Study on Phlebotomine Sand Fly (Diptera: Psychodidae) Fauna in Belo Horizonte, State of Minas Gerais, Brazil

Carina Margonari de Souza, Jose Eduardo Pessanha*, Ricardo Andrade Barata, Érika Michalsky Monteiro, Daniela Carmargos Costa, Edelberto Santos Dias/+

Centro de Pesquisas René Rachou-Fiocruz, Av. Augusto de Lima 1715, 30190-002 Belo Horizonte, MG, Brasil *Secretaria Municipal de Saúde de Belo Horizonte, Departamento de Zoonoses, Belo Horizonte, MG, Brasil

A study on the phlebotomine sand fly fauna in Belo Horizonte city, state of Minas Gerais, Brazil, was carried out. From April 2001 to March 2003, monthly systematic collections were performed in three houses from each of the nine regions of the city, using CDC light traps for four consecutive days. The traps were set into the houses and in peridomestic areas totaling 54 traps. A number of 3871 sand fly specimens of the genera Lutzomyia *and* Brumptomyia *were collected. Sixty eight percent of the specimens were* L. longipalpis *and 16%* L. whitmani, *insect vectors of visceral and American cutaneous leishmaniasis, respectively. Environmental factors such as temperature, humidity, and frequency of precipitation suggest that the number of insects increases after rainy periods. During the same period mentioned above, seasonal captures were carried out in parks and green areas of Belo Horizonte, using Shannon trap. A total of 579 phlebotomine sand flies were collected from which 398 (68.7%) were females with the predominance of* L. whitmani *and* L. monticola. *Those specimens were used for natural infection examination, by polymerase chain reaction. No* Leishmania *DNA was present in any of the specimens tested.*

Key words: Phlebotominae - leishmaniasis - epidemiology - Belo Horizonte - Brazil

Nowadays, leishmaniasis has been observed in large urban centers, showing a wide diversity and adaptation of parasites (*Leishmania*) and vectors to the environmental changes caused by man (Marzorchi & Marzorchi 1994, Ashford 2000, Travi et al. 2002, Azevedo et al. 2002). Such factors enable the existence of several clinic-epidemiological manifestations of the disease and make control measures difficult in those areas.

The first records of leishmaniasis cases in Belo Horizonte (BH) date from 1940 with the detection of 13 human cases of American cutaneous leishmaniasis (ACL) (Orsini 1940). Afterwards, several investigators have studied human and canine cases in the city (Passos et al. 1998, Silva et al. 2001).

Passos et al. (1998) performed an entomological survey in the Boa Vista quarter, a suburban area of BH, and in the district of Venda Nova, where the presence of *Lutzomyia whitmani* and *L. longipalpis*, the insect vectors of ACL and visceral leishmaniasis (VL), respectively, had been reported. Such studies associated with recent publications have confirmed VL (Silva et al. 2001) and ACL (Passos et al. 1993, 2001) urbanization in BH.

The present work intends to provide data for optimization of control measures, upon knowing seasonal fluctuations, habitat and the behavior of phlebotomine sand flies.

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+Corresponding author. Fax: +55-31-3295.3115. E-mail: edel@cpqrr.fiocruz.br
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MATERIALS AND METHODS

Study area - The city of Belo Horizonte (BH), in the state of Minas Gerais, Brazil, has a population of 3,420,000 inhabitants (IBGE 2000) and great part of the population live in substandard accommodations, without basic sanitation and hygiene conditions. Lack of infra-structure associated with environmental factors enables the dissemination of several diseases, including leishmaniasis.

Systematic collections - BH is formed by nine regional areas: Barreiro, Centro Sul (Center-South), Leste (East), Nordeste (Northeast), Noroeste (Northwest), Norte (North), Oeste (West), Pampulha, and Venda Nova. Three residences were chosen for each region totaling 27 houses for insect collection (Fig. 1). Selected residents signed a consent term in order to assure their participation and collaboration during the period of the study. Systematic collections began in April 2001 after a detailed evaluation of the nine regions comprising BH. Several ecological and environmental factors were observed for a posterior selection of the houses to be part of the present study. The presence of a favorable environment for reproduction and development of sand flies was one of the pre-requisites required for the selection of the target-houses. The houses under study had the following features: large back yards, orchard with plants and domestic animals (dogs, chickens, birds, and others). Orientation about the selection of the collection sites of each region was provided by the Municipal Health Secretary of BH (MHSBH), through which data on leishmaniasis prevalence in dogs and man was assessed. Regions with higher leishmaniasis prevalence rates were determined as being searching areas of residences that fit the demands of the present investigation.

Two CDC light traps (Sudia & Chamberlain 1962) were placed for each house: one into the house and another in

the peridomiciliary area, totaling 54 traps. Collections were performed on the last four days of each month, from April 2001 to March 2003. The traps were put at 5:00 pm and removed at 7:00 am of the following day. The insects collected each night were killed in chambers with 70% ether P./a solution and then they were placed in hemolysis tubes with 70% alcohol solution, labeled and taken to the laboratory. All specimens collected were identified based on their morphological characteristics, according to the identification-key proposed by Young and Duncan (1994).

Non-systematic collections - Four seasonal collections were carried out in different parks and green areas of each region, totaling 36 collections in two consecutive years. All collection sites were georeferred by GPS (global positioning system) and located in the map of BH city (Fig. 1). Luminous Shannon traps (Shannon 1939) were used between 5:30 pm and 10:00 pm, totaling 162 h of collection. The insects were collected alive with the help of a manual suction tube and then transferred to chambers with humidified plaster bottoms and covered with a cloth. Afterwards, they were taken to the laboratory, killed in the freezer, identified and then submitted to DNA extraction according to Margonari et al. (2004). DNA samples were used for *Leishmania* spp. search by polymerase chain reaction (PCR).

PCR - A volume of 2 µl DNA (10ng/ ml) was added to the amplification mix with 10 µl buffer solution (100 mM TRIS-HCl, 500 mM KCl, 15 mM $MgCl_2$, pH 9.0), 5 µl dNTPs (2 mM each), 2 µl of each primer (200 ng/µl), 0.5 ml Taq polymerase (2.5 U/µl), and 26.5 µl mili-Q water in a final volume of 60 ml. Specific primers for the genus *Leishmania* were used: 5'GGG GAG GGG CGT TCT GCG AA3', 5'CCG CCC CTA TTT TAC ACC AAC CCC3', 5'GGC CCA CTA TAT TAC ACC AAC CCC3'. Amplification cycles were: at 94°C for 4 min, followed by 35 cycles at 94°C for 30 s; 60°C for 30 s; and finally at 72°C for 30 s in the thermocycler GeneAmp PCR System 2400 (Perkin Elmer) (Michalsky et al. 2002).

PCR products were visualized on 2% ethidium bromide stained agarose gels.

Statistical analysis - It was performed with the software SigmaStat (Jandel 1995) by which likely environmental interferences were determined for the phlebotomine sand fly fauna in BH.

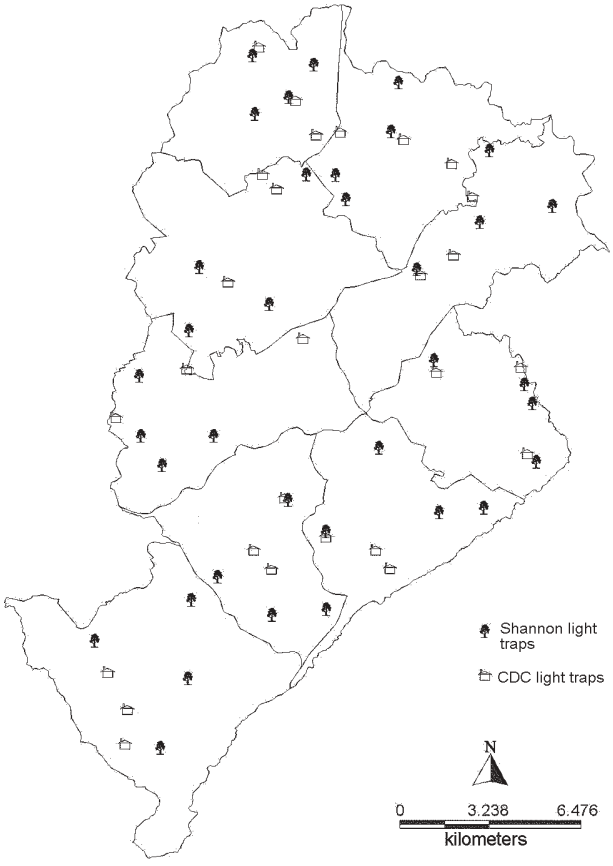

Fig. 1: sites of CDC light and Shannon traps for phlebotomine sand fly collections from April 2001 to March 2003 in Belo Horizonte, Minas Gerais.

RESULTS

A number of 3871 specimens of phlebotomine sand flies were collected in of BH from April 2001 to March 2003. The phlebotomine fauna collected comprised 15 different species and the most frequent were: *L. longipalpis* (68.23%) and *L. whitmani*, (16.04%). The least frequent species were: *L. sallesi* (6.77%), *L. intermedia* (1.42%), *L. firmatoi* (0.75%), *L. pessoai* (0.46%), *L. monticola* (0.34%), *L. quinquefer* (0.34%), *L. termitophila* (0.15%), *L. lenti* (0.09%), *Brumptomyia* sp. (0.08%), *L. longipennis* (0.05%), *L. misionensis* (0.05%), *L. aragaoi* (0.03%), and *L. migonei* (0.03%). Four percent out of the total amount of collected insects could not be identified due to mutilation caused by the traps or technical problems (Table I).

Although the target-houses showed very similar ecological characteristics, a heterogeneous entomological collection was observed both in the number of insects (Table II) and in the species diversity: Barreiro – 146 phlebotomine sand flies (3.8%), Centro Sul – 34 (0.9%), Leste – 282 (7.3%), Nordeste – 1114 (28.8%), Noroeste – 60 (1.5%), Norte – 565 (14.6%), Oeste – 991 (25.6%), Pampulha - 475 (12.3%), and Venda Nova – 204 (5.3%) (Table II).

Concerning diversity, *L. longipalpis* was the predominant species in the following regions: Leste, Nordeste, Noroeste, Oeste, Pampulha, and Venda Nova. The species *L. lenti* was the most collected one in Barreiro whereas *L. sallesi* and *L. monticola* showed to be numerous in Centro Sul. *L. whitmani* was the most frequent in (the North region) Norte (Table III).

In general terms, the number of collected phlebotomine sand flies showed to be higher in the peridomiciliary areas than into the houses. The species *L. whitmani* had similar concentrations in both environments (Tables III, IV).

Analysis on the phlebotomine sand fly populations according to the variables, monthly average temperature, relative humidity and frequency of precipitation, showed no significant statistical differences (Fig. 2).

Analysis on the insects which were proven to be ACL vectors (*L. intermedia, L. pessoai, L. whitmani*) versus the number of ACL human cases, during the period of our investigation, suggests that there is an increasing trend

TABLE I

Monthly number of phlebotomine sand flies separated by gender and species, collected in Belo Horizonte, MG, with the CDC light trap between April 2001 and March 2003

Year	Month	*Brumptomyia* sp.		*L. aragoi*		*L. firmatoi*		*L. intermedia*		*L. lenti*		*L. longipalpis*		*L. longipennis*		*L. migonei*		*L. misionensis*		*L. monticola*		*L. pessoai*		*L. quinquefer*		*L. sallesi*		*L. termitophila*		*L. whitmani*		*Luzomyia* sp.		
		F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	%
2001	April	1	0	0	0	0	0	8	5	0	0	11	77	0	0	0	0	0	0	4	0	0	1	0	0	6	6	0	0	15	34	15	6	4.9
	May	0	0	0	0	0	0	0	0	0	0	15	141	0	0	0	0	0	0	0	0	0	0	0	0	3	8	0	1	7	10	5	4	5.0
	June	0	0	0	0	0	0	0	3	0	0	11	173	0	0	0	0	0	0	0	0	0	0	0	0	7	4	0	0	11	13	5	1	5.9
	July	0	0	0	0	0	0	0	4	0	1	9	106	0	0	0	0	0	0	0	0	2	3	0	0	7	3	0	0	5	13	11	0	4.2
	August	0	0	0	0	0	0	1	0	1	0	3	66	0	0	0	0	0	0	0	0	1	2	0	0	10	1	0	1	27	22	3	2	3.6
	September	0	0	0	0	0	0	0	2	0	0	6	14	0	0	0	0	0	0	1	0	1	4	0	0	10	6	0	0	12	11	6	0	1.9
	October	0	0	0	0	0	0	1	0	0	0	6	22	0	0	0	0	0	0	0	0	0	0	0	0	6	3	0	1	1	3	3	0	1.2
	November	0	0	0	0	0	0	2	1	0	2	7	103	0	1	0	0	0	0	0	0	1	0	1	1	9	8	0	0	3	5	5	6	4.0
	December	1	0	0	0	2	2	1	0	8	17	12	109	0	0	0	0	0	0	0	0	0	0	0	0	14	8	1	0	5	2	1	2	4.8
2002	January	0	0	0	0	1	0	0	0	0	0	18	128	0	0	0	0	0	0	0	0	0	1	2	0	6	5	0	2	1	5	1	2	4.4
	February	1	0	0	0	0	3	0	2	0	0	27	303	0	0	0	0	0	1	1	0	0	0	1	0	8	4	0	0	1	7	3	12	9.7
	March	0	0	0	0	0	0	2	3	0	0	23	256	0	0	0	0	0	0	1	0	0	0	0	0	3	1	0	0	6	13	20	6	8.6
	April	0	0	0	0	0	0	0	0	0	0	16	170	0	0	0	0	0	0	1	0	0	0	0	0	5	1	0	0	7	9	0	0	5.4
	May	0	0	0	0	0	0	4	0	0	0	13	120	0	0	0	0	0	0	0	0	0	0	0	0	6	1	0	0	43	34	3	0	5.8
	June	0	0	0	0	0	0	1	1	0	0	6	32	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	16	11	0	3	1.9
	July	0	0	0	0	1	0	2	4	0	0	2	34	0	0	0	0	0	0	0	0	0	0	0	0	4	2	0	0	25	63	6	0	3.7
	August	0	0	0	0	5	2	2	0	0	0	12	59	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	22	28	3	3	3.6
	September	0	0	0	0	0	0	1	0	0	0	3	40	0	0	0	0	0	0	0	0	0	1	0	0	3	1	0	0	6	11	0	1	1.7
	October	0	0	0	0	0	0	0	3	0	1	10	68	0	0	0	0	0	0	0	0	0	0	0	0	17	2	0	0	3	28	2	3	3.5
	November	0	0	0	0	1	1	0	1	0	0	18	44	0	0	0	0	0	0	0	0	0	1	0	0	13	5	0	0	6	28	8	3	3.3
	December	0	0	0	0	1	5	0	0	0	0	6	27	0	0	0	0	0	0	1	0	0	0	1	0	6	6	0	0	9	6	0	0	1.8
2003	January	0	0	0	0	2	0	0	0	0	2	34	117	0	1	1	0	0	0	0	0	0	0	4	0	10	3	0	0	1	16	6	3	5.2
	February	0	0	0	0	1	0	0	0	1	0	26	89	0	0	0	0	0	0	4	0	0	0	1	0	11	11	0	0	1	13	1	6	4.3
	March	0	0	0	1	1	1	1	0	0	1	16	33	0	0	0	0	0	1	0	0	0	0	2	0	4	0	0	0	0	3	0	0	1.7
	Total	3	0	0	1	15	14	26	29	10	24	310	2331	0	2	1	0	0	2	13	0	5	13	12	1	172	90	1	5	233	388	107	63	

M: males; F: females

TABLE II

Number of phlebotomine sand flies collected in Belo Horizonte, MG, with the CDC light trap between April 2001 and March 2003

Year	Month	Barreiro		South Center		East		Northeast		Northwest		North		West		Pampulha		Venda Nova		
		F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	Total
2001	April	4	8	8	0	2	26	2	7	0	2	28	25	2	27	5	21	5	7	179
	May	0	2	0	0	2	9	4	34	2	1	7	6	11	98	2	13	2	1	194
	June	2	1	0	0	0	7	3	10	1	0	10	14	8	155	4	4	6	3	228
	July	3	3	0	0	0	2	5	24	2	1	14	17	11	76	0	2	0	4	164
	August	5	1	0	1	1	7	0	13	1	1	30	19	9	47	0	4	0	1	140
	September	1	4	0	0	2	10	4	3	2	2	13	7	4	3	4	1	6	7	73
	October	0	1	0	0	1	7	1	2	1	2	3	2	3	11	4	3	4	1	46
	November	0	2	0	2	1	11	10	74	0	0	11	12	2	17	1	3	3	6	155
	December	12	21	0	0	2	3	8	24	0	2	8	4	7	78	5	3	3	5	185
2002	January	1	6	0	0	4	23	9	54	1	0	6	2	3	52	1	1	4	5	172
	February	2	4	0	0	6	31	17	258	2	2	3	6	3	4	2	16	6	11	373
	March	3	0	3	2	4	41	5	43	1	1	5	15	7	102	20	64	7	11	334
	April	0	4	0	0	4	24	2	23	3	1	2	1	9	98	7	29	6	6	219
	May	0	1	0	1	1	1	49	41	2	1	1	2	7	100	5	6	4	2	224
	June	0	0	1	0	0	1	6	32	1	0	11	5	0	1	5	7	2	1	73
	July	1	0	0	0	0	0	3	24	0	0	33	73	0	5	1	0	2	1	143
	August	6	2	0	0	3	9	6	43	0	1	26	20	0	10	2	1	2	7	138
	September	0	0	0	0	0	3	7	40	0	0	6	4	0	4	0	1	0	2	67
	October	1	1	5	0	2	4	3	37	1	4	3	27	1	0	16	30	0	2	137
	November	1	1	0	1	0	5	10	29	0	2	10	30	0	0	24	13	1	2	129
	December	1	7	2	1	3	19	4	7	0	0	7	7	1	1	2	1	4	1	68
2003	January	14	2	0	0	1	0	4	59	4	2	5	13	3	3	22	46	6	17	201
	February	7	3	7	0	0	0	8	56	1	3	1	8	1	7	11	31	10	11	165
	March	4	4	0	0	0	0	1	6	3	4	1	2	0	0	9	23	6	1	64
	Total	68	78	26	8	39	243	171	943	28	32	244	321	92	899	152	323	89	115	3871

M: males; F: females

TABLE III

Number of phlebotomine sand flies collected into houses (I) and in peridomiciliary (P) areas of Belo Horizonte, MG, separated by species and region, with the CDC light trap between April 2001 and March 2003

	Brumptomyia sp.		*L. aragoi*		*L. firmatoi*		*L. intermedia*		*L. lenti*		*L. longipalpis*		*L. longipennis*		*L. migonei*		*L. misionensis*		*L. monticola*		*L. pessoai*		*L. quinquefer*		*L. sallesi*		*L. termitophila*		*L. whitmani*		*Lutzomyia* sp.		Total
	I	P	I	P	I	P	I	P	I	P	I	P	I	P	I	P	I	P	I	P	I	P	I	P	I	P	I	P	I	P	
Barreiro	1	2	0	0	10	17	0	0	16	13	4	1	0	0	1	0	0	2	1	1	8	4	1	7	18	8	2	1	4	15	4	5	146
South Center	0	0	0	0	0	0	0	0	0	0	5	5	0	0	0	0	0	0	4	4	0	1	0	0	8	2	0	0	0	0	3	2	34
East	0	0	0	0	1	0	1	1	1	0	4	232	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	3	17	1	18	282
Northeast	0	0	0	0	1	0	3	8	0	1	137	756	0	0	0	0	0	0	0	0	0	0	0	0	23	24	0	0	26	94	15	26	1114
Northwest	0	0	0	0	0	0	0	0	0	0	17	11	0	0	0	0	0	0	0	0	0	0	0	0	14	9	0	0	2	5	2	0	60
North	0	0	0	0	0	0	28	10	0	1	39	4	1	1	0	0	0	0	1	1	2	3	3	1	25	18	0	1	242	139	22	23	565
West	0	0	0	0	0	0	0	2	0	0	15	905	0	0	0	0	0	0	0	0	0	0	0	0	4	33	1	0	0	17	1	13	991
Pampulha	0	0	0	0	0	0	0	0	0	1	259	129	0	0	0	0	0	0	0	0	0	0	0	0	19	24	0	0	12	7	19	5	475
Venda Nova	0	0	0	1	0	0	0	2	0	1	47	71	0	0	0	0	0	0	0	0	0	0	0	0	7	26	0	0	4	34	6	5	204
Total	1	2	0	1	12	17	32	23	17	17	527	2114	1	1	1	0	0	2	6	7	10	8	4	9	118	144	4	2	293	328	73	97	3871

TABLE IV

Total of phlebotomine sand fly males (M) and females (F) collected with the CDC light trap into the houses and in peridomiciliary areas of Belo Horizonte, MG, between April 2001 and March 2003

	Environment				
	Inside the houses		Peridomiciliary		
Species	F	M	F	M	%
Brumptomyia sp.	1	0	2	0	0.08
Lutzomyia aragoi	0	0	0	1	0.03
L. firmatoi	5	7	10	7	0.75
L. intermedia	15	17	11	12	1.42
L. lenti	7	10	3	14	0.09
L. longipalpis	111	416	199	1915	68.23
L. longipennis	0	1	0	1	0.05
L. migonei	1	0	0	0	0.03
L. misionensis	0	0	0	2	0.05
L. monticola	6	0	7	0	0.34
L. pessoai	3	7	2	6	0.46
L. quinquefer	3	1	9	0	0.34
L. sallesi	78	40	94	50	6.77
L. termitophia	0	4	1	1	0.15
L. whitmani	117	176	116	212	16.04
Lutzomyia sp.	57	16	50	47	4.39
Sub-total	404	695	504	2268	
Total	1099	2772	100		

of ACL cases just after the highest population densities of the vectors (Fig. 3). The same was observed when the number of *L. longipalpis* specimens and VL human cases were assessed (Fig. 4).

Collections performed with the Shannon traps in the parks and green areas of BH comprised 579 phlebotomine sand flies of the following species: *L. whitmani* (60.6%), *L. monticola* (22.3%), *L. pessoai* (5.2%), *L. longipalpis* (2.6%), *L. misionensis* (2.1%), *L. intermedia* (1.7%), *L. sallesi* (1.4%), *L. fischeri* (0.5%), *L. shannoni* (0.5%), *L. termitophila* (0.3%), *L. bianchigalatiae* (0.2%), and *L. sordellii* (0.2%). Out of the, insects collected, a rate of 2.4% could not be identified (TableV).

Females collected – 398 specimens (69.3%) – and submitted to an evaluation regarding natural infection by *Leishmania* had their results negative through the PCR technique.

DISCUSSION

A favorable environment to phlebotomine reproduction associated with the presence of domestic animals in peridomiciliary areas may explain the high number of insects found compared to inner houses under study. The presence of domestic animals in the houses was recorded as follows: 66.7% with dogs; 48.2% with poultry; 18.5% with rodents; 7.4% with cats; 3.7% with skunk, goats and horses, and equine.

The highest population density was represented by the species *L. longipalpis* in BH during the period studied. Such species has been described in several endemic areas of VL in Brazil and in several Latin American coun-

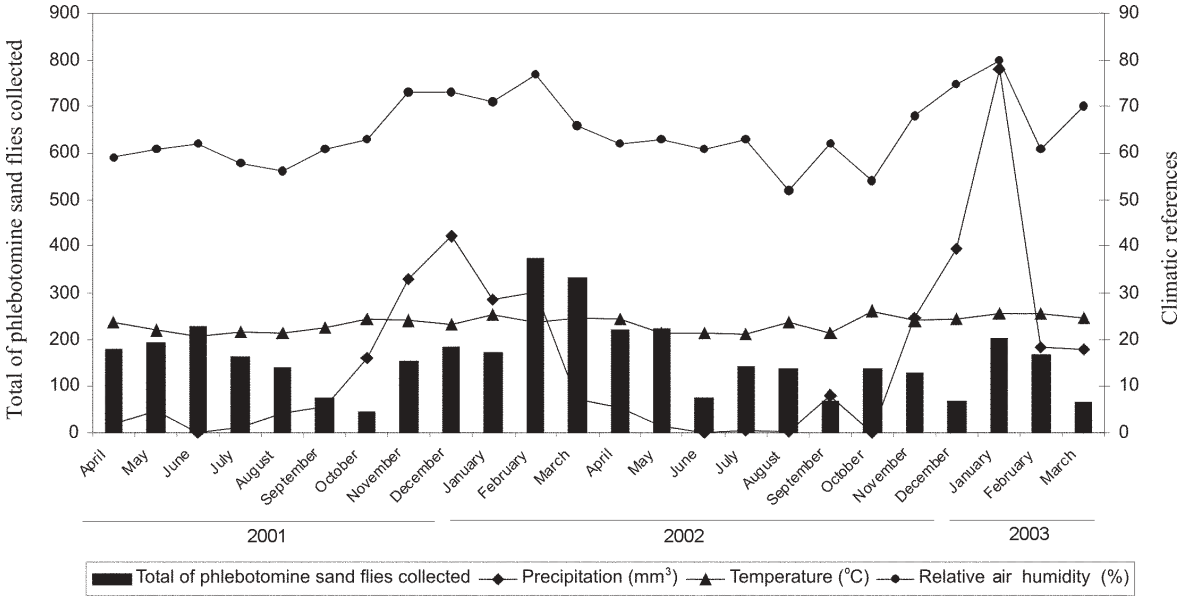

Fig. 2: total of phlebotomine sand flies collected between April 2001 and March 2003 in Belo Horizonte according with monthly average temperature, relative air humidity, and precipitation.

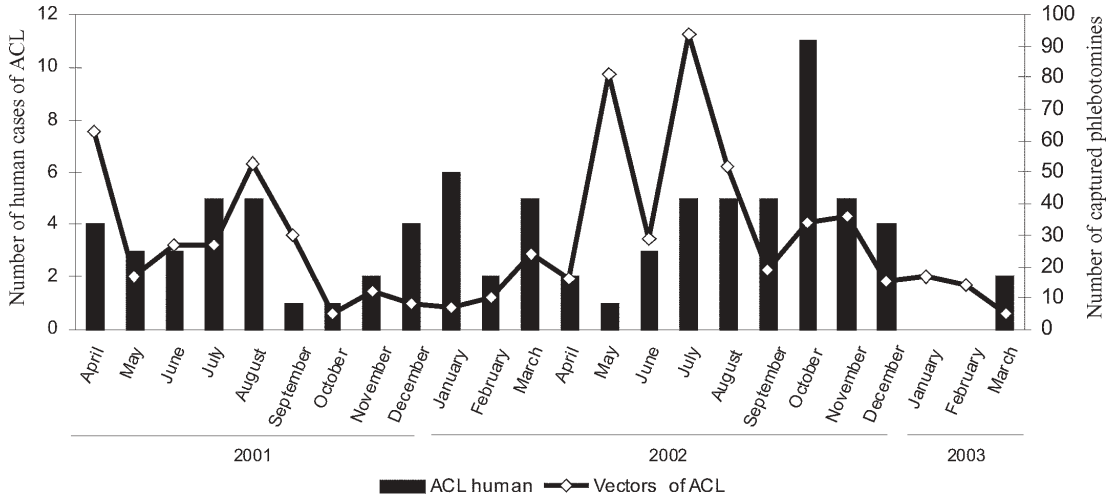

Fig. 3: total of phlebotomine sand flies, vectors of American cutaneous leishmaniasis (ACL) (*Lutzomyia whitmani*, *Lutzomyia pessoai*, *Lutzomyia intermedia*), collected between April 2001 and March 2003 in Belo Horizonte and the number of human cases.

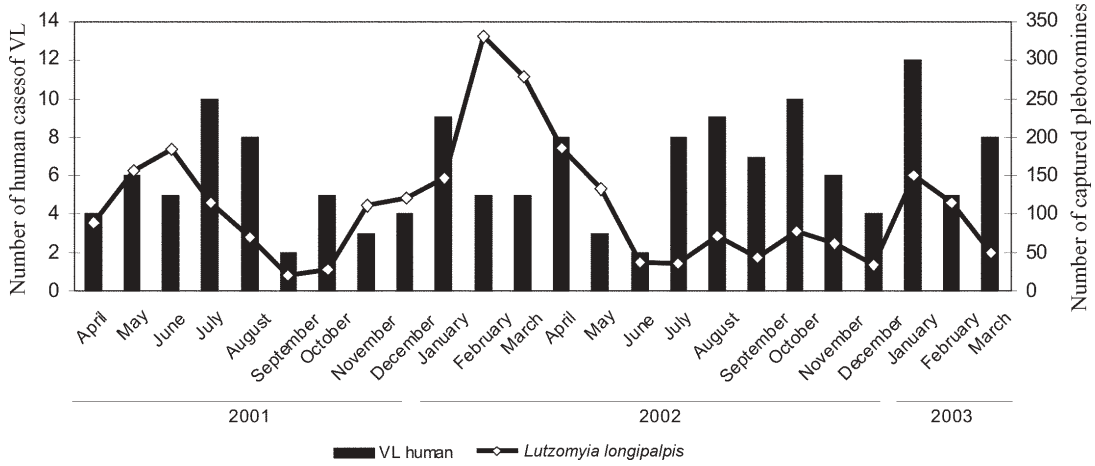

Fig. 4: total of phlebotomine sand flies, vectors of visceral leishmaniasis (VL) (*Lutzomyia longipalpis*), collected between April 2001 and March 2003 in Belo Horizonte and the number of human cases.

TABLE V

Species of phlebotomine sand flies collected with the Shannon trap in parks and green areas of Belo Horizonte, MG, between April 2001 and 2003

Species	Species of phlebotomine		
	Females	Males	Total (%)
L. bianchigalatiae	1	0	1 (0.2)
L. fischeri	1	2	3 (0.5)
L. intermedia	2	8	10 (1.7)
L. longipalpis	5	10	15 (2.6)
L. misionensis	9	3	12 (2.1)
L. monticola	127	2	129 (22.3)
L. pessoai	10	20	30 (5.2)
L. sallesi	8	0	8 (1.8)
L. shannoni	3	0	3 (0.5)
L. sordelli	1	0	1 (0.2)
L. termitophila	2	0	2 (0.3)
L. whitmani	218	133	351 (60.6)
Lutzomyia sp.	11	3	14 (2.4)
Total	398	181	579 (100)

tries. Its high vectorial capacity (Deane 1956, Killick-Kendrick 1990) associated with its high anthropophilia and the presence of the insect in peridomiciliary areas turn the species *L. longipalpis* as the greatest medical importance phlebotomine sand fly (Lainson & Shaw 1998, Soares & Turco 2003). This species has been collected through several punctual studies carried out in BH. In 1997, aimed at knowing habits and behaviour of phlebotomine sand flies, entomological collections were undertaken by the Municipal Department of Health in some sites of the city. The results showed a predominance of *L. longipalpis* (Resende et al. 2004). Afterwards, Passos et al. (1998) also demonstrated the presence of *L. longipalpis* and *L. whitmani* in other locations of Belo Horizonte.

Probably due to the adaptability to urban micro environments, the species *L. longipalpis* was the most frequently found in six of the nine regions under study.

L. whitmani, however, has been regarded to be a species "under adaptation" to the urban environment by several investigators due to the fact that it is collected in high concentrations in thickly forested areas and in peridomiciliary areas of large centers (Camargo-Neves et al. 2002, Dos Santos et al. 2003, Salomón et al. 2003, Oliveira et al. 2003) and it has been associated with peridomiciliary transmissions in Brazilian Northeastern and Southeastern areas (Cuba-Cuba et al. 1985, Azevedo & Rangel 1991, Rangel et al. 1996). Due to this fact, several studies have been undertaken in order to better understand the biology of *L. whitmani*. Particular features regarding genetics and adaptation of the species allow it to have different feeding and behavioral habits, depending on the habitat they live in (Rangel et al. 1996, Ready et al. 1997, 1998, Campbell-Ledrum et al. 2000, Azevedo et al. 2002, Margonari et al. 2004). Upon collecting *L. whitmani* in BH, it could be observed that the species is present in peridomiciliary areas as well as inside the houses (Tables IV, V), besides being predominant in thickly forested areas (Table V). Such observation has revealed that *L. whitmani* may be considered totally adapted to large urban areas being able to live and reproduce in this environment. These results also suggest that ACL has been transmitted not only in peridomiciliary areas but also into the houses.

Therefore, although *L. intermedia* is considered to be the most important ACL vector in the Southeast of Brazil (Marcondes et al. 1997, Laison & Shaw 1998), *L. whitmani* is probably the responsible insect vector for ACL transmission in BH.

In literature data, climatic factors have been reported to influence phlebotomine sand fly populations, depending on the region under study. Usually, phlebotomines are found in high densities during rainy summer months (Gomes & Galati 1987, Salomón et al. 2003). On the other hand, Condino et al. (1998) have reported no relationship between such factors and the phlebotomine sand fly density. In Porterinha (north of the state of Minas Gerais), frequency of precipitation showed to affect directly the amount of phlebotomine collected in the region (Barata et al. 2004).

Rebêlo (2001a) have showed that there was a high frequency of *L. longipalpis* during the whole year in São Luis Island, with an increasing trend in rainy periods when compared to the dry periods. Temperature, humidity, and precipitation showed no influence in monthly insect density. However, precipitation affected seasonal distribution of *L. longipalpis*.

In the states of Ceará, Bahia, and Mato Grosso do Sul, a higher population density of phlebotomine sand flies has been observed during the rainy periods (Deane 1956, Sherlok & Guilton 1969, Galati et al. 1997), while in Costa Rica, higher densities occur in the dry periods (Zeledón et al. 1984) In Amazônia Maranhense, there have been abundant phlebotomine species during the rainy periods, others in dry periods and several species irregularly distributed throughout the year, *L. longipalpis* and *L. whitmani* (Rebêlo et al. 2001b).

Analysis on climate influences in phlebotomine sand fly populations in BH showed no statistically significant results. However, there is an increasing trend in the number of phlebotomine insects just after rainny periods (Fig. 2).

The higher population densities of phlebotomine sand flies may be associated with leishmaniasis cases in the city under study. This association was reported by Rebêlo (2001a) for the municipality of Buriticupu (state of Maranhão) where high densities of *L. whitmani* were coincident with increasing ACL human cases. In the present work, a similar analysis provided information on the same trend, i.e, increasing numbers of ACL human cases just after higher population densities of insect vectors (Figs 3, 4).

The CDC traps have been systematically used in studies on phlebotomine populations. Previous investigations have proven that the single use of CDC traps does not allow the identification of phlebotomine fauna concerning its quantitative and qualitative features (Azevedo et al. 2002, Oliveira et al. 2003). Based on the results of the current work, the use of Shannon traps allowed us to evaluate the presence of some species that were not collected

by CDC traps such as *L. bianchigalatiae*, *L. fischeri*, *L. shannoni*, and *L. sordellii*, corroborating previous reports.

Females collected by Shannon traps and submitted to examination of possible *Leishmania* sp. natural infection, showed negative results through PCR. This technique has been widely used for diagnosis purposes (Passos et al. 1993) and it has been shown to be highly sensitive in detecting protozoa parasites into phlebotomine sand flies (Rodriguez et al. 1999, Miranda et al. 2002, Michalsky et al. 2002). Nevertheless, previous reports have shown that phlebotomine infectivity rates in nature are low, 0.2 to 2%, making the parasite finding difficult (Rodriguez et al. 1999, Miranda et al. 2002).

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