Distribution of Sand Flies (Diptera: Psychodidae) at Different Altitudes in an Endemic Region of American Cutaneous Leishmaniasis in the State of Espírito Santo, Brazil

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The involvement of different sand fly species in the transmission of American cutaneous leishmaniasis (ACL) at different altitudes was evaluated in the municipality of Afonso Cláudio in the State of Espírito Santo, Brazil, from November 1995 to February 1997. CDC light traps and Shannon traps baited with human volunteers were hung simultaneously at three altitudes of a river valley: (a) 650-750 m in an area of active ACL transmission; (b) 750-850 m in a transitional area; and (c) 850-950 m in an area where no ACL transmission occurred. A total of 13,363 specimens belonging to 28 species was collected. The five most abundant man-biting species were Lutzomyia intermedia, *which constituted 24.3% of the total,* Lu. migonei *(22.3%),* Lu. whitmani *(15.4%),* Lu. fischeri *(14.9%) and* Lu. monticola *(5.8%). Analysis of the distribution of these species at the three altitudes provided evidence that* Lu. fischeri *and* Lu. monticola *were not involved in ACL transmission, whereas* Lu. migonei *and* Lu. whitmani *might act as secondary vectors of* Leishmania (Viannia) braziliensis *and* Lu. intermedia *was probably the principal vector of the parasite. These results reinforce those published in the existing literature, which indicate that* Lu. intermedia *is the main vector of* Le. (V.) braziliensis *in Southeast Brazil, while* Lu. migonei *and* Lu. whitmani *are of secondary importance.*

Key words: altitude - sand flies - cutaneous leishmaniasis - Espírito Santo - Brazil

The distribution of American cutaneous leishmaniasis (ACL) is influenced by little-known geographical and climatic factors that determine the distribution of different sand fly vectors, parasites and reservoirs. Tropical climatic conditions and altitudes of up to 800 m above sea level (asl) favor transmission of *Leishmania* (*Viannia*) *braziliensis* (Mogollón et al. 1977, Bonfante-Garrido 1983, Añez et al. 1988), including in Brazil (Forattini et al. 1972, Aguiar et al. 1987, Azevedo & Rangel 1991, Passos et al. 1993, Hermeto et al. 1994, Domingos et al. 1998, Gomes & Neves 1998, Rangel et al. 1999).

In same areas of Northeast and Southeast Brazil that were first colonized by Europeans, the disease is transmitted predominantly in the domestic environment, affecting people of both sexes and of all age groups (Mayrink et al. 1979, Barros et al. 1985, Queiroz et al. 1991, Lainson et al. 1994). Dogs and horses appear to be the domestic reservoirs of *Le. (V.) braziliensis*, the main etiological agent of ACL in these areas (Falqueto et al. 1986, Aguilar et al. 1989). Among the species of sand flies that have probably adapted to habitats modified by human activities, *Lutzomyia intermedia*, *Lu. whitmani* and *Lu. migonei*, are proven or probable vectors of *Leishmania* (Rangel et al. 1984, Hoch et al. 1986, Azevedo et al. 1990a, b, Marzochi 1992, Sessa et al. 1994, Marzochi & Marzochi 1997).

The municipality of Afonso Cláudio in the State of Espírito Santo, Brazil, is an important endemic area of ACL. Sessa et al. (1994) noted that in Liberdade, a village in this municipality, most transmission of the disease was in the peridomicile of houses situated between 600-750 m asl and none occurred above 850 m. Falqueto (1995) collected five man-biting species of sand flies in the village, i.e., *Lu. intermedia*, *Lu. whitmani*, *Lu. migonei*, *Lu. fischeri* and *Lu. monticola*. Although this author considered that most transmission of *Le. braziliensis* occurred in the peridomiciliary environment, with dogs acting as reservoirs of the parasite, he suggested that a residual sylvatic cycle might also occur, with the armadillo (*Euphractus sexcinctus*) and the paca (*Agouti paca*) acting as secondary reservoirs of the parasite.

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Given that man and his domestic animals, as well as wild mammals are present at all altitudes in this area, the absence of ACL above a certain altitude may be attributed to changes in the sand fly fauna. This hypothesis was tested during the present study by systematic sampling of phlebotomines at different altitudes, with the aim at incriminating possible vector species.

MATERIALS AND METHODS

The village of Liberdade (20º10'S; 41º05'W) is located at the head of the Guandu river, a tributary of the Rio Doce in the midwestern region of Espírito Santo, about 100 km from the Atlantic coast. The area was first colonized about 85 years ago, when coffee plantations were established. Few remnants of the original forest remain and the space between houses in the village is generally occupied by plantations.

The valley in which Liberdade lies presents an altitudinal range of 650-1,000 m over a distance of approximately 6 km, extending in a Southeast-Northeast direction. The relatively short length of the valley made it possible to do simultaneous collections of sand flies at three altitudes. The lowest of these (650-750 m asl) is an active transmission area for ACL. The intermediate level (750-850 m) presented fewer cases of the disease and there was no transmission at the head of the valley (850-950 m).

Three modified Shannon traps (Shannon 1939) of dimensions 1 m X 1 m X 1.9 m (topped with a overhanging square roof of side 1.6 m) and three CDC traps (Sudia & Chamberlain 1962) were used to sample sand flies. One trap of each type was installed in each of the three altitudes and sampling was performed simultaneously. Shannon traps were installed in the peridomicile of houses while CDC traps were hung in nearby forest, at least 100 m from the houses. The three volunteers who manned the Shannon traps were randomly assigned to the different altitudes in order to avoid systematic error due to differences in their individual abilities to collect sand flies. Collections were made during the first 4 h after sunset on 3-5 consecutive nights and repeated at intervals of 4-6 weeks, until each trap had occupied each of the habitats sampled for 100 h.

Clearing and mounting of the insects followed the technique described by Barretto and Coutinho (1940). Specimens were identified according to Young and Duncan (1994).

In order to verify the occurrence of ACL among the human population, the number of cases of the disease recorded in the area between 1993 and 1998 was surveyed by checking local health service archives, the patients having been treated by our group.

The total number of insects collected in the peridomicile or forest during the first 4 h of darkness at each altitude was considered as the basic sampling unit used in statistical analysis of the results for the most abundant sand fly species.

Levene's homogeneity test and Kolmogorov-Smirnov's normality test were applied to the data in order to determine whether parametric or nonparametric tests should be applied to the data. Friedman's ANOVA was then applied in multiple samples and, when significant results were obtained, Wilcoxon's test was applied to pairs of samples. Spearman's nonparametric correlation test (Sokal & Rohlf 1979) was used to evaluate the correlation between the altitudes and the most abundant man-biting sand fly species.

The χ^2 (chi-square) test, with binomial approximation and a "*post-hoc*" Tuckey's test for multiple comparisons of proportions (Zar 1996) were used to study the relative proportions of clinical ACL cases and apparently healthy members of the human population.

RESULTS

Sand fly sampling was carried out over 50 nights, between November 1995 and February 1997. A total of 13,363 specimens belonging to 28 species was collected. The distribution of these insects at each altitudinal range and in each habitat type is shown in Table I. Among the man-biting species, *Lu. intermedia*, *Lu. migonei* and *Lu. whitmani* predominated in the peridomicile, *Lu. fischeri* was equally numerous in peridomiciliary and forest collections, and *Lu. monticola* was more abundant in the forest.

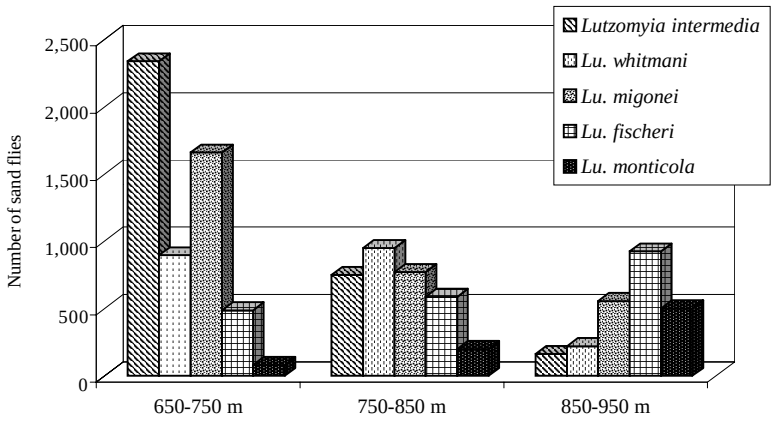
The altitudinal distributions of the five most abundant *Lutzomyia* species are shown in the Figure. Both *Lu. intermedia* and *Lu. migonei* presented distribution patterns inversely proportional to the altitude. The former was the most abundant species at the altitudes at which active transmission of ACL occurred, its numbers progressively decreasing up to the transmission-free level. Although *Lu. migonei* was the second most abundant species at the active transmission level, it was also the second most numerous sand fly at the ACL-free altitude. *Lu. whitmani* was slightly more numerous at the intermediate altitude than at the level at which active transmission occurred, and was scarce at the transmission-free altitude. Both *Lu. fischeri* and *Lu. monticola* showed inverse distribution patterns to those of *Lu. intermedia* and *Lu. migonei*. The most abundant species at the highest (transmission-free) altitude was *Lu. fischeri* and the number of specimens collected at this level was slightly higher than at lower altitudes. At the active transmission level *Lu. monticola* was the least abun-

TABLE I

Phlebotomine sand fly species collected in Liberdade, municipality of Afonso Cláudio, State of Espírito Santo, by Shannon and CDC traps, from November1995 to February 1997, distributed according to altitudes and the catching site

Species	Altitudinal range						Total	%
	650-750 m		750-850 m		850-950 m			
	Pd	Fr	Pd	Fr	Pd	Fr		
Lutzomyia intermedia (Lutz & Neiva)	2,149	189	718	33	159	4	3,252	24.3
Lu. migonei (França)	1,467	194	741	32	524	32	2,990	22.3
Lu. whitmani (Antunes & Coutinho)	609	288	910	41	193	28	2,069	15.4
Lu. fischeri (Pinto)	248	240	307	283	560	366	2,004	14.4
Lu. monticola (Costa Lima)	10	72	24	177	51	451	785	5.8
Lu. alencari Martins, Souza & Falcão	3	-	10	11	4	1	29	0.2
Lu. amarali (Barretto & Coutinho)	1	3	1	1	-	3	9	< 0.1
Lu. callipyga Martins & Silva	-	9	-	1	-	-	10	< 0.1
Lu. costalimai (Mangabeira)	-	2	-	-	-	-	2	< 0.1
Lutzomyia spp.[a]	11	50	7	12	1	1	82	0.6
Lu. edwardsi (Mangabeira)	36	144	5	18	6	12	221	1.6
Lu. ferreirana (Barretto, Martins & Pelegrino)	138	181	50	43	27	72	511	3.8
Lu. firmatoi (Barretto, Martins & Pelegrino)	243	315	28	23	1	17	627	4.6
Lu. hirsuta (Mangabeira)	-	1	-	-	-	-	1	< 0.1
Lu. lenti (Mangabeira)	21	7	7	1	9	-	45	0.3
Lu. matosi (Barretto & Zago)	-	2	-	-	-	-	2	< 0.1
Lu. microps (Mangabeira)	3	9	1	-	-	2	15	0.1
Lu. misionensis (Castro)	-	-	-	-	-	1	1	< 0.1
Lu. pascalei (Coutinho & Barretto)	-	1	-	2	2	3	8	< 0.1
Lu. pessoai (Coutinho & Barretto)	3	2	-	3	-	-	8	< 0.1
Lu. pestanai (Barretto & Coutinho)	-	-	1	-	-	-	1	< 0.1
Lu. quinquefer (Dyar)	406	62	112	14	28	3	625	4.6
Lu. sallesi (Galvão & Coutinho)	4	-	2	1	-	1	8	< 0.1
Lu. schreiberi Martins, Falcão & Silva	9	12	5	3	4	4	37	0.2
Lu. shannoni (Dyar)	-	-	-	-	-	5	5	< 0.1
Lu. termitophila Martins, Falcão & Silva	-	-	-	-	1	-	1	< 0.1
Brumptomyia avellari (Costa Lima)	1	-	-	-	-	-	1	< 0.1
B. cunhai (Mangabeira)	-	1	-	-	-	1	2	< 0.1
B. nitzulescui (Costa Lima)	-	3	-	-	1	-	4	< 0.1
Brumptomyia spp.[b]	2	4	1	1	1	-	9	< 0.1

a: *Lutzomyia* spp. refers to female specimens of the *migonei* group; *b*: *Brumptomyia* spp., to female specimens of the genus *Brumptomyia,* which can not be distinguished based on morphological characters; Pd: peridomicile; Fr: forest



Distribution of the most frequent man-biting sand fly species in relation to altitude, in the Liberdade valley, municipality of Afonso Cláudio, State of Espírito Santo, collected between November 1995 and February 1997.

dant of man-biting species, its numbers progressively increasing with altitude, until at the highest level it was more numerous than both *Lu. intermedia* and *Lu. whitmani*.

The results of the Levene's homogeneity tests, Kolmogorov-Smirnov's normality test, Friedman's ANOVA multiple samples test, Wilcoxon's sample pairs test and Spearman's nonparametric correlation test, applied to the data on the five most abundant man-biting species are shown in Table II. Among these species, *Lu. intermedia* presented a significantly negative correlation with altitude. Based on Wilcoxon's test, the number of specimens collected at the lowest altitude was significantly lower than that collected at the intermediate level. A significant drop was also seen between the latter and the highest level. By contrast, the numbers of *Lu. migonei* presented a significantly negative correlation with increasing altitude between the two lowest levels although the difference between the intermediate and highest levels was not significant according to Wilcoxon's test. A significantly negative correlation was seen between the numbers of *Lu. whitmani* and increasing altitude for the two highest levels but results were not significant when the lowest and intermediate levels were compared. *Lu. monticola* showed a significantly positive correlation with increasing altitude only when the lowest and highest levels were compared. Although a positive correlation was observed between numbers of *Lu. fischeri* sampled and altitude, this was not significant. This was confirmed using Friedman's analysis.

With respect to human disease, 22 cases of ACL were registered in the area from 1993-1998. The distribution of the cases according to the altitude is shown in Table III, and the number of healthy people in each level is represented by present residents who did not acquire the disease. The relative proportions of ACL cases at the three altitudes were significantly different ($\chi^2 = 7.78$; $p < 0.05$) and according to Tuckey's test, the proportion of ACL cases and apparently healthy individuals in the population only differed significantly between the lowest and highest levels.

DISCUSSION

The distribution of ACL in the study area seems to be influenced by altitude, the number of cases progressively decreasing from the lowest level, in which active transmission occurs, to the highest altitude, where the disease is absent. Sessa et al. (1994) surveyed residents of the area using the Montenegro skin test, which revealed that 29% were positive for *Leishmania* infection. The authors carried out this survey among individuals who lived below 700 m and no cases of leishmaniasis have been reported above this altitude since then. Other

TABLE II

Results of statistical tests applied to the data on the five most frequent species of man-biting sand flies

Species	Levene's Homogeneity	Kolmogorov-Smirnov's Normality	Friedman's Anova	Wilcoxon Low/ Intermediate			Spearman's Correlation	
				Low/ Intermediate	Intermediate/ High	Low/High		
Lutzomyia intermedia	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P = 0.04	P ≤ 0.01	R = - 0.44	P ≤ 0.01
Lu. migonei	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P > 0.05	P ≤ 0.01	R = - 0.33	P ≤ 0.01
Lu. whitmani	P ≤ 0.01	P ≤ 0.01	P ≤ 0.01	P > 0.05	P = 0.02	P ≤ 0.01	R = - 0.38	P ≤ 0.01
Lu. fischeri	P ≤ 0.01	P ≤ 0.01	P > 0.05	-	-	-	R = 0.15	P > 0.05
Lu. monticola	P = 0.02	P ≤ 0.01	P = 0.04	P > 0.05	P > 0.05	P ≤ 0.01	R = 0.27	P ≤ 0.01

TABLE III

Numbers (percentages) of clinical cases of American cutaneous leishmaniasis (ACL) and apparently healthy individuals at three altitudinal ranges in the Liberdade valley, municipality of Afonso Cláudio, State of Espírito Santo, 1993-1998

Altitude (m asl)	Clinical condition		Total
	ACL cases	Apparently healthy	
650-750 m[a]	19 (18.6)	83	102
750-850 m[ab]	3 (6.2)	45	48
850-950 m[b]	0	20	20
Total	22 (12.9)	148	170

Different letters represent significant differences

authors refer to the occurrence of ACL predominantly at elevations below 800 m (Bonfante-Garrido 1983, Gomes & Neves 1998) in tropical and subtropical regions of South America.

ANOVA of the altitudinal distribution of the five most abundant man-biting species indicated the possible role of each in the transmission of *Le. (V.) braziliensis* to man in the study area.

Among these five species, *Lu. monticola* appears to be better adapted to higher altitudes where ACL does not occur and therefore is probably not involved in *Le. braziliensis* transmission. Hermeto et al. (1994) noted a high degree of anthropophily and attraction to light by this sand fly in an ACL-endemic area situated at 680 m asl in the Brazilian State of Minas Gerais, where *Lu. monticola* comprised 43 (39.8%) of 108 sand flies collected. Falqueto (1995) collected large numbers of this species in the municipality of Venda Nova do Imigrante, Espírito Santo, (750 m asl) an area from which ACL has not been reported. Small numbers of *Lu. monticola* have been collected in ACL-endemic areas of the Brazilian States of São Paulo, Minas Gerais and Rio de Janeiro (Aguiar et al. 1987, Passos et al. 1993, Mayo et al. 1998).

The distribution of *Lu. fischeri* was similar at the three altitudes, suggesting that this species was not involved in the transmission of *Le. braziliensis*, although it is abundant in several areas where the parasite is endemic (Aguiar et al. 1987, Gomes & Neves 1998).

The uniform distribution of *Lu. migonei* at the intermediate and high levels indicates that this species may not be an important vector of *Le. (V.) braziliensis* in the study area. It is noteworthy that large numbers of this sand fly have been collected in domestic environments in several ACL-endemic areas of both Northeast and Southeast Brazil (Azevedo & Rangel 1991, Falqueto 1995, Gomes & Neves 1998). The dog, considered to be a reservoir of the parasite by some authors, is highly attractive to *Lu. migonei*, so that this species is suspected of maintaining canine ACL (Falqueto et al. 1986, 1991, Falqueto 1995, Marzochi & Marzochi 1997). There are also records of natural infection of *Lu. migonei* by *Le.* (*V.*) *braziliensis* in different regions of Brazil (Azevedo et al. 1990a, Queiroz et al. 1991).

It appears that *Lu. whitmani* is a secondary vector in the study area, since it occurred in similar numbers at the active and intermediate transmission levels. Although this species showed a low predisposition to enter houses in the Afonso Cláudio area, it was highly attracted to dogs and was the most man-biting species (Falqueto 1995). Working in this same area, Sessa et al. (1994) reported a high prevalence of natural infection by *Le. (V.) braziliensis* among dogs. In addition, there are many records of natural infection of *Lu. whitmani* by *Le. (V.) braziliensis* (Hoch et al. 1986, Azevedo et al. 1990b, Queiroz et al. 1991). This sand fly is therefore considered to be an important peridomiciliary vector in the main endemic areas of ACL in the Northeast of Brazil and in parts of the Southeast region (Mayrink et al. 1979, Azevedo & Rangel 1991, Lainson et al. 1994). In Espírito Santo, *Lu. whitmani* is abundant in only six municipalities of the midwest region, including Afonso Cláudio (Falqueto 1995).

Lu. intermedia appears to be the most important vector in the study area, since its decreasing frequency in samples from increasingly higher altitudes, coincides with the distribution of ACL cases. The results of other studies suggest that this species is the main vector of *Le. (V.) braziliensis* in Southeast Brazil, at least in areas situated below 800 m (Marzochi 1992, Lainson et al. 1994, Rangel et al. 1999). *Lu. intermedia* is abundant in many endemic areas of ACL, where it has adapted to habitats modified by human activity, showing an increased predisposition to enter houses and bite both man and dogs (Falqueto 1995, Marzochi & Marzochi 1997, Domingos et al. 1998). There are also records of natural infection with *Leishmania* (Forattini et al. 1972, Rangel et al. 1984). *Lu. intermedia* is the most abundant sand fly species in the principal ACL foci of Espírito Santo and other states of Southeast Brazil, providing further evidence of its role in the transmission of *Le. (V.) braziliensis* (Barros et al. 1985, Falqueto 1995, Gomes & Neves 1998, Rangel et al. 1999).

Finally, in considering the possible participation of *Lu. intermedia*, *Lu. migonei* and *Lu.*

whitmani in the transmission of *Le. (V.) braziliensis*, it should be taken into account that all three species were relatively abundant at the intermediate altitude, where ACL cases are rarely observed. This could be explained by the fact that a poor parasitism was found in dogs infected with *Le.* (*V.*) *braziliensis*, so that higher densities of the vector would be required to maintain the transmission cycle (Falqueto et al. 1986). The results of this study could thus assist in the development of new strategies to control the disease, based on continuous efforts to maintain sand fly populations below levels that permit transmission of *Le. (V.) braziliensis.*

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