Transmission of *Leishmania* in Coffee Plantations of Minas Gerais, Brazil

Bruce Alexander/+, Emerson Barbosa de Oliveria*, Emily Haigh, Lourenço Leal de Almeida**

Centro de Pesquisas René Rachou-Fiocruz, Av. Augusto de Lima 1715, 30190-002 Belo Horizonte MG, Brasil *Universidade de Alfenas, Alfenas, MG, Brasil **Emater-MG, Machado, MG, Brasil

Transmission of Leishmania *was studied in 27 coffee plantations in the Brazilian State of Minas Gerais. Eighteen females and six males (11.6% of the people tested), aged between 7-65 gave a positive response to the Montenegro skin test. Awareness of sand flies based on the ability of respondents to identify the insects using up to seven predetermined characteristics was significantly greater among inhabitants of houses occupied by at least one Mn+ve individual. Five species of phlebotomine sand fly, including three suspected* Leishmania *vectors, were collected within plantations under three different cultivation systems. Four of these species* i.e., Lu. fischeri *(Pinto 1926),* Lu. migonei *(França 1920),* Lu. misionensis *(Castro 1959) and* Lutzomyia whitmani *(Antunes & Coutinho 1939) were collected in an organic plantation and the last of these was also present in the other two plantation types. The remaining species,* Lu. intermedia *(Lutz & Neiva 1912), was collected in plantations under both the* "adensado" *and* "convencional" *systems. The results of this study indicate that transmission of* Leishmania *to man in coffee-growing areas of Minas Gerais may involve phlebotomine sand flies that inhabit plantations.*

Key words: *Leishmania* - Phlebotominae - coffee - Minas Gerais - Brazil

American cutaneous leishmaniasis (ACL) occurs in a wide variety of ecological settings throughout Brazil (Lainson 1989) but no information exists on transmission of *Leishmania* by phlebotomine sand flies in coffee plantations. This habitat is important in Colombia, with approximately 30% of ACL cases being reported from coffee-growing municipalities (Alexander et al. 2001). Brazil is the world's principal coffee producer, the southeastern state of Minas Gerais being responsible for approximately 50% of national production (Anon 1996). Unlike in Colombia, where about a third of all coffee is still grown on relatively small plantations under shade, in Brazil the crop is generally produced in more extensive monocultures (mean area 10 ha) in full sunlight. Although such plantations have been shown to harbour several species of sand fly in Colombia and *Leishmania* transmission occurs under this system, the factors involved in coffee-growing in Brazil differ in a number of respects, including the varieties grown, greater use of agrochemicals and more marked fluctuations in seasonal temperatures, particularly in the southeast of the country where most coffee is produced.

ACL was first recorded in the coffee-growing southwestern region of Minas Gerais in 1986, with a total of 264 cases reported from the 26 municipalities of the Alfenas administrative region between 1986-1997, including 52 from the municipality of Machado (19.6% of the total). Although ACL thus occurs in coffee-producing municipalities of the region, no information is available on the relationship between coffee-growing and *Leishmania* transmission. A pilot study was therefore carried out to (a) determine the prevalence of human *Leishmania* infection in a coffee-growing area, (b) sample the sand fly fauna under different coffee-growing systems and (c) gather information on agricultural practices that might influence transmission, as well as knowledge of sand flies and ACL among growers and their families.

MATERIALS AND METHODS

Skin testing - The study was done on 27 coffee plantations of the municipality of Machado (21º39'S, 45º55'W, elevation 820 masl) in January 1998. A total of 207 residents was subjected to the Montenegro (leishmanin) skin test (WHO 1990). All subjects were asked to read and sign a consent form prior to testing. The antigen concentration used was 40 µg N/ml of promastigotes of *Le. (Le.) amazonensis*, determined as the optimum for cutaneous response by Melo et al. (1977) Clinical examination of the participants was restricted to asking whether they had active lesions or scars that might have been due to ACL.

Participants were also asked several questions on the characteristics of the farms on which they lived, including materials of which houses were constructed, presence of animal shelters within 100 m, variety of coffee grown and use of pesticides. These characteristics could affect *Leishmania* transmission by reducing sand fly populations or limiting contact between humans and vectors. The respondents were usually unable to give accurate information on the area and density of the coffee plantations.

Sand fly sampling - Sand fly collections were restricted to three consecutive nights, one each on plantations of a particular coffee-growing system*: convencional*, where coffee bushes of the varieties *mundo novo* and *catuai*

+Visiting researcher supported by CNPq and corresponding author. Fax: +55-31-3295-3566. E-mail: bruce@cpqrr.fiocruz.br
Received 8 August 2001
Accepted 6 December 2001

were grown in a monoculture at densities of up to 3,500 bushes/ha, *adensado* (density 3,500-5,000 bushes/ha); and *orgânico* (organic), where coffee was grown under shade trees (principally *Grevillea* spp.) in a biologically diverse plantation. Collections were made using an illuminated Shannon trap manned by one person (Alexander 2000) and three CDC light traps (Sudia & Chamberlain 1962). All traps were placed at least 50 m within the plantations and not at the boundaries with other habitats.

Awareness of sand flies - Awareness of vectors was based on the ability of people interviewed to describe up to seven characteristics of phlebotomine sand flies, each of which was given a score: nocturnal biting activity (1 point), size about 3 mm (2), painful bite (3), silent (4), movement around host by series of hops (5), whitish coloration in reflected light (6) and erect, V-shaped wings at test (7). Thus scores of between 1-28 were possible. Although the first four characters could also be used to describe other haematophagous Diptera present in this habitat, including Culicidae (1), Ceratopogonidae (3 and 4), Simuliidae (2, 3 and 4) and even Tabanidae (3) the latter three are exclusive to phlebotomine sand flies and the seven features taken together describe no other biting insect. These characteristics explain the common names given to the insects in other parts of Brazil, such as "*asa branca*" (white wing) and "*cangalinha*" (little yoke, referring to the resemblance between the posture of the wings at rest and a Y-shaped yoke used to prevent livestock from passing through fences). The respondents were also asked what name they gave to the insects that bothered them. Scores obtained for people who lived with at least one Mn+ve individual were compared with those for people who lived in houses where all the occupants were Mn-ve using the Kruskal-Wallis test at the 0.05 significance level.

RESULTS

Skin testing - Twenty-four people (11.6%) gave a positive response to the Montenegro skin test, manifested by the appearance of a swelling ≥ 5mm in diameter at the injection site 48-72 h after application, or else reported having had cutaneous leishmaniasis in the past. Three people were encountered during the survey who had antecedents of the disease and had received treatment at the local heath centre. All the positive individuals were residents of the farms and had not spent a night elsewhere during the previous six months. Positive individuals included six males and 18 females, with ages ranging from 7 to 65. There was no significant difference between the median ages of Mn+ve individuals and those who gave a negative response to skin testing.

Most (76.8%) of the houses had animal shelters within 100 m, including chicken houses (58.9% of dwellings visited), pigsties (28.6%) and corrals containing horses or cattle (14.3%). No significant correlation was found between proximity of animal shelters and presence of Mn+ve individuals. With respect to the other variables that might affect contact between man and infected sand flies, one category was usually predominant. Thus *Mundo Novo* was the coffee variety grown around 76.8% of houses; the crop was always situated within 100 m (or within 10 m in 32.1% of cases), i.e., well within the flight range of sand flies (Alexander 1987, Alexander & Young 1992); brick and cement were the only construction materials used; and pesticides of various types were applied to the crop on at least 69.2% of the farms visited.

Sand fly collections - Five species of phlebotomine sand fly were collected on the three nights of sampling (Table). Four of these, i.e., *Lu. fischeri* (Pinto 1926), *Lu. migonei* (França 1920), *Lu. misionensis* (Castro 1959) and *Lu. whitmani* (Antunes & Coutinho 1939) were collected in the organic (shaded) plantation. Two (*Lu. misionensis* and *Lu. whitmani*) were also collected on the "*adensado*" plantation, while the sample from the "*convencional*" plantation consisted of *Lu. whitmani* and *Lu. intermedia* (Lutz & Neiva 1912). At least one anthropophilic species (*Lu. whitmani*) therefore occurred on all three types of plantation.

Awareness of sand flies - Sixteen (15.4%) of the plantation residents interviewed reported no problems with biting insects while 24 (23.1%) gave scores of 11 or more, having used at least one of the three characteristics that

TABLE

Species of phlebotomine sand fly (*Lutzomyia* spp.) captured in different types of coffee plantation in Machado, Minas Gerais, January 1998

			Sand flies collected	
Farm	Plantation type	Collection type	*Lutzomyia* sp.	No. Sex
Jacarandá	Shade/organic	CDC light trap	*Lu. whitmani*	1M
		Shannon trap	*Lu. whitmani*	7M/4F
			Lu. migonei	5M
			Lu. fischeri	1F
			Lu. misionensis	1M
Serranha	Adensado	CDC light trap	*Lu. whitmani*	2M/1F
		Shannon trap	*Lu. whitmani*	1M/2F
			Lu. misionensis	1F
Colônia	Convencional	CDC light trap	-	-
		Shannon trap	*Lu. whitmani*	2F
			Lu. intermedia	5M/14F

distinguish sand flies from other Diptera to describe the flies that bothered them. None of the people interviewed gave any of the common names generally used for sand flies in Brazil, all of them using the generic term "*pernilongo*" which also applies to mosquitoes. Awareness scores were also compared among individuals who lived in houses where the occupants were skin tested. None of these individuals was able to give all seven characteristics but in spite of the small sample size (n = 27), the scores obtained for people who lived with at least one Mn+ve individual were significantly higher than those who lived in houses whose occupants were all Mn-ve (Kruskal-Wallis H = 4.046, p = 0.04).

DISCUSSION

The first human cases of ACL were recorded in the Alfenas region as recently as 1986. This may explain the relative lack of awareness of the local population regarding the disease, compared with coffee-growing regions of Colombia where the disease is long-established (Alexander et al. 1992). All of the sand fly species collected during the present study had already been recorded in the area (Vianna Martins et al. 1978) and the relatively recent appearance of the disease could be attributed to the arrival of infected reservoirs or farms and/or changes in the biting behaviour of the sand fly vectors, perhaps due to some recent environmental modification.

Most species of *Lutzomyia* are associated with forested areas and coffee plantations often represent the only suitable habitat in regions where the original vegetation cover has been extensively cleared. This is particularly true of plantations in which the crop is protected from strong winds and direct sunlight by shade trees. The tree species planted and left in place are often deciduous, losing all their leaves during the dry season and contributing to a rich carpet of leaf litter on the plantation floor. Fruit and seeds from these species sustain a variety of rodents and other small mammals that are bitten by sand flies and may be involved in the transmission of *Leishmania* (Alexander et al. 1998). The trunks of shade trees are used as diurnal resting sites for sand flies and the insects are also found during the day in other dark, humid microhabitats such as under leaf litter and in animal burrows. Alexander et al. (1992) found 17 sand fly species, including *Lu. spinicrassa* (Morales, Osorno-Mesa, Osorno & Hoyos) infected with *Le. braziliensis*, in a shaded plantation of only 2 ha in Colombia. Although these plantations would seem to offer a more propitious habitat for sand flies than those exposed to full sunlight, Alexander et al. (2001) found a total of 15 species in unshaded monocultures of coffee. Sand flies captured in plantations and exposed to a variety of plants in the laboratory took sugar meals by perforating the leaves, including those of coffee itself (Alexander & Usma 1994). Although there have been no direct observations of sand fly breeding sites in coffee plantations, *Lu. migonei* has been reared in the laboratory through several generations on a diet that includes powdered coffee leaves (E Nieves, pers. commun.). This is noteworthy, since the leaves contain caffeine and other alkaloids that protect them from herbivorous insects. Thus sand flies appear to obtain all their vital requirements within the confines of coffee plantations.

As discussed earlier, coffee in Colombia and Brazil is grown under somewhat different conditions and none of the sand fly species collected in the Alfenas area appear to occur in Colombian plantations. Existing knowledge of the species sampled during the present study is briefly summarised: (a) *Lu. (Nyssomyia) whitmani* s.s is the suspected or confirmed vector of three *Leishmania (Viannia)* species in different biogeographical regions of Brazil (Ready et al. 1997). In the southeast of the country it has adapted to habitats disturbed by man, including the peripheries of cities such as Belo Horizonte (Passos et al. 1993); (b) *Lu. (N.) intermedia* has also adapted to disturbed and even urban environments, a phenomenon first recorded by Aragão (1922). It is a suspected vector of *Le. braziliensis* in Brazil, the increasing urbanisation of ACL having been attributed to adaptation of *Lu. intermedia* to habitats affected by human activities (Pereira de Oliveira et al. 1995). Marcondes (1996) suggested that *Lu. intermedia* represents a complex of morphologically similar species and resurrected one of the members, *Lu. neivai* (Pinto 1926). Although the results of studies by Marcondes et al. (1998) indicate that specimens of *Lu. intermedia* s.s. from SW Minas Gerais (including the Machado area) may belong to the latter species, this remains to be confirmed; (c) *Lu. (Pintomyia) fischeri* often bites man in and around houses. It appears to favour tree trunks as diurnal resting sites and trypanosomes have been isolated from wild-caught females (Ryan et al. 1987, Young & Duncan 1994); (d) *Lu. migonei* belongs to the species group of the same name and is a suspected vector of *Le. braziliensis* in Brazil (Azevedo et al. 1990). It has also been collected in coffee plantations in Venezuela (Nieves & Pimenta 2000); (e) *Lu. misionensis* is an ungrouped species under the classification system of Young and Duncan (1994). Little is known of its habits, but it has been recorded biting man in Argentina (Bejarano & Duret 1950). Although this is the first record from SW Minas Gerais it has been collected in several localities near Belo Horizonte (Vianna Martins et al. 1978).

Thus all five species encountered during the present study are opportunistic man-biters and at least three are suspected vectors of *Leishmania.*

Awareness of sand flies among human populations at risk is an important prerequisite to any intervention programme that seeks their participation in control measures (Koirala et al. 1998), whether active (such as the use of impregnated bednets) or passive (e.g. acquiescence in household spraying campaigns carried out by health authorities). Local people may be ignorant of sand flies, even in *Leishmania*-endemic areas, if the insects do not present a significant biting nuisance. On the other hand, in certain areas people may be able to describe sand flies in considerable detail. Alexander et al. (1995) found a strong correlation between the percentage of households able to list the seven characteristics mentioned above and the prevalence of Mn+ve individuals in riverine communities of SW Colombia.

Transmission of *Leishmania* therefore seems to be occurring on coffee plantations of south Minas Gerais

and at least three highly anthropophilic sand fly species that are suspected vectors of the parasites are present. Further studies are now being carried out to identify the vector, *Leishmania* species and mammalian reservoirs involved, as well as determine whether contact between man and vectors occurs in intra-, peri-, or extradomiciliary situations.

ACKNOWLEDGEMENTS

To Dr Luiz Gonzaga of Emater-MG and Marisa Cenízio dos Santos of the University of Alfenas for logistic assistance. To Profs Wilson Mayrink and Odair Genero of the Federal University of Minas Gerais for donating the Montenegro skin test antigen.

REFERENCES

Alexander JB 1987. Dispersal of phlebotomine sand flies (Diptera: Psychodidae) in a Colombian coffee plantation. *J Med Entomol 24*: 552-558.

Alexander B 2000 Sampling methods for phlebotomine sand flies (Diptera: Psychodidae) *Med Vet Entomol 14*: 11-13.

Alexander B, Usma MC 1994. Potential sugar sources of *Lutzomyia youngi* (Diptera: Psychodidae) in a Colombian coffee plantation. *Ann Trop Med Parasitol 88*: 543-549.

Alexander B, Young DG 1992. Dispersal of phlebotomine sand flies (Diptera: Psychodidae) in a Colombian focus of *Leishmania (Viannia) braziliensis*. *Mem Inst Oswaldo Cruz 87*: 123-130.

Alexander B, Agudelo LA, Navarro F, Ruiz F, Molina J, Aguilera G, Quiñones ML 2001. Phlebotomine sand flies (Diptera: Psychodidae) in Colombian coffee plantations under two systems of cultivation. *Med Vet Entomol 15*: 1-11.

Alexander B, Ferro C, Young DG, Morales A, Tesh RB 1992. Ecology of phlebotomine sand flies in a focus of *Leishmania braziliensis* in Northeastern Colombia. *Mem Inst Oswaldo Cruz 87*: 387-395.

Alexander B, Lozano C, Barker DG, McCann SHE, Adler GH 1998. Detection of *Leishmania braziliensis* in wild animals from Colombian coffee plantations by PCR and DNA hybridization. *Acta Trop 69*: 41-50.

Alexander B, Morales AL, Becerra J, Rojas CA 1995. Entomological aspects of a leishmaniasis control project based on community participation in riverine communities of SW Colombia. *Bol Dir Malariol San Amb 35* (Suppl. 1): 29-40.

Anon 1996. O Café em Minas Gerais. *Estado de Minas* (Belo Horizonte),19th August.

Aragão HB 1922. Transmissão da leishmaniose no Brasil pelo *Phlebotomus intermedius*. *Bras Med 36*: 129-130.

Azevedo ACR, Rangel EF, Queiroz RG 1990. *Lutzomyia migonei* (França, 1920) naturally infected with peripylarian flagellates in Baturité, a focus of leishmaniasis in Ceará State, Brazil. *Mem Inst Oswaldo Cruz 85*: 479.

Bejarano JFR, Duret JP 1950. Contribución al conocimiento de los flebótomos argentinos (Dipt. Psych.) *Rev San Milit Argent XLIX 4*: 327-336.

Koirala S, Parija SC, Karki P, Das ML 1998. Knowledge, attitudes, and practices about kala-azar and its sandfly vector in rural communities of Nepal. *Bull WHO 76*: 485-490.

Lainson R 1989. Demographic changes and their influence on the epidemiology of the American leishmaniaises. In MW Service, *Demography and Vector-Borne Disease*, CRC Press, Boca Raton, FL, p. 85-106.

Marcondes CB 1996. A redescription of *Lutzomyia (Nyssomyia) intermedia* (Lutz & Neiva 1912) and resurrection of *L. neivai* (Pinto 1926) (Diptera, Psychodidae, Phlebotominae). *Mem Inst Oswaldo Cruz 91*: 457-462.

Marcondes CB, Lozovei AL, Vilela JH 1998. Distribuição geográfica de flebotomíneos do complexo *Lutzomyia intermedia* (Lutz & Neiva, 1912) (Diptera, Psychodidae). *Rev Soc Bras Med Trop 31*: 51-58.

Melo MN, Mayrink W, da Costa CA, Magalhães PA, Dias M, Williams P, Araujo FG, Coelho MV, Batista SM 1977. Padronização do antígeno de Montenegro. *Rev Inst Med Trop São Paulo 19*: 161-164.

Nieves E, Pimenta P 2000. Development of *Leishmania (Viannia) braziliensis* and *Leishmania (Leishmania) amazonensis)* in the sand fly *Lutzomyia migonei* (Diptera: Psychodidae). *J Med Entomol 37*: 134-140.

Passos VMA, Falcão AL, Marzochi MCA, Gontijo CMF, Dias ES, Barbosa-Santos EGO, Guerra HL, Katz N 1993. Epidemiological aspects of American cutaneous leishmaniasis in a periurban area of the metropolitan region of Belo Horizonte, Minas Gerais, Brazil. *Mem Inst Oswaldo Cruz 88*: 103-110.

Pereira de Oliveira SM, Afonso RC de H, Giordiano Dias CM, Brazil RP 1995. Estudo da fauna de flebotomíneos (Diptera: Psychodidae) em Santa Cruz, município de Rio de Janeiro, Brasil. *Rev Bras Entomol 39*: 547-551.

Ready PD, Day JC, de Souza AA, Rangel EF, Davies CR 1997. Mitochondrial DNA characterization of populations of *Lutzomyia whitmani* (Diptera: Psychodidae) incriminated in the peri-domestic and silvatic transmission of *Leishmania* species in Brazil. *Bull Entomol Res 87*: 187-195.

Ryan L, Shaw JJ, Braga RR, Ishikawa EAY 1987. Leishmaniasis in Brazil XXV. Sandfly vectors of *Leishmania* in Pará State, Brazil. *Med Vet Entomol 1*: 383-395.

Sudia WD, Chamberlain RW 1962. Battery light trap, an improved model. *Mosquito News 22*: 126-129.

Vianna Martins A, Williams P, Falcão AL 1978. *American Sand Flies (Diptera: Psychodidae, Phlebotominae)*, Academia Brasileira de Ciências, Rio de Janeiro, 195 pp.

WHO 1990. *The Leishmaniases*. Technical Report Series 701, World Health Organization, Geneva, 140 pp.

Young DG, Duncan MA 1994. Guide to the identification and geographic distribution of *Lutzomyia* sand flies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). *Mem Am Entomol Inst 54*: 1-881.