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Risk factors associated with asymptomatic infection by *Leishmania chagasi* in north-east Brazil

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Abstract

Various factors have been associated with a predisposition to the development of clinical American visceral leishmaniasis (AVL). However, little information is available about the factors that predispose to asymptomatic infection. To identify the risk factors associated with asymptomatic infection, a study was carried out between July 1997 and June 1998 on children aged 0–5 years in the districts of Vila Nova and Bom Viver in the municipality of Raposa in the island of São Luís, State of Maranhão, Brazil. A questionnaire containing socioeconomic, demographic and epidemiological data was used. The delayed-type hypersensitivity (DTH) test was carried out on 639 children in the first phase, and on 572 in the second, 7 months after the first survey, using *Leishmania amazonensis* antigen. Infection was determined by enzyme-linked immunosorbent assay (ELISA) in 638 children during the first phase, and in 572 during the second. Six outcome measures were used: initial prevalence, final prevalence and incidence, each determined by DTH and ELISA. The incidence of infection was 10·8% when determined by DTH and 28·5% when determined by ELISA. After adjustment for confounding variables using Cox regression, infection by *L. chagasi* was associated with child's age (≥2 years), location of the dwellings (Vila Nova) and reporting of relatives with AVL. Bathing outside the house and playing outdoors between 18:00 and 20:00 were identified as risk factors in some analyses but not in others. Presence of intra- and peridomestic *Lutzomyia* sandflies and animals such as dogs or chickens in the house or in the neighbourhood appeared as risk factors in some analyses but in others they unexpectedly seemed to protect from infection. Malnutrition was not found to be associated with infection.

Keywords: visceral leishmaniasis, *Leishmania chagasi*, asymptomatic infection, children, prevalence, incidence, risk factors, Brazil

Introduction

American visceral leishmaniasis (AVL) is an infectious disease causing significant immunological changes that lead to alterations in both cellular and humoral immunity. Of these, alterations in humoral immunity have been used as a criterion for diagnosis of the infection owing to the presence of elevated titres of specific antibodies (BADARÓ *et al.*, 1986a; WHO, 1990; FNS, 1996).

AVL is caused by the parasite *Leishmania chagasi* and is transmitted by the bite of a *Lutzomyia* sandfly. It has been recently determined that large numbers of individuals in endemic areas are infected with the parasite but do not develop the classical signs and symptoms of the disease. A recent advance in our ability to identify persons in this group has been the development of a *Leishmania*-specific antibody test and skin test for the detection of leishmanial infection (BADARÓ *et al.*, 1986b; REED *et al.*, 1986).

An enzyme-linked immunosorbent assay (ELISA) has been used in both Brazil and Kenya to monitor asymptomatic and subclinical disease in addition to diagnosing acute AVL, and epidemiological studies have shown that the ratio of infected people, either asymptomatic or with subclinical disease, to those with classical symptoms of AVL ranges from 4 to 30:1 (BADARÓ *et al.*, 1986a; JAHN *et al.*, 1986; EVANS *et al.*, 1992).

In endemic areas of north-east Brazil, it is estimated that 7·5% of individuals aged <15 years are infected by *Leishmania* each year, and about 20% of those infected by *L. chagasi* develop the classical form of the disease (BADARÓ *et al.*, 1986a; WHO, 1990). The highest prevalence of human AVL occurs in children aged 0–9 years, who account for 80% of cases; of these, 60% are aged <5 years (BADARÓ *et al.*, 1986a).

Various factors have been associated with a predisposition to the development of AVL disease, such as young age, malnutrition (BADARÓ *et al.*, 1986a; CERF *et al.*, 1987), poor lymphocyte proliferation, low production of gamma interferon (CARVALHO *et al.*, 1992, 1994), and exposure to *L. chagasi*. However, little information is available about the factors that predispose to asymptomatic infection. According to EVANS *et al.* (1992), the factors that determine the development of infection after transmission of *L. chagasi* by *Lutzomyia* sandflies have not been completely clarified. The island of São Luís in Maranhão is currently experiencing an epidemic of AVL, accounting for 65% of the total cases for the state (FNS, 1996). To identify possible risk factors that may lead to asymptomatic infection by *L. chagasi* after exposure, the delayed-type hypersensitivity (DTH) and ELISA tests were used in a population of north-east Brazil.

Subjects and Methods

A prospective study was carried out between July 1997 and June 1998 on all children aged 0–5 years in the localities of Vila Nova and Bom Viver in the municipality of Raposa, Maranhão.

Description of the area

The island of São Luís has an area of 905 km^2 and contains the municipalities of São Luís, São José de Ribamar, Paço do Lumiar and Raposa. The municipality of Raposa is situated 28 km from the city of São Luís, the state capital of Maranhão. The area of the municipality is 63·9 km^2 and the population consists of 15 075 inhabitants distributed among 21 districts, including Vila Nova and Bom Viver, the focus of the present study. The districts of Vila Nova and Bom Viver originated from recent land occupations, with respective populations of 2600 and 4307 inhabitants. The economic activity of the 2 localities is based on fishing and crafts. These localities were selected because they are endemic for AVL and have poor populations with little information concerning the disease (GAMA *et al.*, 1997). In the past 3 years, the incidence of AVL observed in children aged 0–5 years has varied in these districts: 22·8 cases per 1000 children in 1995,

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9·4 cases per 1000 children in 1996 and 13·3 cases per 1000 children in 1997 (unpublished data obtained from the National Health Foundation).

Study design

The study was started in July 1997 with a population survey and then divided into 2 phases. The first (September–October 1997) involved a cross-sectional study using a standardized questionnaire applied to mothers/guardians and examination by the study group during which children's height was measured and the DTH and ELISA tests were performed. In the second phase (April–May 1998) the DTH and ELISA tests, and height measurements, were repeated 7 months after the first phase.

The mothers/guardians gave written informed consent for the children to participate in the study. The wishes and decisions of the mothers were always respected.

In the choice of variables, associations found in other endemic areas were taken into account (BADARÓ *et al.*, 1986c; CERF *et al.*, 1987; EVANS *et al.*, 1992). The variables contained in the questionnaire were: child's age (in months) and gender, father's and mother's literacy, type of housing, bathroom (indoors/outdoors), chronic malnutrition, place where the child plays in the early evening (indoors/outdoors), presence of animals (chickens, dogs or both), insecticide spraying during the previous year, AVL in relatives and neighbours, and *Lutzomyia* sandflies in the house.

Description of measurements and tests

The DTH was performed with *L. amazonensis* promastigotes (MHOMBR-88-BA-125) as previously described (REED *et al.*, 1986). The test was applied intradermally to the anterior forearm, and readings were taken at 48–72 h after application (CUBA *et al.*, 1985; WHO, 1990), with the diameter of the induration being measured with a millimetre ruler. One or both diameters of ⩾5 mm was considered a positive reaction. The test was carried out on 639 children aged 0–71 months in the first phase, and on 572 children aged 7–78 months in the second phase. The difference in the number of children between phases was due to migration occurring in these areas.

Antileishmanial antibodies were detected by ELISA using antigen prepared with *L. chagasi* in Bahia, Brazil. These antigens show high sensitivity (98%) and specificity (96%) for the detection of antibodies in sera from AVL patients (BADARÓ *et al.*, 1986b). The test was performed on 638 children in the first phase and on 572 in the second. Eight children with hepatosplenomegaly and unexplained fever of more than 1 week's duration were considered to be potential cases of leishmaniasis, as confirmed with bone-marrow aspirate, and were excluded from the study. Blood (2–4 mL) was collected from each child, placed in tubes without heparin and left to coagulate at room temperature. The samples were then centrifuged and serum was separated. The serological reaction was considered positive when the level of absorbance (cut-off) was ⩾0·045, this figure representing 2 SD above the average absorbance level of a sample of 20 sera from healthy, non-exposed individuals.

Child's height was measured using a portable anthropometer and recorded to the last 0·1 cm. Children younger than 2 years were measured in the supine position and the others in the upright position. Data regarding the nutritional status of the children were analysed using the National Center for Health Statistics (NCHS) standards. *Z*-scores for height-for-age were calculated using the EpiInfo EpiNut software. Chronic malnutrition (stunting) was assessed by height-for-age *Z* scores compared to the reference standards (WATERLOW, 1972; WATERLOW *et al.*, 1977; WHO, 1986).

Statistical analysis

Unadjusted and adjusted relative risks (RRs) for initial prevalence, final prevalence and incidence of infection detected by DTH and ELISA were calculated with 95% confidence interval. The variables were classified into 2 or more categories, always taking the first category or that with the least risk as the baseline. Initial prevalence was defined as children with positive results in the first phase and final prevalence as children with positive results in the second. Incidence was calculated from the number of children with a negative result in the first phase and a positive result in the second phase, i.e., the number of children infected during the study period divided by the total number in the exposed population.

All variables presented in the unadjusted analysis were included in the multivariate analysis. A backward stepwise procedure was used with a *P*-value of 0·10 for retaining a variable in the final model. The results were adjusted for confounders by the Cox regression (COX, 1972) technique using the STATA (1997) program, modified for sectional design (LEE, 1994), considering 7 months as the follow-up time for all children, with no removals from the study or losses to follow-up. Because the standard errors of the coefficients tend to be overestimated when Cox regression is applied to sectional designs, the robust method of calculating the variance-covariance matrix (LIN & HEI, 1989) was used instead of the conventional inverse-matrix-of-second-derivatives method. In the calculation of the standard errors of the coefficients, we also took clustering into account because on some occasions more than 1 child per household participated in the study.

Results

Vila Nova consisted of 361 families and Bom Viver of 574 families. Each family had on average 5·9 persons and 3·9 children. Family income was lower than 1 Brazilian minimum wage in most cases (89%) and almost all children have lived in the 2 districts since birth: 48% were males and 52% females. The incidence of *L. chagasi* infection was 10·8% when determined by DTH and 28·5% when determined by ELISA.

Unadjusted analysis of infection

Regarding initial prevalence measured by DTH, children aged 24–71 months, who used to bathe outside in the backyard and who had relatives with AVL were at increased risk of acquiring AVL infection. According to the ELISA test, children who lived in Vila Nova and who had relatives with AVL were at higher risk for AVL infection. The presence of chickens around the house or in the neighbourhood appeared to protect them from infection (Table 1).

Using final prevalence as the outcome, according to the DTH test, children aged 48–78 months who lived in Vila Nova district were at higher risk for infection. Reporting *Lutzomyia* sandflies in the house, AVL in relatives and the presence of chickens or dogs in or around the house were associated with increased risk of infection. As measured by the ELISA test, only 3 risk factors were detected: age, playing in the backyard, and district of residence. Presence of *Lutzomyia* sandflies and dogs in the house appeared as protective factors (Table 2).

In terms of incidence of asymptomatic infection measured by DTH, children who lived in Vila Nova district and who reported *Lutzomyia* sandflies or dogs in the house/neighbourhood or AVL in relatives were at increased risk of infection. According to ELISA, child's age, playing around the house between 18:00 and 20:00 and residence in Vila Nova district were associated with infection (Table 3).

The male sex was not found to be a risk factor of infection. The houses in Bom Viver were in better conditions than those in Vila Nova. However, living in

Table 1. Unadjusted analysis of the initial prevalence of asymptomatic *L. chagasi* infection measured by DTH and ELISA (Raposa–Maranhão, Brazil, 1997)

	DTH ($n = 639$)				ELISA ($n = 638$)			
Variable	n	Positive (%)	RR (95% CI)	P	n	Positive (%)	RR (95% CI)	P
Age (months)				0·027				0·500
0–23	231	13	1·00		227	12	1·00	
24–47	225	21	1·64 (1·08–2·49)		226	14	1·24 (0·76–2·01)	
48–71	183	22	1·73 (1·13–2·64)		185	15	1·32 (0·81–2·14)	
Gender				0·665				0·605
Female	334	18	1·00		332	14	1·00	
Male	305	19	1·08 (0·77–1·51)		306	13	0·90 (0·60–1·34)	
Chronic malnutrition				0·970				0·752
No	477	19	1·00		480	14	1·00	
Yes	162	19	0·99 (0·67–1·47)		158	13	0·92 (0·55–1·54)	
Play				0·915				0·295
Indoors	587	19	1·00		590	13	1·00	
Outdoors	52	19	1·04 (0·54–1·97)		48	19	1·44 (0·73–2·83)	
Bathing place				0·002				0·406
Indoors	111	7	1·00		111	16	1·00	
Outdoors	528	21	2·92 (1·46–5·82)		527	13	0·80 (0·46–1·36)	
Father's literacy				0·279				0·909
Can read and write	350	20	1·00		352	14	1·00	
Cannot read or write	289	17	0·82 (0·57–1·18)		286	13	0·97 (0·62–1·52)	
Mother's literacy				0·921				0·438
Can read and write	443	19	1·00		442	14	1·00	
Cannot read or write	196	18	0·98 (0·66–1·45)		196	12	0·82 (0·50–1·35)	
Districts				0·148				<0·001
Bom Viver	348	16	1·00		348	7	1·00	
Vila Nova	291	21	1·30 (0·91–1·86)		290	22	3·29 (1·94–5·56)	
Type of housing				0·141				0·427
Bricks/tiles/cement/ceramic	55	11	1·00		57	9	1·00	
Mud walls/straw/earthen	584	19	1·77 (0·83–3·80)		581	14	1·59 (0·51–4·98)	
Lutzomyia sandflies in house				0·603				0·413
No	219	17	1·00		220	12	1·00	
Yes	420	19	1·11 (0·75–1·66)		418	14	1·21 (0·76–1·94)	
Spraying last year				0·205				0·629
Yes	536	20	1·00		537	14	1·00	
No	103	14	0·69 (0·39–1·22)		101	12	0·86 (0·47–1·57)	
Animals in house/neighbourhood				0·804				0·011
None	249	20	1·00		253	16	1·00	
Chickens	181	17	0·87 (0·56–1·36)		180	7	0·42 (0·21–0·83)	
Dogs	121	21	1·05 (0·65–1·69)		119	21	1·33 (0·80–2·21)	
Chickens and dogs	88	16	0·81 (0·44–1·49)		86	11	0·66 (0·31–1·40)	
AVL in relatives				0·019				0·014
No	618	18	1·00		618	13	1·00	
Yes	21	38	2·12 (1·13–3·98)		20	30	2·32 (1·18–4·54)	
AVL in neighbours				0·794				0·913
No	610	19	1·00		610	13	1·00	
Yes	29	21	1·12 (0·49–2·56)		28	14	1·06 (0·36–3·16)	

DTH, delayed type hypersensitivity skin test with *L. amazonensis* antigen; ELISA, enzyme-linked immunosorbent assay for serum antibodies against *L. chagasi* antigen; RR (95% CI), relative risk (95% confidence interval); AVL, American visceral leishmaniasis.

a mud house with a straw roof and an earthen floor was not a risk factor for infection.

With respect to parental literacy, 31% of mothers and 46% of fathers did not know how to read or write. Nevertheless, illiteracy was not shown to be associated with increased risk of infection. The prevalence of chronic malnutrition among the children in the 2 phases of the study was high (25·5% and 26·0%, respectively), but this was not shown to be a risk factor in either analysis.

Adjusted analysis of infection

Using initial prevalence as the outcome, bathing outside the house and reporting AVL cases in relatives were risk factors for infection as measured by DTH. In addition, children who lived in Vila Nova district had a higher risk of infection according to ELISA, whereas the presence of chickens in the house or in the neighbourhood seemed to protect them from infection (Table 4). Regarding final prevalence as measured by DTH, child's age, residence in Vila Nova district, reporting AVL in relatives, presence of chickens or dogs in the house or in the neighbourhood and reporting the presence of *Lutzomyia* sandflies in the house were associated with increased risk of infection. When ELISA tests were considered, playing outdoors was also

Table 2. Unadjusted analysis of the final prevalence of asymptomatic *L. chagasi* infection measured by DTH and ELISA (Raposa–Maranhão, Brazil, 1998)

	DTH ($n = 572$)				ELISA ($n = 572$)			
Variable	n	Positive (%)	RR (95% CI)	P	n	Positive (%)	RR (95% CI)	P
Age (months)				0·270				<0·001
0–23	137	13	1·00		137	21	1·00	
24–47	214	20	1·53 (0·94–2·49)		214	33	1·57 (1·10–2·24)	
48–78	221	25	1·93 (1·19–3·13)		221	44	2·07 (1·46–2·95)	
Sex				0·989				0·165
Female	293	20	1·00		293	37	1·00	
Male	279	20	1·00 (0·72–1·38)		279	32	0·85 (0·67–1·07)	
Chronic malnutrition				0·273				0·876
No	410	19	1·00		410	35	1·00	
Yes	162	24	1·22 (0·86–1·73)		162	34	0·98 (0·76–1·26)	
Play				0·718				<0·001
Indoors	523	21	1·00		523	32	1·00	
Outdoors	49	18	0·89 (0·47–1·68)		49	59	1·84 (1·41–2·40)	
Bathing place				0·502				0·170
Indoors	100	18	1·00		100	28	1·00	
Outdoors	472	21	1·17 (0·75–1·82)		472	36	1·28 (0·90–1·82)	
Father's literacy				0·342				0·411
Can read and write	307	19	1·00		307	33	1·00	
Cannot read or write	265	22	1·18 (0·84–1·65)		265	36	1·10 (0·88–1·39)	
Mother's literacy				0·832				0·147
Can read and write	391	20	1·00		391	32	1·00	
Cannot read or write	181	21	1·04 (0·73–1·48)		181	39	1·19 (0·94–1·51)	
Districts				<0·001				<0·001
Bom Viver	312	14	1·00		312	28	1·00	
Vila Nova	260	29	2·07 (1·44–2·96)		260	43	1·55 (1·23–1·96)	
Type of housing				0·052				0·784
Bricks/tiles/ cement/ceramic	52	10	1·00		52	33	1·00	
Mud walls/straw/ earthen	520	22	2·24 (0·99–5·04)		520	35	1·06 (0·70–1·59)	
Lutzomyia sandflies in house				0·005				0·012
No	190	13	1·00		190	42	1·00	
Yes	382	24	1·83 (1·21–2·78)		382	31	0·74 (0·59–0·94)	
Spraying last year				0·293				0·096
Yes	479	21	1·00		479	33	1·00	
No	93	16	0·76 (0·45–1·27)		93	42	1·27 (0·96–1·69)	
Animals in house/ neighbourhood				0·004				0·087
None	218	15	1·00		218	38	1·00	
Chickens	164	23	1·54 (0·99–2·39)		164	34	0·89 (0·67–1·18)	
Dogs	106	32	2·19 (1·41–3·39)		106	25	0·65 (0·45–0·95)	
Chickens and dogs	84	17	1·14 (0·62–2·08)		84	41	1·08 (0·80–1·45)	
AVL in relatives				<0·001				0·594
No	552	19	1·00		552	34	1·00	
Yes	20	60	3·15 (1·99–5·00)		20	40	1·17 (0·66–2·07)	
AVL in neighbours				0·547				0·734
No	545	20	1·00		545	34	1·00	
Yes	27	26	1·28 (0·57–2·90)		27	37	1·08 (0·69–1·68)	

For explanation of the abbreviations, see the footnote to Table 1.

identified as a risk factor for infection. In contrast to DTH results, the presence of dogs and *Lutzomyia* sandflies in the house appeared to confer protection against *L. chagasi* infection according to the ELISA test.

Using incidence as a measure of infection, according to DTH, residence in Vila Nova district, presence of dogs in the house or in the neighbourhood and reporting AVL in relatives conferred a higher risk of infection. By the ELISA test, children aged 24–48 months who played outside the house between 18:00 and 20:00 and lived in Vila Nova district were at increased risk for infection. In contrast, presence of *Lutzomyia* sandflies in the house appeared to protect children from infection (Table 4).

Discussion

In this study, 6 outcome measures were used. The variables that were most relevant and may thus predict the risk of asymptomatic infection by *L. chagasi* were district of residence, reporting AVL in relatives and child's age. Some variables appear to increase the risk of infection in some analyses but not in others, as was the case for playing outdoors in the early evening. Use of an outdoor bathroom predicted infection only once.

Presence of *Lutzomyia* sandflies in the house was

Table 3. Unadjusted analysis of the incidence of asymptomatic *L. chagasi* infection measured by DTH and ELISA (Raposa–Maranhão, Brazil, 1998)

	DTH ($n = 469$)				ELISA ($n = 485$)			
Variable	n	Positive (%)	RR (95% CI)	P	n	Positive (%)	RR (95% CI)	P
Age (months)				0·194				<0·001
0–23	123	11	1·00		118	19	1·00	
24–47	169	11	0·94 (0·50–1·74)		184	31	1·66 (1·09–2·52)	
48–78	177	17	1·49 (0·82–2·71)		183	44	2·37 (1·58–3·57)	
Sex				0·710				0·367
Female	245	13	1·00		246	35	1·00	
Male	224	14	1·09 (0·68–1·75)		239	31	0·89 (0·68–1·15)	
Chronic malnutrition				0·869				0·590
No	337	13	1·00		347	34	1·00	
Yes	132	14	1·04 (0·62–1·75)		138	31	0·92 (0·69–1·23)	
Play				0·147				0·001
Indoors	429	14	1·00		448	31	1·00	
Outdoors	40	5	0·36 (0·09–1·44)		37	54	1·73 (1·24–2·41)	
Bathing place				0·955				0·059
Indoors	92	13	1·00		82	23	1·00	
Outdoors	377	13	1·02 (0·57–1·82)		403	35	1·51 (0·98–2·32)	
Father's literacy				0·144				0·196
Can read and write	247	11	1·00		260	31	1·00	
Cannot read or write	222	16	1·44 (0·88–2·36)		225	36	1·18 (0·92–1·53)	
Mother's literacy				0·370				0·117
Can read and write	319	12	1·00		329	31	1·00	
Cannot read or write	150	15	1·25 (0·76–2·06)		156	38	1·23 (0·95–1·60)	
Districts				<0·001				0·001
Bom viver	263	7	1·00		287	27	1·00	
Vila Nova	206	21	3·12 (1·79–5·45)		198	41	1·52 (1·18–1·97)	
Type of housing				0·054				0·419
Bricks/tiles/ cement/ceramic	46	2	1·00		47	28	1·00	
Mud walls/straw/ earthen	423	14	6·63 (0·97–45·59)		438	34	1·21 (0·76–1·94)	
Lutzomyia sandflies in house				0·028				0·069
No	159	8	1·00		164	38	1·00	
Yes	310	16	1·93 (1·07–3·48)		321	30	0·79 (0·61–1·02)	
Spraying last year				0·924				0·157
Yes	388	13	1·00		407	32	1·00	
No	81	14	1·03 (0·53–2·02)		78	40	1·25 (0·92–1·72)	
Animals in house/ neighbourhood				0·032				0·636
None	180	10	1·00		181	35	1·00	
Chickens	135	14	1·41 (0·75–2·63)		150	33	0·94 (0·69–1·28)	
Dogs	84	23	2·26 (1·23–4·17)		81	27	0·78 (0·52–1·18)	
Chickens and dogs	70	9	0·86 (0·35–2·08)		73	36	1·02 (0·72–1·46)	
AVL in relatives				0·042				0·889
No	457	13	1·00		472	33	1·00	
Yes	12	33	2·63 (1·04–6·66)		13	31	0·93 (0·34–2·54)	
AVL in neighbours				0·963				0·578
No	447	13	1·00		464	33	1·00	
Yes	22	14	1·03 (0·26–4·15)		21	38	1·16 (0·68–1·98)	

For explanation of the abbreviations, see the footnote to Table 1.

found to be a risk factor for infection in one analysis (final prevalence as measured by DTH), whereas in 2 other analyses it appeared to be protective, as was the case for final prevalence and incidence according to the ELISA test. Presence of animals in the house or in the neighbourhood showed the same behaviour. In 2 analyses (final prevalence and incidence according to DTH) the presence of dogs or chickens tended to increase the risk of asymptomatic infection. However, according to ELISA, presence of chickens appeared to protect children from infection in the case of initial prevalence and the presence of dogs seemed to decrease the risk of infection in the final prevalence analysis.

What may be the reasons for these inconsistencies between the 6 analyses? At first, 3 different endpoints of infection were used: initial prevalence, final prevalence and incidence. Asymptomatic infection was identified by 2 different tests: DTH and ELISA. These 2 tests measure different types of the immune response and thus are not likely to produce the same results. In the case of presence of *Lutzomyia* sandflies and animals in the house, DTH identified these variables as risk factors, whereas ELISA identified them as protective factors. Other reasons for this might be random error or confounding by other factors not accounted for in the analysis. In addition, past insecticide spraying and

Table 4. Adjusted analysis of the initial prevalence, final prevalence and incidence of asymptomatic *L. chagasi* infection measured by DTH and ELISA (Raposa–Maranhão, Brazil, 1998)

	DTH		ELISA	
Variable	RR (95% CI)	*P*	RR (95% CI)	*P*
Initial prevalence				
Bathing place		0·002		
Indoors	1·00			
Outdoors	2·97 (1·49–5·90)			
Districts				<0·001
Bom Viver			1·00	
Vila Nova			2·98 (1·75–5·07)	
AVL in relatives		0·012		
No	1·00			
Yes	2·23 (1·19–4·27)			
Animals in house/neighbourhood				0·062
None			1·00	
Chickens			0·51 (0·26–0·99)	
Dogs			1·31 (0·80–2·16)	
Chickens and dogs			0·75 (0·37–1·53)	
Final prevalence				
Age (months)		0·053		<0·001
0–23	1·00		1·00	
24–47	1·55 (0·97–2·48)		1·56 (1·10–2·21)	
48–78	1·81 (1·12–2·92)		2·19 (1·56–3·05)	
Play				<0·001
Indoors			1·00	
Outdoors			1·69 (1·29–2·22)	
Districts		<0·001		<0·001
Bom Viver	1·00		1·00	
Vila Nova	2·02 (1·42–2·87)		1·58 (1·26–1·97)	
AVL in relatives		0·008		
No	1·00			
Yes	1·98 (1·19–3·29)			
Animals in house/neighbourhood		0·014		0·017
None	1·00		1·00	
Chickens	1·65 (1·07–2·54)		0·94 (0·72–1·24)	
Dogs	1·98 (1·29–3·05)		0·64 (0·44–0·91)	
Chickens and dogs	1·26 (0·71–2·22)		1·19 (0·88–1·60)	
Lutzomyia sandflies in house		0·019		0·009
No	1·00		1·00	
Yes	1·65 (1·08–2·51)		0·75 (0·60–0·93)	
Incidence				
Age (months)				<0·001
0–23			1·00	
24–47			1·66 (1·10–2·51)	
48–78			2·38 (1·60–3·56)	
Play		0·076		0·015
Indoors	1·00		1·00	
Outdoors	0·26 (0·06–1·15)		1·51 (1·08–2·10)	
Districts		<0·001		0·001
Bom Viver	1·00		1·00	
Vila Nova	3·10 (1·77–5·44)		1·52 (1·18–1·95)	
Lutzomyia sandflies in house				0·033
No			1·00	
Yes			0·77 (0·60–0·98)	
Animals in house/neighbourhood		0·027		
None	1·00			
Chickens	1·67 (0·88–3·16)			
Dogs	2·15 (1·21–3·82)			
Chickens and dogs	0·92 (0·40–2·14)			
AVL in relatives		0·034		
No	1·00			
Yes	2·61 (1·08–6·34)			

For explanation of the abbreviations, see the footnote to Table 1.

presence of *Lutzomyia* sandflies were determined using the questionnaire. Even though the 2 communities had been recently exposed to a comprehensive programme of health education regarding prevention of AVL, differential misclassification might have distorted some results. Residual confounding is another possibility due to this probable misclassification in the assessment of these 2 variables. However, if differential misclassification had affected the results it would have been expected to produce distortion in only one direction. This

is not completely in agreement with what was observed, since presence of *Lutzomyia* sandflies was found to be a risk factor in some analyses but not in others. These inconsistencies are most probably linked to the type of test used to detect infection. Our results are in agreement with some reports that have revealed that DTH is more sensitive in detecting early infection whereas ELISA is more sensitive in identifying symptomatic infection (BADARÓ *et al.*, 1986b; DYE *et al.*, 1993; FNS, 1996). Underreporting of past spraying was more likely to have occurred and may have produced some degree of non-differential misclassification and a bias towards the null.

Children aged ⩾2 years had a higher relative risk for infection than those aged <2 years. This finding is consistent with those obtained by BADARÓ *et al.* (1986a) in Jacobina, Bahia, Brazil, where the frequency of infection rose after 1 year of age, and with those of PAMPIGLIONE *et al.* (1975) obtained in Mediterranean regions, and is also in agreement with observations by ALI & ASHFORD (1993) in Ethiopia. It is important to note that prevalence of infection tends to increase with age since there is little or no immunological recovery from infection as measured by DTH. However, recovery from ELISA may be significant. This may explain why age was not associated with infection when the outcome measure was initial prevalence, whereas it was associated with infection when final prevalence was considered. On this basis, variation in incidence with age is more elucidative and age was also associated with increased risk of infection as measured by ELISA.

It was also noted that Vila Nova children experienced a relative risk of infection 3 times greater than Bom Viver children. This difference was probably due to a more intense migratory process or to a higher *Lutzomyia longipalpis* density in Vila Nova than in Bom Viver. With increased migration, more susceptible individuals enter the area, some taking with them their infected dogs, and are at greater risk of becoming infected and presenting as new cases of the illness (EVANS *et al.*, 1992; FNS, 1996).

The study showed that living conditions in Vila Nova and Bom Viver are precarious. Studies carried out in the state of Pará have shown that children living in areas of land occupations are at increased risk for AVL due to the large number of susceptible people settling in the area, increases in population density of *Lu. longipalpis* and living conditions that may encourage the vector's entry, especially in the more open-built houses (SILVEIRA *et al.*, 1997). In our study, however, the type of dwelling did not represent a risk factor. The *Lutzomyia* sandfly is found throughout all of São Luís city (REBÊLO *et al.*, 1996), including zones at the periphery of urban conglomerations. In Bom Viver it predominates over other species (97% of all species captured were *Lu. longipalpis*), and has as easy targets young children who tend to sleep wearing little or no clothing, leaving the body's surface exposed (ARAÚJO *et al.*, 2000).

Reporting AVL in relatives was associated with infection, in agreement with PAMPIGLIONE *et al.* (1975) who detected a higher infection rate in those who had reported cases in relatives or in neighbours in Emilia Romagna and Sicily in the Mediterranean region. ABRAMSON *et al.* (1995) showed that infection among those who had experienced contact with AVL cases can suggest the occurrence of subclinical infections in many people in endemic areas.

It is known that transmission of AVL occurs through the bite of a *Lutzomyia* sandfly, and therefore in some analyses the presence of these insects in the peri- and intradomestic areas was a risk factor for infection by *L. chagasi*, as also was the presence of dogs and chickens. The presence of chickens circulating around the house may increase the population density of *Lu. longipalpis*. The domestication of the vector *Lu. longipalpis* can be stimulated by factors such as recent urbanization with the derangement of the ecology, by the food sources of humans and animals (dogs, chickens), by abundant tree planting in backyards and by the accumulation of waste (WHO, 1990), characteristics that were common in the study area. The role of chickens in the epidemiology of AVL is a possibility raised by one of our analyses that remains open to debate. However, the lack of consistency of this association found in the present study argues against this possibility. Moderate or severe malnutrition was not found to be linked with infection either in the adjusted or non-adjusted cases for the 2 parameters used. BADARÓ *et al.* (1986a), while studying risk factors associated with AVL in Jacobina, Bahia, verified that in areas of highest incidence of the disease a 2-year-old child had a 1 in 10 chance of being infected, and, if infected, a 1 in 4 chance of developing the disease. If a similar child suffered from malnutrition, the probability of developing the disease was 1:2. CERF *et al.* (1987) reported that children with severe or moderate malnutrition were exposed to an 8·7-fold risk of developing the disease.

Our data, compared to previous studies, illustrate that risk factors for visceral leishmaniasis may vary among different settings and populations. This observation is not unexpected in a disease with multiple risk factors. In such cases, the role of a single factor is dependent on the role played by other components. Studies in different areas will contribute to a clearer worldwide picture of the most relevant risk factors for visceral leishmaniasis. In addition, new research may contribute to the elucidation of the reasons for some unexpected associations found in this study.

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References

Abramson, M. A., Dietze, R., Frucht, D. M., Schwantz, R. & Kenney, R. T. (1995). Comparison of New and Old World leishmanins in an endemic region of Brazil. *Clinical Infectious Diseases*, **20**, 1292–1297.

Ali, A. & Ashford, R. W. (1993). Visceral leishmaniasis in Ethiopia. I. Cross-sectional leishmanin skin test in an endemic locality. *Annals of Tropical Medicine and Parasitology*, **87**, 157–161.

Araújo, J. A. C., Rebêlo, J. M. M., Carvalho, M. L. & Barros, V. L. L. (2000). Composição dos flebotomíneos (Diptera, Psychodidae) do municipio de Raposa–MA, Brasil. Area endêmica de leishmanioses. *Entomologia y Vectores*, 7, 33–47.

Badaró, R., Jones, T. C., Carvalho, E. M., Pedral-Sampaio, D., Reed, S. G., Barral, A., Teixeira, R. & Johnson, W. D. Jr (1986a). New perspectives on a subclinical form of visceral leishmaniasis. *Journal of Infectious Diseases*, **154**, 1003–1011.

Badaró, R., Reed, S. G., Barral, A., Orge, G. & Jones, T. C. (1986b). Evaluation of the micro enzyme-linked immunosorbent assay (ELISA) for antibodies in American visceral leishmaniasis: antigen selection for detection of infection specific responses. *American Journal of Tropical Medicine and Hygiene*, **35**, 72–78.

Badaró, R., Jones, T. C., Lourenço, R., Cerf, B. J., Sampaio, D., Carvalho, E. M., Rocha, H., Teixeira, R. & Johnson, W. D. Jr (1986c). A prospective study of visceral leishmaniasis in an endemic area of Brazil. *Journal of Infectious Diseases*, **154**, 639–649.

Carvalho, E. M., Barral, A., Pedral-Sampaio, D., Barral-Netto, M., Badaró, R., Rocha, H. & Johnson, W. D. Jr (1992). Immunological markers of clinical evolution in children recently infected with *L. donovani chagasi*. *Journal of Infectious Diseases*, **165**, 535–540.

Carvalho, E. M., Bacellar, O., Brownell, C., Regis, I., Coffman, R. L. & Reed, S. G. (1994). Restoration of IFN-γ

production and lymphocyte proliferation in visceral leishmaniasis. *Journal of Immunology*, **152**, 5949–5956.
Cerf, B. J., Jones, T. C., Sampaio, D., Teixeira, R. & Johnson, W. D. Jr (1987). Malnutrition as a risk factor for severe visceral leishmaniasis. *Journal of Infectious Diseases*, **156**, 1030–1033.
Cox, D. R. (1972). Regression models and life tables. *Journal of the Royal Statistical Society B*, **34**, 187–220.
Cuba, C. A. C., Marsden, P. D., Barreto, A. C., Jones, T. C. & Richards, F. (1985). The use of different concentrations of leishmanial antigen in the skin testing to evaluate delayed hypersensitivity in American cutaneous leishmaniasis. *Revista da Sociedade Brasileira de Medicina Tropical*, **18**, 231–236.
Dye, C., Vidor, E. & Dereure, J. (1993). Serological diagnosis of leishmaniasis: on detecting infection as well as disease. *Epidemiology and Infection*, **103**, 647–656.
Evans, T. G., Vasconcelos, I. A. B., Lima, J. W., Teixeira, J. M., McAullife, I. T., Lopes, U. G., Pearson, R. D. & Vasconcelos, A. W. (1992). Epidemiology of visceral leishmaniasis in northeast Brazil. *Journal of Infectious Diseases*, **166**, 1124–1132.
FNS, Fundação Nacional de Saúde (1996). *Controle, Diagnóstico e Tratamento de Leishmaniose Visceral (Calazar)*. Brasília: Ministério da Saúde, Brasil.
Gama, M. E. A., Barbosa, J. S., Pires, B., Cunha, A. K. B., Freitas, A. L., Ribeiro, I. R. & Costa, J. M. L. (1997). Avaliação do nível de conhecimento que a população residente em áreas endêmicas periurbana e rural tem sobre leishmaniose visceral, Estado do Maranhão, Brasil. *Cadernos de Saúde Pública*, **14**, 109–118.
Jahn, A., Lelmett, J. L. & Diesfeld, H. J. (1986). Seroepidemiological study on kala-azar in Baringo District, Kenya. *Journal of Tropical Medicine and Hygiene*, **89**, 91–104.
Lee, J. (1994). Odds ratio or relative risk for cross-sectional data? *International Journal of Epidemiology*, **23**, 201–203.
Lin, D. Y. & Hei, L. J. (1989). The robust inference for the Cox proportional hazards model. *Journal of the American Statistical Association*, **84**, 1074–1078.
Pampiglione, S., Manson-Bahr, P. E. C., La Placa, M., Borgatti, M. A. & Musumeci, S. (1975). Studies in Mediterranean leishmaniasis. 3. The leishmanin skin test in kala-azar. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **69**, 60–68.
Rebêlo, J. M. M., Mendes, W. A., Costa, J. M. L. & Cavaleiro, N. (1996). Lista preliminar das espécies do gênero *Lutzomyia* França, 1924 (Psychodidae, Phlebotominae) do Estado do Maranhão, Brasil. *Cadernos de Saúde Pública*, **12**, 545–549.
Reed, S. G., Badaró, R., Mansur, H., Carvalho, E. M., Lourenço, R., Lisboa, A., Teixeira, R., Johnson, W. D. Jr & Jones, T. C. (1986). Selection of a skin test antigen for American visceral leishmaniasis. *American Journal of Tropical Medicine and Hygiene*, **35**, 79–85.
Silveira, F., Shaw, J. J., Bichara, C. N. C. & Costa, J. M. L. (1997). Leishmaniose visceral americana. In: *Doenças Infecciosas e Parasitárias: Enfoque Amazônico*, Leão, R. N. C. (coord.), Belém, Cejup: UEPA, **40**, 631–644.
Stata Corporation (1997). *Stata Reference Manual release 5*. Texas: Stata corporation.
Waterlow, J. C. (1972). Classification and definition of protein–calorie malnutrition. *British Medical Journal*, **iii**, 566–569.
Waterlow, J. C., Buzina, R., Keller, W., Lane, J. M., Nichaman, M. Z. & Tanner, J. M. (1977). The presentation and use of height and weight data for comparing the nutritional status of groups of children under the age of 10 years. *Bulletin of the World Health Organization*, **55**, 489–498.
WHO (1990). *Control of Leishmaniasis*. Geneva, Switzerland: World Health Organization, Technical Report Series, no. 793.
WHO Working Group (1986). Use and interpretation of anthropometric indicators of nutritional status. *Bulletin of the World Health Organization*, **64**, 924–941.

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Book Review

Malaria in Sri Lanka: Current Knowledge on Transmission and Control. F. Konradsen, F. P. Amerasinghe, W. van der Hoek & P. H. Amerasinghe. Colombo: International Water Management Institute, 2000. xiv+77pp. Price US$20.00 (US$10.00 in developing countries). ISBN 92-9090-406-2.

This useful volume sets out to provide a comprehensive account of malaria in Sri Lanka for students, researchers and the malaria-control community in that country. This book can also provide profitable reading for the malariologist whose geographical focus lies elsewhere, as Sri Lanka has a famous history of providing high-quality primary health care (PHC), and thus is an important model of the role that PHC can play in malaria-endemic regions. Sri Lanka is also considered perhaps the prime example of a country that achieved the virtual eradication of malaria in the 1960s, only to see its resurgence in the latter years of that decade.

The authors of *Malaria in Sri Lanka* are most comfortable approaching malaria from the vector-control perspective. There is a strong chapter on the biology of Sri Lankan anopheline vectors, and informative discussion, in the 'Malaria control' chapter, of the peculiarities of the ancient Sri Lankan irrigation systems and its 'tanks' as vector breeding sites. Some otherwise obscure work, such as that on evidence for multiple blood feeding by *Anopheles culicifacies*, is given a mostly deserved airing in stand-alone 'boxes' interspersed throughout the text. My preference would have been for more depth in the area of parasite biology. Further discussion of the development and spread of resistance to chloroquine and sulfadoxine–pyrimethamine as it relates to the specific epidemiology of malaria (vivax and falciparum) on the island would also have been welcome.

In contrast to many familiar African endemic settings where the acquisition of immunity is almost universal, Sri Lanka's malaria patients are very likely to be adults, and asymptomatic infections are rare. The chapter on immunity is thus of general interest to malariologists, and the important contribution of K. N. Mendis and her colleagues is heavily drawn upon. Some of the most important investigations of transmission-blocking immunity, for example, are from Sri Lanka. Whereas both *Plasmodium vivax* and *P. falciparum* are discussed in the context of particular studies, there is unfortunately no consideration of interactions between these 2 species in Sri Lanka, such as those observed in endemic areas in the Western Pacific.

This slim, readable book will be particularly valuable for students specializing in malaria and disease control, providing in one volume a useful treatment of most aspects of malaria. For researchers and professionals working outside Sri Lanka its main interest lies in the contrast between the island's particular experience of malaria, and that of the other endemic regions in which we work.

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