmaniasis in Pará in 1934, however, there were no roads linking that state with Northeastern or Southern Brazil. Access was possible only by boat or small aircraft and, at that time, this would have severely limited any migration of families and their dogs to northern Brazil from those regions. Moreover it is highly likely that previous cases of AVL in Pará must have gone undiagnosed long before Penna's chance discovery, and at a time, therefore, when importation of the disease into the forested north of Brazil, in this way, was even more unlikely. Relatively recent eco-epidemiological studies in some areas of northern Pará have shown cases to be sporadic, widely separated and not significantly associated with immigrant families. Furthermore, infections registered in men sleeping in lumber camps in or near forest, and far from fixed habitations, suggested a feral source of the parasite (Lainson, Shaw, Silveira, and Souza, unpublished observations).

In the Old World it has been suggested that the origin of human visceral leishmaniasis, due to parasites of the *L. (L.) donovani* complex, was a rural enzootic in wild canids such as foxes, jackals and wolves, and that it later spread to dogs (Lysenko 1971). Wild canids have been present in South America since the Pleistocene era some 2-3 million years ago, and a similar origin from such animals has been postulated (Lainson 1989). In support of this is the predominantly benign infection commonly found in foxes, which does suggest an ancient well balanced host-parasite relationship, as opposed to the usually virulent nature of infection in the dog. In Venezuela, and doubtless impressed by the Deanes' record of infected foxes in Ceará, Torrealba and Torrealba (1964) inoculated a specimen of *Cercopithecus thous* with *L. i. chagasi*. The animal showed no signs of infection, but amastigotes were encountered in its bone marrow seven months later. In Amazonian Brazil, workers of the Instituto Evandro Chagas examined 23 *C. thous* from agricultural land close to both primary and secondary forest on the outskirts of Belém, Pará, and isolated *L. i. chagasi* from three of them (Lainson et al. 1969, 1987).

None of the animals showed outward signs of infection, and neither canine nor human visceral leishmaniasis had ever been recorded in that locality, which was very sparsely inhabited. Turning their attention to foci of AVL in rural areas of the Island of Marajó, Pará, the same laboratory (Silveira et al. 1982, Lainson et al. 1987) isolated the parasite from 11 of 26 *C. thous* (42.3%) by the inoculation of hamsters with triturates of liver and spleen from these animals. In addition, it was shown that 22 other specimens (54.6%) were serologically positive by the indirect fluorescent antibody test (IFAT): none of the parasitologically or serologically positive animals showed signs of infection. A similar occult infection of *C. thous* has also been recorded in a focus of AVL in Corumbá, in the state of Mato Grosso do Sul, Brazil (Mello et al. 1988).

Although the Deanes (1954c) had shown that *Lu. longipalpis* could readily be infected when fed on a fox suffering from an acute infection with *L. i. chagasi*, it remained to show that apparently healthy foxes with an

occult infection could also serve as a source of infection for these sandflies. Lainson et al. (1990) infected laboratory-bred *Lu. longipalpis* with a fox strain of the parasite by feeding them on a blood-suspended triturate of heavily infected hamster spleen, through a chick-skin membrane. On the sixth day post-infection, and following oviposition, only four of the sandflies remained alive, and attempts were made to feed these on a young fox which was serologically negative at the time of the experiment and for seven weeks previously. Only two of the flies fed on the animal, but subsequent dissection showed them both to be heavily infected. Five weeks later the IFAT titre of the fox was 1: 1280, and when 60 clean *Lu. longipalpis* were fed on the animal after a further 10 weeks, four of the 54 surviving flies (7.4%) showed promastigote infections. Infection of the fox was confirmed by isolation of the parasite from skin, spleen, liver and bone marrow.

Other wild animal hosts of *L. i. chagasi*

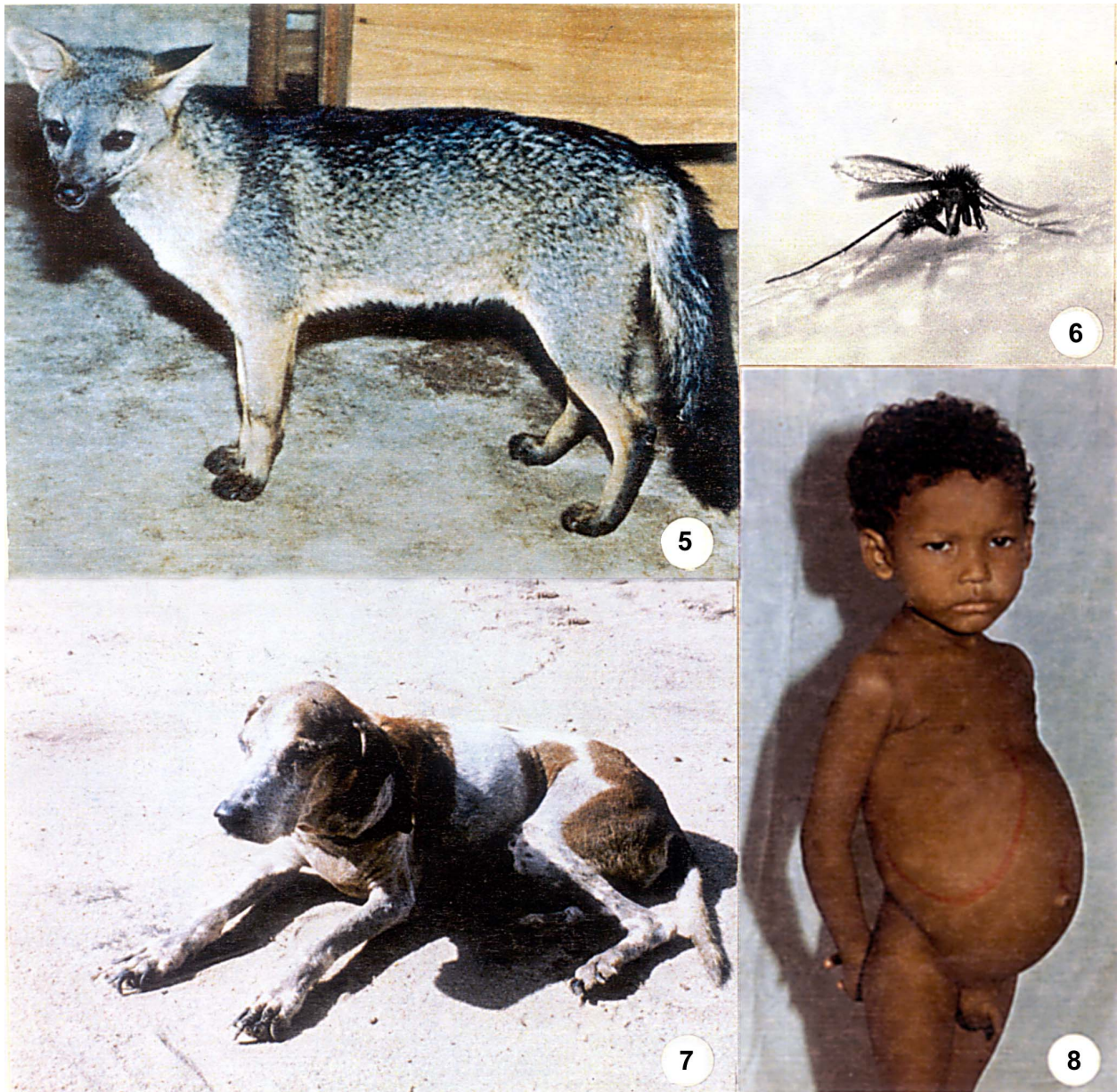
Sherlock et al. (1984, 1988) isolated *L. i. chagasi* from two opossums, *Didelphis albiventris*, captured in a focus of AVL in Jacobina, Bahia, but considered that it was unlikely that this animal represented an important reservoir of the parasite because of the very low infection-rate (two of 84 examined).

Workers in Colombia (Corredor et al. 1989a,b, Travi et al. 1994) registered the isolation of the parasite from the common opossum *Didelphis marsupialis* following the in vitro culture of spleen, liver and skin in various media and the intraperitoneal inoculation of hamsters. In one focus of AVL the infection-rate of the opossums was as high as 12/37 (32%), and it was concluded that this animal is an important reservoir of *L. i. chagasi*. Travi et al. (1998a) followed up these findings by experimentally infecting *D. marsupialis* with both amastigotes and promastigotes of *L. i. chagasi* (dog strain). No parasites could be detected by culture of the opossums' blood and only very few *Lu. longipalpis* were infected when fed on these animals. They nevertheless considered that xenodiagnosis with the sandfly *Lu. longipalpis* was a more sensitive method for detecting infection than was the polymerase chain reaction (PCR). Travi et al. (1998b) then studied a variety of small mammals captured in both undisturbed and degraded, dry forest in northern Colombia, using the PCR and dot-blot hybridization techniques: they made no attempt to isolate the parasite. Positive PCR/hybridization results for *L. i. chagasi* DNA were obtained for 3/21 (14.3%) *D. marsupialis* caught in undisturbed forest and 13/137 (9.5%) of this animal from the degraded forest. Positive results were also recorded for 3/34 specimens of the rodent *Proechimys canicollis* from undisturbed forest and in 2/4 from degraded forest, and the authors considered these results to indicate active infections of these rodents with *L. i. chagasi*. No foxes were examined in these surveys, although the authors state that *C. thous* was present in the study areas and "...might contribute to the maintenance of *L. chagasi*". The very high percentage of *C. thous* infected in foci of AVL in North Brazil and experimental infection of *Lu. longipalpis* fed on an infected fox, together suggest this to be highly likely.

As a result of the positive PCR-hybridization tests for *L. i. chagasi* in wild-caught *Proechimys canicollis*, the Colombian workers investigated the susceptibility of another spiny rat, *Proechimys semispinosus*, to experimental infection with *L. i. chagasi* by the intracardial and intradermal inoculation of promastigotes (Travi et al. 2002). No parasites could be isolated from these spiny rats by the periodic culture of liver aspirates, but at autopsy they were isolated in cultures of splenic material from 5/10 of the animals. No parasites could be found in stained spleen smears, however, and repeated xenodiagnosis (*Lu. longipalpis*) failed to reveal parasites. Finally, PCR-hybridization examination of skin (ears) were all negative.

The authors concluded that “The inability to infect *P. semispinosus* experimentally with *L. chagasi* indicates that it is not highly susceptible to this *Leishmania* species....”; that “.... *L. chagasi* infection in *Proechimys semispinosus* is contained and compartmentalized.”; and that “*Proechimys canicollis*, which is naturally infected with *L. chagasi* in Northern Colombia, may be a more capable reservoir host than *P. semispinosus*”

During studies on leishmaniasis in the Amazon region of north Brazil by workers in the Instituto Evandro Chagas, a total of 2637 wild animals were examined for leishmanial infection, including rodents, marsupials, procyonids, canids and edentates (Lainson et al. 1987): this list in-



Figs 5-8: four fundamental factors in the establishment of a focus of Amazonian visceral leishmaniasis in the state of Pará, Brazil. Fig. 5: a natural reservoir host of the causative parasite *Leishmania infantum chagasi*, the fox *Cercopithecus thous*. Fig. 6: the sandfly vector *Lutzomyia longipalpis*. Fig. 7: infected domestic dogs, which become the major source of human infection (Fig. 8).

cluded large numbers of the opossum *D. marsupialis* and the spiny rat *P. guyannensis*, many of which were captured near the houses of patients with AVL and, in the case of opossums, frequently in the backyards of such houses. No infections with *L. i. chagasi* were detected in any animal other than the fox *C. thous*, following the culture of spleen and liver tissue and the inoculation of this material intraperitoneally into hamsters. At the time of these studies the PCR/hybridization technique had not been developed and, in view of the finding of Travi et al. (1998b) that tissues of wild-caught *P. canicollis* gave a positive PCR to *L. i. chagasi* DNA in Colombia, Lainson et al. (2002) examined the susceptibility of laboratory-bred *P. guyannensis* to experimental infection with a canine strain of *L. i. chagasi* from north Brazil. The animal proved to be totally resistant to infection by way of promastigotes and amastigotes after massive intraperitoneal inoculation of the parasite, and subsequent PCR/hybridization tests made on liver and spleen tissue were negative. This, failure in attempts to feed laboratory-bred *Lu. longipalpis* on *P. guyannensis* or to capture this sandfly in traps baited with the rodent and placed in or near houses infested by *Lu. longipalpis*, led to the conclusion that this species of spiny rat plays no part in the eco-epidemiology of AVL in north Brazil.

At the 3rd World Congress on Leishmaniasis in April, 2005, workers at the Adolfo Lutz Institute, São Paulo, Brazil, presented the results of an examination of wild animals for evidence of *Leishmania* infections in two localities of endemic cutaneous leishmaniasis in the state of São Paulo. Each animal was examined by "...one or more of the following methods: detection of rK39 antibody in whole blood; intradermal inoculation of hamsters with skin biopsies from lesions and/or hipocromic spots or culture and/or DNA extraction for PCR and RFLP tests". Among the positive results were "*L. (L.) chagasi* in 1 *Akodon* sp. and 2 *D. marsupialis*". Unfortunately, the published abstract of the presentation (Tolezano et al. 2005) does not indicate by which method these results were obtained or, more importantly, if the parasite was isolated from these three animals.

The finding of a benign infection of *L. i. chagasi* in marsupials and rodents similar to that commonly found in foxes, does tend to support the hypothesis that the parasite is indigenous to the Americas, in spite of genomic evidence to the contrary (Mauricio et al. 1999). Of greater importance, however, is the fact that it raises the question as to what extent these animals may act as reservoirs of infection for the sandfly vector and thus play a role in the epidemiology of human AVL.

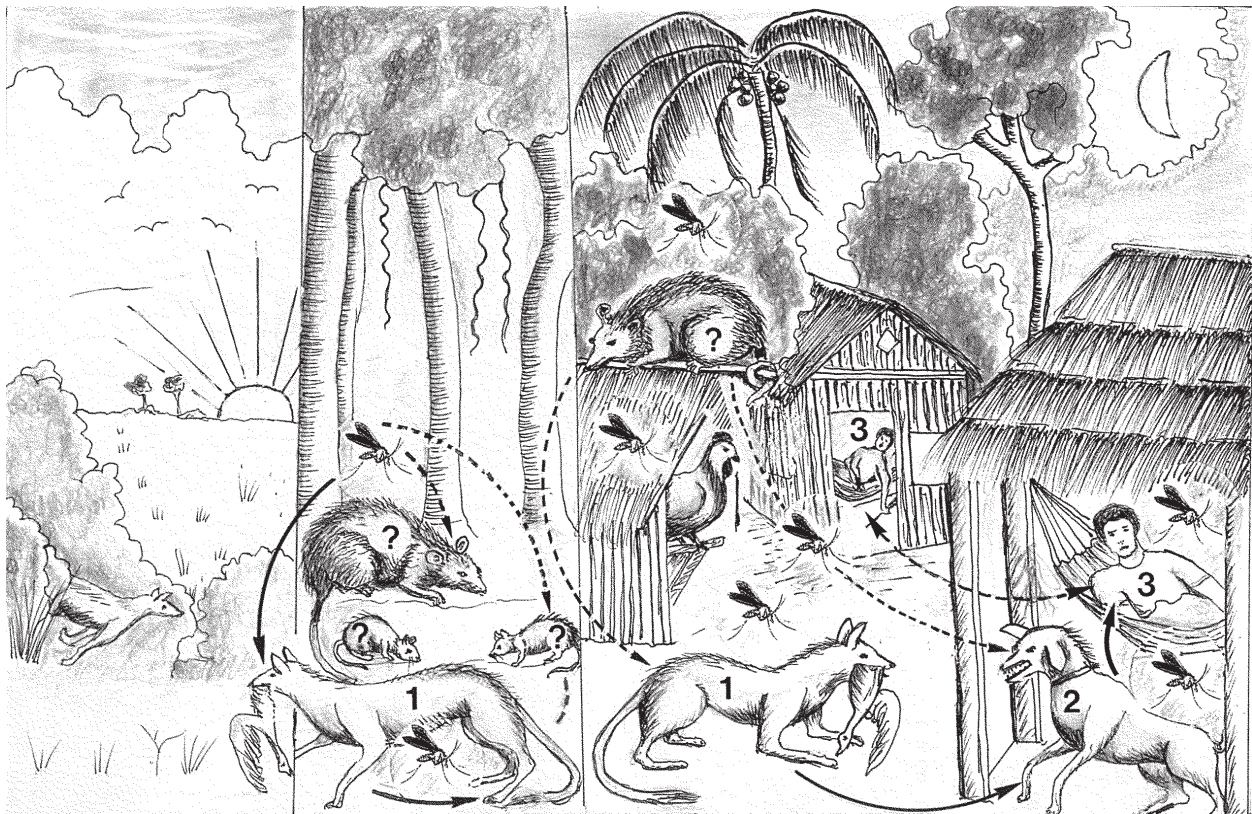


Fig. 9: suggested eco-epidemiology of American visceral leishmaniasis in the state of Pará, North Brazil. The parasite *L. i. chagasi*, originating from a silvatic enzootic in foxes and possibly other wild animals (1), is maintained by a silvatic population of the sandfly *Lutzomyia longipalpis*. Invasion of dwelling places on the edge of the forest by this sandfly enables the establishment of canine and human infection (2, 3), and the domestic dog now becomes the major source of the parasite. Unbroken lines indicate definite routes of transmission. Broken lines represent possible transmission with other wild animals, and possibly man himself, serving as a source of infection for sandflies (Modified from Lainson, 1989).

An effective reservoir-host of any parasite is one which can participate in the maintenance and dissemination of that parasite in nature, and when parasites are dependant on haematophagous vectors for their transmission it is clearly necessary to show that these can be infected when fed on the host in question. Until this is done the infected animal is best referred to as a potential reservoir. The isolation of *L. i. chagasi* from numerous specimens of the fox *C. thous* and the opossum *D. marsupialis*, and the experimental infection of *Lu. longipalpis* fed on these animals, places them firmly in the category of natural reservoirs of *L. i. chagasi* (Lainson et al. 1990, Travi et al. 1998a). On the other hand, although positive results of PCR tests on the tissues of some wild rodents do suggest that these may also represent reservoirs, isolation of the parasite and experimental infection of *Lu. longipalpis* fed on the infected animals are needed to confirm this.

Control

In the areas of high endemicity, such as in the north-eastern states of Brazil, past attempts to control visceral leishmaniasis had the form of a three-pronged attack, with the treatment of patients, destruction of infected dogs and regular insecticide spraying of houses (Deane 1956). Although this effectively reduced the number of human cases of AVL, the system was costly and could rarely be maintained for a sufficiently long period to totally eliminate the disease and, with recent criticism of culling infected dogs, the control of AVL still presents a serious problem for the health authorities throughout the vast geographic range of the causative parasite.

Destruction of serologically and parasitologically positive dogs - Although this may temporarily affect the cumulative incidence of seroconversion in these animals and may also diminish the incidence of human cases of AVL (Ashford et al. 1998), it inevitably meets with opposition on the part of dog owners, who understandably find it difficult to see why their apparently healthy (but serologically positive) dogs are condemned to death. For this reason, dogs tend to be hidden during control measures; there is the constant problem of innumerable strays, and there remains the potential danger of new or revitalized foci of AVL from the wild animal enzootic. Mathematical models regarding the three methods of control (Dye 1996) suggest that the destruction of serologically positive dogs is far less likely to solve the problem of AVL than insecticide spraying or (if and when available) the vaccination of these animals (Tesh 1995).

Vaccination of dogs - An efficient vaccine would be of immense help in the control of AVL and its application could coincide with anti-rabies vaccination, thus cutting the costs considerably. Preliminary experiments with a vaccine prepared from *L. (V.) braziliensis* combined with BCG gave promise (Mayrink et al. 1996), but the Phase III trials led to the conclusion that the vaccine "did not appear to protect the dogs against visceral leishmaniasis" (Genaro et al. 1996). The "Fucose-Mannose ligand" (FML), a complex glycoprotein fraction isolated from an aqueous extract of *L. (L.) donovani*, has been used in conjunction with a saponin adjuvant in attempts to vaccinate dogs

against *L. i. chagasi* (Silva et al. 2000). The authors regarded the vaccine as a promising tool in the control of canine visceral leishmaniasis in endemic areas of AVL. However, the Brazilian Ministry of Health is still not using this vaccine in the National Programme for Control of Visceral Leishmaniasis.

Use of insecticides - The development of chemical insecticides in the 1940's resulted in extensive use of DDT against mosquito vectors of malaria and this, at the same time, had a fortuitous and dramatic effect on the peridomestic sandfly vectors of visceral leishmaniasis, in particular in the endemic areas of kala-azar due to *L. (L.) donovani* in India. In Brazil it was first used in the 1950s to spray the internal and external walls of houses in the state of Ceará, specifically to combat AVL, and it resulted in a considerable reduction in the number of cases in some localities (Deane et al. 1955, Deane 1958, Alencar 1961, 1962). In others, however, there were perplexingly poor results which were probably due to a failure to apply the insecticide at the right time of year (Alencar 1961).

In spite of evidence militating against the use of DDT, due to both environmental side effects and human health hazards, this insecticide was still recommended as the one of choice by the World Health Organization up to 1990, because of its low cost, high efficacy and long residual action (WHO 1990). It is still used to this day in Brazil, but attention has now been focused on the synthetic pyrethroids. Although some have described the results as rather inconsistent (Silans et al. 1998), Le Pont et al. (1989), and Marcondes and Nascimento (1993) found the spraying of deltamethrin (DM) to be effective against *Lu. longipalpis* in Bolivia and Brazil, respectively.

A novel approach to the control of canine visceral leishmaniasis due to *L. i. infantum* in the Cévennes, Southern France, utilized PVC plastic dog collars impregnated with deltamethrin (Killick-Kendrick et al. 1997). The collars protected dogs from 96% of the bites of sandflies (laboratory-bred *Phlebotomus perniciosus*) and this activity was maintained for up to 34 weeks. It was concluded that "at least in the Mediterranean subregion, this insecticidal collar would protect a dog from the majority of sandfly bites and retain a killing effect for a complete sandfly season. Moreover, it seems likely that the use of the collars on all dogs in a focus of *L. infantum* would reduce contact between sandfly vectors and canine reservoir hosts sufficiently to diminish the risk of infection in humans as well as dogs". DM treatment of dogs by way of an aqueous bath has been found to be highly effective in protecting the animals against sandfly bites in China (Xiong et al. 1994, 1995). DM impregnated collars have been used in the same country (Chen et al. 2001), Italy (Maroli et al. 2001), and Brazil (David et al. 2001, Reithinger et al. 2004). Among the great advantages of DM is its repellent and killing action of long duration. No side effects have been detected, and it is considered to offer no human or canine health risk (WHO 1967). Following the results of field trials with DM impregnated dog collars in Brazil, it would seem that an extensive and governmentally managed programme for the control of canine AVL in Latin America might well utilize either this

method or the periodic bathing of dogs in aqueous solutions of the insecticide.

Biological control

In addition to *Leishmania*, *Trypanosoma*, and *Endotrypanum* spp., and possibly other trypanosomatids, neotropical sandflies may harbour other parasites including microsporidians (Lainson et al. 1977b), gregarines (Adler & Mayrink 1961, Ayala 1971, Brazil et al. 1996, Lewis et al. 1970), some *Plasmodium* spp. of lizards (Klein et al. 1987), and even nematodes (Brazil & Ryan 1984). There is little information on the pathological effects these parasites may produce in their sandfly hosts, but the gregarine *Ascogregarina chagasi* is known to reduce longevity and egg production and can effectively destroy a laboratory colony of *Lu. longipalpis* (Dougherty & Ward 1991): microsporidial infection may sometimes be extremely heavy, and these parasites are known to be highly pathogenic in some insects (Kudo 1960). To what extent *A. chagasi* and microsporidian spp., could be employed in biological control of wild populations of sandflies, particularly *Lu. longipalpis*, is questionable. One serious obstacle to such control is that the precise locality of the breeding sites of sandfly vectors of both visceral and cutaneous leishmaniasis are poorly known. When these are in the forest, application of biological control is likely to be particularly difficult and possibly of environmental risk.

Epilogue

The present-day enzootology and epidemiology of AVL as seen in the more remote parts of Latin America possibly represents a recapitulation of what transpired when the early colonists first commenced their ecological upheavals. Native animals harbour *L. i. chagasi* as an enzootic maintained by sylvatic sandflies (*Lu. longipalpis* and/or *Lu. evansi*) and the parasite inevitably gains entrance into newly established human settlements. This may be by way of infected sandflies that migrate there from the nearby sylvatic enzootic, or when infected scavengers such as foxes or opossums invade human habitations and are fed on by the sandflies from peridomestic populations. Transmission of the parasite to dogs or man is then only a question of time and a single infected dog, often with a vast supply of amastigotes for these sandflies in its skin, sets the scene for a small focus of canine or human visceral leishmaniasis. When human habitations become overcrowded, with conditions of poor hygiene and an abundance of domestic animals, particularly dogs, the concentration of the vector *Lu. longipalpis* (and/or *Lu. evansi* in some parts of Latin America) may reach a very high level and there is the risk of a serious outbreak of canine and human disease. At this stage of events the dog becomes the major reservoir of infection for man, and the wild animal host may, in fact, no longer be present in the immediate area. Control measures in such foci of infection will certainly reduce the number of human cases of AVL but, unfortunately, total elimination of the disease is unlikely, due to a persistent source of *L. i. chagasi* in the wild animal enzootic - a problem equally difficult to resolve in control of the cutaneous leishmaniasis.

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