Original article

Visceral leishmaniasis in southern Ethiopia I. Environmental and behavioural risk factors

Ahmed Ali[1]

Abstract: The role of socio-economic, behaviourial and environmental factors in the transmission of VL and the relation of the disease to other endemic parasites was examined in a case control study. The household risk factors included unplastered house, keeping animals in human dwellings at night and proximity of residence to termite hills, with marginal effect of low family income. Overnight stays in an implicated site of intense VL transmission and ill-health prior to onset of VL were also indicated as major individual risk factors. The assessment of the relation of VL to other parasitic diseases showed that cases appeared to be prone to infection with *T. saginata* and *G. lamblia*. [*Ethiop. J. Health Dev.* 1997;11(2):131-137]

Introduction

Most of the people in rural areas of developing countries are victims of endemic parasitic diseases. The diseases are generally associated with poverty, poor sanitation and high risk of exposure to environmental and biological hazards. Socio-economic and behaviourial factors play important roles in the transmission of vector-borne diseases (1). There is, however, paucity of information on controlled study of risk assessment in the transmission of visceral leishmaniasis.

Visceral leishmaniasis is often found together with a host of other vector-borne and directly transmitted diseases. The relation of VL to other diseases in the same host has not, however, been demonstrated beyond doubt. Earlier, Kork-hill (2) made a remark on the clinical association of Kala-azar with malaria, typhoid and relapsing fever and speculated that the specified diseases may activate Kala-azar from latency into manifest clinical form, and that probably other diseases will do likewise. Fendall (3) reported a fatal case of Kala-azar associated with *Brucella* infection and further noted (4) concomitant diseases like malaria, chicken pox, pyogenic infection, pertussis, tape worm, brucellosis, yaws and pulmonary tuberculosis. The co-existence of Kala-azar and brucellosis was also indicated later (5).

Visceral leishmaniasis is usually accompanied by immunosuppression (5-6). The specific and nonspecific immuno-suppression in VL is reported as a predisposing factor to inter current viral, bacterial, fungal and parasitic infections (6-10). The absence of *S. mansoni* (8) and malaria (8,1112) from patients with VL was, nevertheless, unexpected.

An unfamiliar feature of leishmaniasis has also recently been reported in immuno-compromised patients, with growing numbers of reports on the association of VL to HIV (13-17) and the reactivation of latent leishmaniasis with immuno-suppression (18-21). Some in fact have gone far enough to recommend that *L. donovani* should be added to the growing list of opportunistic infections (21). In this paper, the likely risk factors in the transmission of VL are reported and the association of visceral Leishmaniasis to other endemic parasitic diseases is highlighted.

[1]Institute of Pathobiology and Department of Community Health, Addis Ababa University, P.O.Box 1176, Addis Ababa, Ethiopia.

Methods

The study area was the Aba Roba Peasant's Association in southern Ethiopia described in relative depth elsewhere(22).

To explore in general how VL cases might differ from matched controls in terms of certain antecedent risk factors leading to the development of the disease in cases compared to controls, a case-control study was conducted. Data from case and control house- holds and individual cases and controls were collected using a questionnaire. Relevant medical history and physical findings of cases and controls were recorded. Cases and controls were also investigated for parasitic diseases. *Identification of Cases and Controls*: A case was defined as an individual susceptible to VL and proved to be a case on positive spleen/bone marrow smear or culture for *L. donovani*. Old and newly diagnosed cases of VL residing in the area during the study were recruited as cases.

Households with cases were designated as case house holds. Both the case households and the individual cases were identified with code numbers.

A control was defined as an individual who has not been suspected to be a case of VL during life time. Households with controls were labled as control house holds. However, due to logistic and technical reasons, not all control households have been free of cases. Individual and household controls were also identified with specific identification numbers. The cases and controls were from the six villages of Aba Roba farmer's association.

Sample size: Since the objective of the study was to explore as many risk factors as possible, calculations based on preliminary estimates of exposure to different risk factors would have indicated different sample sizes for each risk factor. Therefore, since it was logistically possible to do so, all available (VL) cases qualifying for the case definition and staying in the area during the study period were studied as cases. Two matched controls for age, sex and village per case were also included in the study; more would have been logistically difficult. The sample size was thus predetermined by the number of available cases and potential controls. From tables in Smith (23) for sample size estimation technique for case control studies, it was shown that the sample size of 110 cases (available cases) and 220 controls was sufficient to detect risk ratios

in being statistically significant, working with Alpha (level of significance) = 0.05; Power = 0.80 (one sided test) as follows:

Proportion exposed in control group											
0.01	0.05	0.10	0.15	0.20	0.25	0.30	0.40	0.50	0.60	0.70	0.80
Risk ratio which is significant	10	4	2.5	2.5	2.0	2.0	2.0	2.0	2.0	2.5	3.0

In the analysis risk ratio was estimated by odds ratio.

The questionnaire and its Administration: A questionnaire was developed to interrogate both cases and controls about certain exposure factors. The interview included questions on major socioeconomic aspects of case and control households and on individual cases and controls. Emphasis was made on assessment of information which was related to VL transmission, as for instance, presence of termite hills within the premises, history of travel and overnight stays in implicated sites of intense VL transmission (eg Segen Valley), etc. Due to difficulties encountered in the assessment of income, annual tax and contributions to the government were taken as indirect parameters to indicate income of house holds. (Tax and other contributions to the government were levied by a committee and there were five distinct strata of tax payers).

The questionnaire was conducted with cases and controls in a consistent manner with one interviewer (AA). The response to the questionnaire was recorded and later cross- tabulated with case and control households and with respective case and control study subjects. The odds ratio, the significance of the difference between case and control responses and the confidence limit were estimated using the matched analysis of the Epi Info epidemiological soft ware. To control confounding effects, certain variables were stratified in the analysis and the combined relative risk estimate and test statistic were used. To control other potentially confounding factors, the conditional logistic regression for matched sets in the SAS (statistical analysis system) programme was used.

Parasitological examination: Stool and blood film specimens were collected from cases and controls. Stool specimen was collected with screw-capped vials containing 10% formalin. Another part of the stool specimen was cultured in the field for larvae of *Strongyloids*, etc. Thin and thick blood smears were collected on standard microscopic glass slides.

A quantitative parasitological technique (24) was employed to count parasite ova in the preserved stool samples. Samples were first shaken and contents were transferred to 15 ml graduated centrifuge tubes. The centrifuge tubes were then filled with water and spun at 2000 rpm for five minutes. Then, the deposits were measured on the tubes' graduations and resuspended to 10 times its volume with distilled water. Egg counts were finally made by examining 0.1 ml of the well mixed specimen under appropriate magnification, parasite ova were reported as "eggs per ml" of faeces by just multiplying the number of eggs counted by the dilution factor. The stool specimen was also scanned in high power for protozoan infections.

Faecal smears were prepared from all stool samples. The smears were air dried and stained with Safranine and Methylene blue as described by Baxby et al (25) and examined for *Cryptospordium*. The thin and thick blood films were stained with Giemsa's stain and examined for haemoparasites. Relevant frequencies, cross tabulations and risk factor estimation were carried out for the parasitological findings.

Results

Household factors: Socio-economic information on certain anticipated risk factors in the transmission of VL was collected from house-holds of 110 cases and 211 age, sex and village matched controls. The data collected comprised of information on occupation, educational status, family size and annual tax and contributions to the government. Data on type of housing, keeping animals in human dwellings (at night) and proximity of the house

to termite hills were also among the household information gathered.

No statistically significant difference was demonstrated in the occupation, educational status and family size between case and control households. Control households were, however, found to be marginally better than case households pertaining to income, indirectly guaged by annual tax and contributions to the government (chi-square = 3.58, P = 0.058, oadds ratio for all strata = 1.56). When type of house was classified as mud - plastered and non-plastered, more control house holds were found to be living in plastered houses compared to cases and the difference was statistically significant (chi-square = 7.97; P-value = 0.0047; odds ratio = 3.7). On assessment of keeping animals in human dwellings at night, more case house- holds compared to controls kept animals in human dwellings. The difference was also found to be statistically significant (chi-square = 7.3; P = 0.0068; adds ratio = 4.6). On examination of proximity of houses to termite hills, more case households reported closer proximity to termite hills with the difference being statistically significant (chisquare = 11.49; P = 0.0007; odds ratio = 2.24).

Individual Factors: Information on occupation, educational status, history and frequency of visit to a near- by valley (Segen), over-night stays in the area and on the general state of health was collected from 110 cases and 211 controls. Due to incomplete data, nine controls were excluded from the analysis. The information sought from cases concentrated on the time just before development of the disease.

There were not significant differences between the occupational and educational status of cases and controls. Frequency of visit to Segen valley did not seem either to influence transmission of visceral leishmaniasis. Over- night stays in Segen valley, however, appeared to play an important role in the transmission of the disease. Cases were found to be spending significantly more nights in the valley

compared to fellow controls (Chi. square = 16.89; P-value = 0.00040; odds ratio = 2.68).

On assessment of the perception of the general status of their health, it was learnt that generally cases were more unhealthy about six months prior to the development of overt VL compared to their respective controls. The difference in health status between cases and controls was found to be highly statistically significant (chi-square = 45.8, P-value = 0.0000-odds ratio = 7.15).

Independence of risk factors: All the statistically significant risk factors were standardised for potentially confounding factors in a model of conditional logistic regression for matched sets in SAS programme. The analysis by SAS was based on 307 matched pairs for whom information was available on all significant factors. The decision to include those factors in the model was based on statistical significance of their effects on the initial analysis as described above which conformed with intuitive reasoning.

All selected factors viz unplastered house, keeping animals in the house, proximity to termite hills, over-night stays in Segen Valley and poor state of health, contributed significantly to the model and were found to be independent risk factors to one another in the transmission of VL. The adjusted relative risks, 95% confidence intervals (CI) for the relative risk (RR) and the P-values for the respective standardised factors are shown in Table 1.

Table 1: **Possible risk factors in the transmission of VL in a case-control study in Aba Roba, southern Ethiopia. 1988-1990.**

Risk factors	Relative risk(RR)	95% CL for RR	P-values
Unplastered house	7.15	1.56 to 32.72	0.0113
Animals in house	8.62	1.13 to 65.87	0.0378
Presence of termite hils	7.70	2.65 to 22.36	0.0002
Poor state of health	14.3	4.8 to 50.0	0.0001
Spending nights in Segen	4.97	1.9 to 13.01	0.0011

Relation of VL to Other Parasitic diseases: The stool and blood specimen collected from 110 cases 211 controls were examined according to the procedures mentioned earlier. Thirteen of the cases (11.8%) and 21 of the controls (10.0%) and 84 of the cases (76.4%) and 162 of controls (76.8%) had helminths and protozoan infections, respectively. A variety of helminth and protozoan parasites were demonstrated in stool specimen of cases and controls, infections with non-pathogenic protozoans, however, made the bulk of the protozoan infections (Table 2). A statistically significant difference pertaining to parasitic infestation between cases and controls were found only with *T. saginata* and *G. lambilia.*

Table 2: **Parasitic infestation in 110 cases of VL and in 211 age and sex matched controls in Aba Roba, Southern Ethiopia, 1988-1990.**

Parasite	"cases" % pos.	Cont-rols % pos	Chi- square	P-value	Odds ratio
Helminth in general	11.8	10.0	0.26	0.6068	1.21
Ascaris lumbricoides	2.7	3.3	0.08	0.7730	0.82
T. Saginata	6.4	1.9	4.35	0.0371	3.52
H. Nana	0.9	2.4	0.84	0.3599	0.38
S. *stercoralis*	1.8	1.4	0.07	0.7858	1.28

Hook worm	0.9	1.9	0.46	0.4987	0.47
Enterobius	0.0	0.5	0.52	0.4703	0.00
Trichuris	0.0	0.5	0.52	0.4703	0.00
Protozoans in general	76.4	76.8	0.01	0.9339	0.98
G. lambilia	25.5	14.2	6.15	0.0132	2.06
E. histolytica	0.9	3.8	2.20	0.1382	0.23
e. coli	54.5	64.5	2.42	0.1197	0.69
I. buetschili	20.9	23.7	0.32	0.5723	0.85
E. nana	4.5	5.7	0.19	0.6651	0.79
Malaria	0.0	0.5	0.52	0.4703	0.00

The stool culture from cases and controls were negative for *Strongyloides*. The Safranine Methylene blue stained stool specimens were also found to be negative for *cryptosporidium*.

Discussion

Farming is a major means of earning a living in the study area and no difference was demonstrated in the occupation of heads of case and control households. Probably due to the prevailing illiteracy rate and the general low level of consciousness, it was also difficult to find any difference of statistical significance in the educational status of heads of case and control households. Large families were the rule in the study locality. A single study in Latin America showed that children with VL came from larger families (26). However, there was no significant difference in the family size of case and control house holds in this endemic focus. It was difficult to get a reliable and acceptable parameter of assessment of income adaptable to the study area. Since tax and other contributions to government were levied by local committees and rates were based on amount of possession, annual tax and contributions were employed as indirect estimates of annual income. Based on this indicator, case households were found to have relatively lower income compared to controls.

Due to the hot climatic conditions and ease of construction, huts were mostly of the open "basket work" type instead of mud walls. The physical structure of a house was found to play a vital role in the transmission of VL and unplastered houses were found to contribute significantly to the risk of developing the disease. The association of the disease with the type of house might suggest the presence of domestic or peridomestic reservoir. Keeping animals in the house was also demonstrated to be an independent risk factor and presence of termite hills in the premise was found to compound the risk. Proximity of house to termite hills was earlier reported to increase risk of the disease (27-28), although Ho et al (29) failed to confirm the association. The finding of infected sandflies in termite hills very close to houses in the study locality (30) make the assertion of proximity to termite hills as a major risk a valid observation.

Individual occupational and educational status per se were not indicated to be risk factors. However, some lesson was learnt on detailed examination of activities and human behaviour in a specific, highly implicated focus of intense VL transmission, i.e. Segen valley. Most farming activities take place in Segen valley. Since the valley is within a walking distance of the villages, people make routine daily trips to the area. Specially older male children and young adults usually spend nights in temporary shelters in Segen Valley to scare away wild animals from their crops and to tend their cattle. Frequency of travel to Segen valley by itself was not associated with increased risk of developing VL. However, an obvious risk was demonstrated with over-night stays in the locality. This could be due to the fact that people could also be bitten by infected vectors while spending the night out in the bush.

The information about the relation of VL to other parasitic diseases is far from being complete. Since VL cases are usually immunocompromised (5-6), they are considered to be prone to diseases of various etiology (2-4, 6-8).

In this study, effort was made to assess the susceptibility of VL cases to other parasitic diseases. When the general health status of cases was examined retrospectively, they were found to suffer more from ill-health compared to matched controls. It was, however, very difficult to speculate beyond doubt the cause of their reduced health. Although more cases had a history of symptomatic treatment for malaria, there was no objective finding to suggest that VL cases run increased risk of malarial infection. The absence of malarial infection from cases of VL was suggested earlier (11) and some even postulated a protective effect of splenomegaly due to chronic malaria against leishmanial infection (12). Only one control had a positive blood film for malaria and the very low malarial infection in the area was un- expected. One possible explanation for the virtual absence of malaria could be the access of the study population to the recently built clinic just for symptomatic treatment of the disease.

The hot, dry climatic condition, the lack of humus in the soil texture and the recent introduction of pit latrines in the study site might have contributed to the low prevalence of specially helminth infections. Unlike the recent report of 73.6% of infection of VL cases with one or more intestinal parasites (10), only 11.8% of cases were infected with one or more intestinal parasites. Although the total number of infected were few, it was interesting to learn that cases were more prone to infections with *T. saginata* and *G. lamblia* compared to controls.

In conclusion, the study highlighted the role certain behaviourial and ecological factors play in the transmission of visceral leishmaniasis. It is recommended that policy makers and health planners encourage and undertake related studies and make use of the available information to control and prevent such a noxious disease. The information generated in this study could be utilised in the control of the disease in areas of related environmental and socioeconomic set up.

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References

1. Buck AA, Anderson RI, Mac Rae AA. Epidemiology of polyparasitism II. Types of combinations, relative frequency and asociations of multiple infections. Tropcical Medicine and parasitology 1978; 29:137-144.
2. Corkhill NL. Activation of latent Kala-azar by Malaria and relapsing fever. Annals of Tropical Medicine and Parasitology 1948;42:230-235.
3. Fendall NRE. Kala-azar in the Kitui Reserve. East African Medicial Journal 1950;27:291-296.
4. Fendall NRE. Kala-azar in East Africa with particularl reference to Kenya and the Kamba country. III. Journal of Tropical Medicine and Hygiene 1952;55:220-233.
5. Manson-Bahr PEC. East African Kala-azar with special reference to the pathology, prophylaxis and treatment. Transactions of the Royal Society of Tropical Medicine and Hygiene 1959;53:123-137.
6. Ho. M, Koech DK, Iha DW, Bryceson ADM. Immunosuppression in Kenyan Visceral leishmaniasis, Clinical and experimental immunology 1983;51:207-214.
7. Chunge CN, Gachihi G, Chulay JD, Spencer HC. Complications of Kala-azar and its treatment in Kenya. East African Medical Journal 1984;61:120-127.
8. Chunge CN, Gachichi G, Muigai R, Wasuna K, Rashid JR, Chulay JD, Anawabi G, Oster CN, Bryceson ADM. Visceral leishmaniasis un responsive to antimonial drugs. III. Successful treatment using combination of sodium stibogluconate plus allopurinol. Transactions of the Royal society of Tropical Medicine and Hygiene 1985;79:715718.
9. Lal AHB. Significance of radiological examination of the chest in cases of visceral leishmaniasis. Bulletin Rejendra Memorial Research institute of Medical Sciences 1978;Vol. VI:23-24.
10. Badaro R, Jones TC, Lorenco R, Cerf. BJ, Sampaio D, Carvalho EM, Rocha H, Teixeira R, Johnson Jr, W.D. A prospective study of visceral leishmaniasis in an endemic area of Brazil. Journal of Infections Diseases 1986a;154:640-649.
11. Kager PA, Rees PH, Mangugu FM, Bhatt KM, Hockmeyer WT, Wellde BT, Lyerly Jr. W.H. Clinical presentation of visceral leishmaniasis in Kenya. A prospective study on 64 patients. Tropical and Geographical Medicine 1983;35:323-331.
12. Jahn A, Lelmett JM, Diesfeld HJ. Seroepidemiological study on Kala-azar in Baringo District. Journal of Tropical Medicine and Hygiene 1986;76:741-746.
13. Le Fichoux Y, Marty P, Fuzibet JG, Bertrand F. Leishmaniose viscerale Mediterraneenne Chez deux malades porteurs, d'anticorps anti-HIV. Bulletin de la societe Francaise de parasitologie 1986;4:201-204.
14. Blazquez J, Alvar J, Najera R. Leishmaniasis in an HIV (LAV/HILVII) serologically positive patient. Journal of infection 1987;14;89-90.
15. Smith DE, Lindley R, Bryceson ADM. Visceral leishmaniasis in a patient with AIDS. Transactions of the Royal Society of Tropical Medicine and Hygiene 1988;82:654.
16. Peters BS, Fish D, Golden R, Evans DA, Bryceson ADM, Pinching AJ. Visceral leishmaniasis in HIV infection and AIDS: Clinical features and response to therapy. Quarterly Journal of Medicine 1990;77:1101-1111.
17. Jeannel D, Tuppin P, Brucker G, Danis M, Gentilini M. Imported and autochthonous Kala-azar in France. British Medical Journal 1991;303:336-312.
18. Ma DDF, Concannon AJ, Hayes J. Fatal leishmaniasis in renal-transplant patient. Lancet 1979;2:311-312.
19. Broechaert VOA, Michielsen P, Vandepitte J. Fatal leishmaniasis in a renal transplant patient. Lancet 1979; 2:740-741.

20. Martinez L, De Letona J, Masa Vazquez C, Perez Maestu R. Visceral leishmaniasis: an opportunistic infection. Lancet 1986;1:1094.

21. Badaro R, Carvalho EM, Rocha R, Queiroz AC, Jones TC. Leishmania donovani. An opportunistic microbe associated with progressive diseasein three immuno-compromised patients. Lancet 1986b;330:647-648.
22. Ali A, Ashford RW. Visceral leishmariasis (VL) in Ethiopia.IV. Prevalence, incidence the relation of infection to disease in an endemic area. Annals of Tropical Medical and parasitology 1994;88:289-293.
23. Smith PG. Sample size determination in epidemiological studies. Workshop on epidemiolocal methods for research in tropical diseases 1983:31.
24. Ashford RW, Hall AJ, Babona D. Distribution and abundance of intestinal helminths in man in western papua New Guinea with special reference to strongyloides. Annals of Tropical Medicine and parasitology 1981;75;269-279. 25. Baxby D, Blundell N, Hart CA. The development and performance of a simple, sensitive method for the detection of cryptosparidium oocysts in faeces. Journal of Hygiene 1984;92:317-323.
26. Harrison LH, Talapala G, Naidu J, Drew S, Alencar JE, Pearson RD. Reciprocal relationships between undernutrition and the parasitic disease visceral leishmaniasis. Review of Infectious Diseases 1986;8:447-453.
27. Southgate BA. Studies in the epidemiology of East African leishmaniasis. 2. The human distribution and determinants. Transactions of the Royal Society of Tropical Medicine and Hygiene 1964;58:377-390.
28. Southgate BA. The structure of foci of visceral leishmaniasis in north eastern Kenya. In: Ecologie desleishmanioses. Colloques internationaux du centre National de la Recherche scientifique 1977;239:241-247.
29. Ho M. Siongok TK, Lyerly WH, Smith DH. Prevalence and disease spectrum in a new focus of visceral leishmaniasis in Kenya. Transactions of the Royal society of Tropical Medicine and Hygiene 1982;76:741-746.
30. Gebre-Michael T. Studies on Phlebotomine vectors of VL in southern Ethiopia. PhD.Thesis Univ. London, U.K. 1992.