Tropical Medicine and International Health

VOLUME 7 NO 1 PP 35–44 JANUARY 2002

Epidemiology and clinical manifestations of *Leishmania donovani* infection in two villages in an endemic area in eastern Sudan

E. A. G. Khalil[1], E. E. Zijlstra[1], P. A. Kager[2] and A. M. El Hassan[1]

1 *Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan*
2 *Department of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands*

Summary We conducted a longitudinal study in an endemic area for visceral leishmaniasis (VL) in eastern Sudan to compare the epidemiology and clinical spectrum of *Leishmania donovani* infection in two populations differing in ethnic background and duration of residence in the area. The study took place in two villages from April 1994 to April 1996. In Um-Salala village, which is inhabited by members of the Masaleet tribe, half of the villagers had previous exposure to cutaneous leishmaniasis (*Leishmaria major*) before moving there. The population of the second village, Mushrau Koka, belong to the Hausa tribe and most were born there. The incidence of VL was 20.4/1000 person-years in 1994/1995 and increased sharply to 38.3/1000 person-years in 1995/1996 in Um-Salala. A rise in the incidence of VL was also observed in Mushrau Koka but with a lower incidence, 3.3/1000 person-years to 4.6/1000 person-years. The incidence rate of confirmed VL reflects only a limited part of the total infection rate which includes various forms of subclinical infection. The ratio of clinical to subclinical infection in Um-Salala was 1.2 : 1 in 1994/1995 compared with 2.6 : 1 in 1995/1996. This ratio was 1 : 11 in 1994/1995 and 1 : 2.5 in 1995/1996 in Mushrau Koka. In both villages the mean age of subclinical cases was higher, but in Mushrau Koka the mean age of subclinical cases also was higher than that of subclinical cases in Um-Salala. The leishmanin skin test (LST) was positive in 56% of individuals in Um-Salala and in 33% in Mushrau Koka. VL only occurred in leishmanin-negative individuals. Post kala-azar dermal leishmaniasis (PKDL) followed in 58% of confirmed VL patients in Um-Salala; the low incidence of VL for Mushrau Koka did not permit to estimate a PKDL rate. The clinical manifestations resulting from exposure to *L. donovani* range from subclinical infection to VL and PKDL. No firm conclusion as to the difference in incidence of VL between the two villages could be reached but differences in exposure to VL and cutaneous leishmaniasis (CL) as well as other factors such as ethnic background and differences in nutritional status may play a role.

keywords Eastern Sudan, epidemiology, Hausa, Masaleet, visceral leishmaniasis

correspondence E. A. G. Khalil, Institute of Endemic Diseases, University of Khartoum, PO Box 45235, Khartoum, Sudan. E-mail: eltahir@usa.net

Introduction

Visceral leishmaniasis (VL) or kala-azar is endemic in an area of eastern Sudan along the Sudanese-Ethiopian border that extends south into Upper Nile state (Siddig *et al*. 1988). The steady-state of endemicity is associated with occasional outbreaks or epidemics that cause great morbidity and mortality (Satti 1958; Perea *et al*. 1989; De Beer *et al*. 1990; El Hassan *et al*. 1993). A major epidemic started in 1988 in Western Upper Nile Province in southern Sudan, an area previously not known to be endemic. Large numbers of immunologically naïve individuals were affected and an estimated 100 000 people died (Seaman *et al*. 1996). The endemic area in eastern Sudan has a typical savannah climate with a short rainy season (July–October) and a dry hot one (November–June). The soil is alluvial clay that forms large cracks during the dry season. The area is characterized by woodland dominated by *Balanites aegyptiaca* and *Acacia seyal* trees. *Balanites*/*Acacia* woodland, the cracked clay

soil and termite hills are the typical biotope of *Phlebotomus orientalis*, the vector of kala-azar in the Sudan (Hoogstraal & Heynemann 1969; Schorscher & Goris 1992; Elnaiem *et al.* 1996). Case detection and drug treatment is the only control measure adopted so far. In a previous study in the same area, we found that incidence rates of VL were rising in the early 1990s after a period of low transmission in villages along the Rahad river (Zijlstra *et al.* 1994; El Hassan *et al.* 1995) with no cases reported in the period 1986–1990. In one sentinel village (Um-Salala) incidence rates for 1991/1992 and 1992/1993 were 38.5 and 38.4 per 1000 person-years, respectively (Zijlstra *et al.* 1994). We found that VL did not occur among individuals with a positive leishmanin skin test (LST), and hypothesized that cross protection by previous exposure to *Leishmaria major* infection in their homeland prior to migration into the area could play a role; but no definite proof could be found (Zijlstra *et al.* 1994). Another important finding was the high post kala-azar dermal leishmaniasis (PKDL) rate (56%) after VL. We therefore decided to compare incidence rates of VL and PKDL in Um-Salala with another village (Mushrau Koka) most of whose inhabitants had been living in the area since birth. Here we report the epidemiological findings and the clinical spectrum of *Leishmaria donovani* in both villages.

Patients and methods

Study area and population

The selected villages, Um-Salala and Mushrau Koka, are located on the eastern bank of the Rahad River, a tributary of the Blue Nile that marks the eastern border of the Dinder National Park in Gedaref state, 400 km south-east of Khartoum. Um-Salala was founded in 1969 by members of the Masaleet tribe who migrated from western Sudan (Darfur state, near El-Geneina town). The migration to the village increased dramatically after the drought that hit Darfur in 1984. A census in 1991 estimated the total number of individuals in the village at 1430. The inhabitants are mainly farm labourers and subsistence farmers and live in grass huts without latrines. The standard of hygiene is poor. Their diet is mainly carbohydrates with meat and fish once or twice a week; lemon and mango are available during the season.

Mushrau Koka is 35 km north of Um-Salala, also situated along the Rahad river. The village was established 50 years ago by members of the Hausa tribe who migrated from northern Nigeria (mainly from the towns of Kanu and Sakatu); the majority of inhabitants can therefore be expected to have been born in the village. The ecology of the area around Mushrau Koka is the same as in Um-Salala village. A 1994 census showed a total population of 970. Houses in the village are thatched grass huts and all have latrines. The village appears much cleaner than Um-Salala. The villagers' diet consists mainly of fish supplemented with sorghum, millet, vegetables and fruit.

Surveys and diagnostic procedures

Surveys were conducted in April and November of each year. From June to November the area is inaccessible because of the bad state of the roads during the rains. All houses in the two villages were numbered and all individuals within a household were registered by name and given an identification number. Informed consent to participate in the study was obtained from all adults and in the case of children from their guardians. An enrolment form containing demographic data, past medical history, present complaints, height and weight was completed for every individual. A general clinical examination was conducted with particular reference to hepato-splenomegaly, enlargement of lymph nodes and presence of scars of previous cutaneous leishmaniasis (CL). Liver size was measured in the mid clavicular line from the costal margin; the spleen size was assessed by measuring the distance between the costal margin in the anterior axillary line to the tip of the spleen. Lymphadenopathy was classified as localized if found only at one site and generalized if at two or more sites. Inguinal lymphadenopathy was not included, as this is a common finding particularly in people who often walk barefoot. The oral and nasal mucous membranes were examined for evidence of mucosal leishmaniasis.

A thick and thin blood film for malaria parasites was examined from all individuals who either had fever, looked ill or had splenomegaly, and those with a positive blood film were treated with chloroquine.

Direct agglutination test (DAT) and LST

From every participant, we took a finger-prick blood sample on Whatmann no. 3 filter paper, and using freeze-dried antigen (Meredith *et al.* 1995) for the estimation of antileishmanial antibodies, all samples were diluted until endpoint ≥1 : 102 400. Based on previous experience, a cut-off titre of ≥1 : 6400 was taken as positive (Zijlstra *et al.* 1997). In April 1994 all inhabitants of both villages underwent a leishmanin skin test by injecting 0.1 ml of the leishmanin antigen (*Leishmaria infantum*, kindly donated by Dr Gramiccia, Istituto Superiore di Sanità, Rome) intradermally on the volar aspect of the left forearm.

Control consisted of 0.1 ml of leishmanin diluent injected at least 10 cm from the test antigen site. Induration of ≥5 mm at the test site and no induration at the control site 48 h later was recorded as a positive leishmanin reaction. In the subsequent surveys (November 1994–April 1996) only those who were negative in the previous survey underwent the leishmanin skin test, but the DAT was performed on all individuals at each survey.

Lymph node and/or bone marrow aspiration

An inguinal lymph node aspiration was performed on those clinically suspected of VL (i.e. all individuals with fever for more than 2 months or left upper quadrant pain or lymphadenopathy or splenomegaly or wasting). Those with a negative result underwent bone marrow aspiration from the superior posterior iliac crest. The smears were fixed with methanol, stained with Giemsa and examined under an 100× oil-immersion lens.

Definitions used in the study

VL confirmed: patients who were clinically suspect for VL (see above) and in whose lymph node or bone marrow aspirate *Leishmania* parasites were found.
VL suspected: patients who were clinically suspect for VL, in whose lymph node and bone marrow aspirates no parasites were found but who had converted in the DAT (i.e. a DAT result of ≥1 : 6400 with a previous result of <1 : 200).
VL diagnosed in retrospect: patients diagnosed and treated on clinical grounds by the medical assistants between our visits and who were found to have converted in the DAT.
VL deaths: these were persons in whom VL was considered the likely cause of death in a postmortem interview with relatives.
Subclinical cases: individuals with a negative LST result in a previous survey and who converted in DAT or LST or both in the absence of clinical symptoms (Zijlstra *et al.* 1994).
LST positive DAT converters: individuals found LST positive in a previous survey and who converted in the DAT.

The ratio clinical/subclinical infection was calculated as the number of confirmed VL cases and VL diagnosed in retrospect to the number of subclinical cases in LST negative individuals. Diagnosis of PKDL was made clinically based on aspect, distribution of skin lesions and a temporal relationship with VL (Zijlstra *et al.* 1994).

Characterization of *Leishmania* parasites

Bone marrow or lymph node aspirates were inoculated into RPMI 1640 culture medium supplemented with 20% foetal calf serum. Twelve positive cultures were referred to the WHO reference centre in Montpellier, France (Dr F. Pratlong and Prof. JP Dedet, Laboratoire de Parasitologie, Centre Hospitalier Universitaire) for characterization by isoenzymes.

Treatment

Patients with confirmed and suspected kala-azar were treated with sodium stibogluconate (Wellcome, UK) 20 mg/kg daily for 15 days. This regimen was chosen based on previous experience (Zijlstra *et al.* 1994) which provided evidence that 15 days of treatment was equally effective as 30 days and would make more efficient use of scanty resources.

Statistical analysis

Data were entered in Epi-Info 6 and analysed using the SPSS statistical package. Rates were compared using the chi-square test and mean values by *t*-test; a level of $P < 0.05$ was considered statistically significant. Incidence rates were calculated as described in Zijlstra *et al.* (1994). The incidence rate for VL was calculated as the number of cases who were either confirmed VL or diagnosed in retrospect per number of person-years follow-up in the total population. Similar incidence rates for subclinical cases were calculated. For 1994–1995, each individual who was seen on the baseline survey in April 1994 and on the two consecutive surveys in November 1994 and April 1995 was counted for 1 person-year; newborns, new arrivals and persons lost to follow-up were counted as 0.5 person/year. Similar calculations were made for 1995–1996 with April 1995 as baseline and November 1995 and April 1996 as consecutive surveys. In all surveys, all individuals including those who had evidence of previous infection were entered in the calculations.

Anthropometric measurements

To compare the nutritional condition in the two study villages, for all children under 5, *Z*-scores for weight-for-age (WAZ), height-for-age (HAZ) and weight-for-height (WHZ) were obtained using the EPINUT anthropometry program of EpiInfo; subsequently the means of these parameters were compared using the *t*-test. For adults, the body mass index (BMI) was calculated and mean values were compared between the two villages.

Results

Demographic data

In Um-Salala we screened 1107 villagers in April 1994, 1266 in November 1994, 1220 in April 1995, 1222 in November 1995 and 1267 in April 1996. In Mushrau Koka these numbers were 879, 897, 887, 848 and 841, respectively. Table 1 shows the comparison between the two villages; in Mushrau Koka 91% of people were born in the village whereas in Um-Salala this was 50%. The age and sex distribution in the two villages is shown in Figures 1 and 2. The age distribution is typical of that of developing countries, with a high proportion of younger age groups. There was a significant difference in sex ratio with apparent under representation of young males in Um-Salala, likely because this group work as casual labourers outside the village. Although some people in Mushrau Koka reported a history of CL and had scars compatible with it, there were more individuals with scars in Um-Salala, despite the fact that few reported a history of CL. There were no VL cases in LST-positive individuals against 69 cases that developed in LST-negative individuals in the two villages.

Table 2 shows further and more detailed comparison between the two villages. Both mean WAZ and WHZ scores were lower in Um-Salala, indicating poorer nutritional state. Also in adults, mean BMI values in Um-Salala were significantly lower. Splenomegaly rates were higher in Mushrau Koka, in particular in those ≤15 years of age. Malaria was significantly more common in Mushrau Koka among those eligible for testing at all surveys except one.

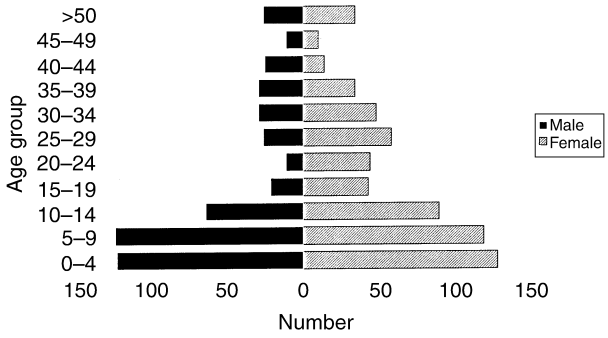

Figure 1 Population structure of Um-Salala village.

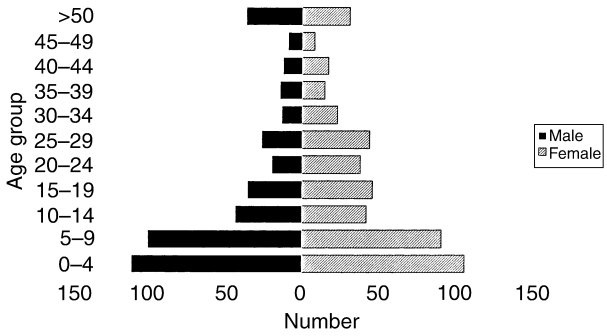

Figure 2 The population structure of Mushrau Koka village.

Leishmanin skin test

In April 1994, the LST positivity rate was 56% in Um-Salala, and 88% of those born in western Sudan (El-Geneina town), where cutaneous leishmaniasis is

Table 1 Demographic data, history and scars of cutaneous leishmaniasis, leishmanin skin test (LST) distribution and prevalence of visceral leishmaniasis (VL) according to LST result and origin in the two study villages

Village	Mushrau Koka		Um-Salala		
Place of birth	Mushrau Koka	Nigeria	Um-Salala	Geneina	*P*-value
Number	754 (91%)	78 (9%)	543 (50%)	550 (50%)	
Mean age (SD)	12.3 (11.2)	55.6 (15.1)	5.7 (4.7)	29.5 (15.5)	0.000
Sex ratio*	368/386	37/41	271/272	208/342	0.000
Years in village (SD)	12.2 (10.7)	31.8 (9.5)	5.5 (4.0)	11.5 (5.5)	0.000
History of CL	3	0	3	12	
Scars of CL	3	0	6	193	
LST pos (%)	192/721 (33%)	69/78 (88%)	109/486 (22%)	457/518 (88%)	0.000
VL† in LST positive	0	0	0	0	
VL† in LST negative	7	0	55	7	
VL† in LST unknown	0	0	9	3	

* Male/female.

† VL confirmed and in retrospect.

Table 2 Nutritional parameters, spleen size and malaria prevalence in the two study villages

	MK	Um-Salala	*P*-value
Z scores			
WAZ	−1.27 (1.47)	−1.88 (1.2)	0.000
HAZ	−2.04 (1.92)	−2.17 (1.7)	0.40
WHZ	+0.09 (1.30)	−0.68 (1.12)	0.000
BMI	22.3 (9.0)	20.1 (2.8)	0.000
Spleen size (age ≤ 15)			
Mean spleen size in cm (SD)	1.37 (2.19)	0.49 (1.59)	0.000
0	329 (64%)	570 (87)	0.000
1–4	129 (25%)	54 (8%)	0.000
5–9	58 (11%)	30 (4.5%)	0.000
10–14	2 (0.4%)	2 (0.3%)	1.000
≥15	0	1 (0.2%)	1.000
Spleen size (age > 15)			
Mean spleen size in cm (SD)	0.47 (1.67)	0.19 (1.68)	0.004
0	327 (91%)	428 (96%)	0.15
1–4	17 (5%)	10 (2%)	0.07
5–9	16 (4%)	7 (1.5%)	0.02
10–14	–	2 (0.5%)	0.50
≥15	1 (0.3%)	0	0.44
Malaria prevalence*			
April 94	498/694 (72%)	161/790 (20%)	0.000
November 94	170/222 (77%)	118/255 (46%)	0.000
April 95	180/227 (79%)	218/264 (83%)	0.42
November 95	75/119 (63%)	79/305 (26%)	0.000

WAZ: Weight-for-age Z-score, HAZ: height-for-age Z-score, WHZ: weight-for-height Z-score.
* In those eligible for testing: fever, ill or splenomegaly.

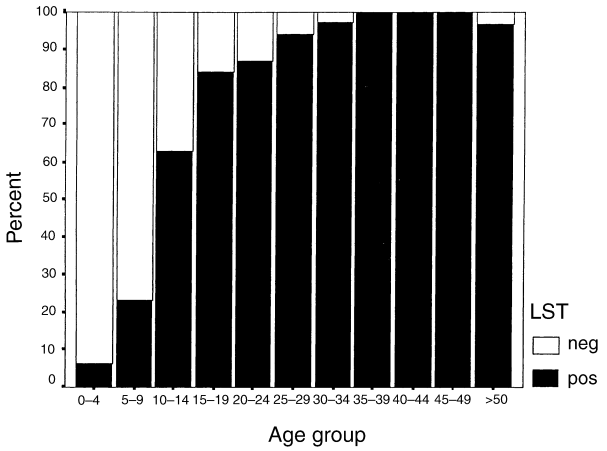

Figure 3 Distribution of the Leishmanin skin test (LST) result according to age group in Um-Salala.

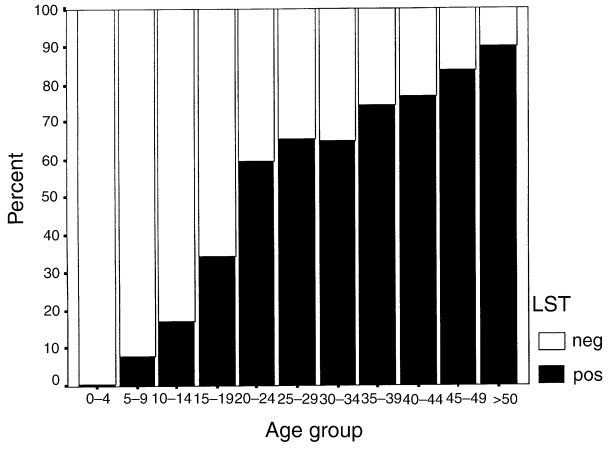

Figure 4 Distribution of the Leishmanin skin test (LST) result according to age group in Mushrau Koka.

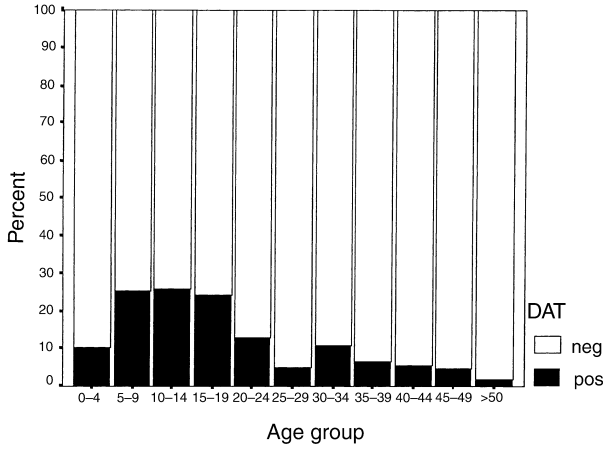

Figure 5 Distribution of the direct agglutination test (DAT) result according to age group in Um-Salala.

common, gave a positive leishmanin reaction, but only 22% of those born in Um-Salala were positive. The first comprehensive population leishmanin test carried out in Mushrau Koka village in April 1994 gave a positivity rate of 33%. Rates of positive LST results were higher in Um-Salala in all age groups up to 50 years. In both villages the rate of leishmanin positivity increased with age, particularly in Um-Salala where in the age group of 35–50 years it reached 100% (Figures 3 and 4).

Results of the DAT

The DAT showed sensitivity of 80% in confirmed kala-azar cases and in those with a positive result the test remained positive for up to 18 months at follow-up.

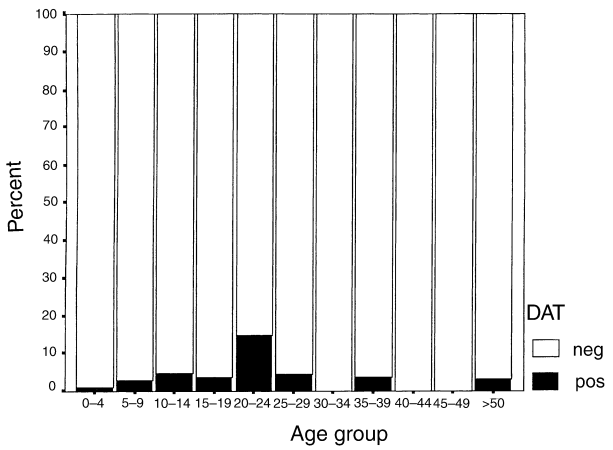

Figure 6 Distribution of the direct agglutination test (DAT) result according to age group in Mushrau Koka.

Um-Salala had higher rates of positive DAT results in all age groups up to 40 years (Figures 5 and 6), and 83% of PKDL patients had a positive DAT at the time of diagnosis. Tables 3 and 4 show a breakdown of all diagnostic categories in the 2 years of observation.

In Um-Salala, there was a marked increase in incidence of VL in 1995/1996 compared with the year before, mainly in VL cases (confirmed and in retrospect), as the ratio of clinical disease to subclinical infection was also higher in 1995/1996 than in 1994/1995. All cases except one were seen in individuals of < 17 years of age. There was a significant difference in mean age between VL and those with subclinical infection [mean age 7.5 (SD 5.1) and 11.0 (SD 8.0), $P < 0.001$, respectively). The incidence rate of VL (confirmed and in retrospect) in Mushrau Koka was low. But subclinical infection is more common than VL. The mean age of those with subclinical infection was higher than in those with VL [21.0 (SD 17.3) and 9.0 (SD 5.2), respectively, $P < 0.001$]. Comparison with Um-Salala shows that there is no difference in mean age in VL cases ($P = 0.46$), but that on average, subclinical cases were older in Mushrau Koka ($P < 0.001$).

Characterization of *Leishmania* parasites by isoenzymes

The 12 isolates that were typed by isoenzymes revealed the presence of three zymodemes: MON 18, MON 30 and MON 82 corresponding to *L. donovani* s.s., *L. infantum* and *Leishmaria archibaldi* (Oskam *et al.* 1998).

Outcome of treatment of patients with confirmed VL

Of the 32 confirmed VL patients diagnosed in both villages, 28 could be followed up for at least 6 months.

Table 3 Clinical spectrum of *L. donovani* infection in Um-Salala village 1994–1995/1995–1996

	1994–1995			1995–1996		
Diagnosis	Number	M/F	Mean age (range)	Number	M/F	Mean age (range)
Clinical						
VL confirmed	8	5/3	7.4 (3–17)	18	11/7	6.6 (1–15)
VL retrospect	16	8/8	9.4 (1–28)	32	12/20	5.4 (0.7–15)
VL suspect	3	3/0	6.3 (6–7)	3	0/3	4.7 (2–7)
VL deaths	12	6/6	5.3 (1–23)	2	1/1	5 (4–6)
Subclinical						
LST-negative persons						
DAT conversion	2	2/0	2 (1–3)	5	1.0/4	6 (0.8–13)
LST conversion	10	6/4	12.5 (3–33)	13	4/9	9.5 (1/5–27)
DAT and LST conversion	8	3/5	14.3 (4–35)	1	0/1	3
LST-positive persons with DAT conversion	18	5/13	27 (10–68)	12	8/4	30.7 (3–58)
Ratio clinical/subclinical*	1.2/1			2.6/1		
Incidence rates/1000 person-years						
VL confirmed and retrospect		20.4			38.3	
VL all		33.2			42.1	
Subclinical (in LST-negatives)		29.0			19.1	
Subclinical all†		43.4			28.3	
VL all and subclinical all		76.7			70.4	

* Clinical includes visceral leishmaniasis (VL) confirmed and in retrospect; subclinical leishmanian skin test (LST) negative only.
† Includes subclinical and LST-positive DAT seroconverters.

Table 4 Clinical spectrum of *L. donovani* infection in Mushrau Koka village 1994–1995/1995–1996

	1994–1995			1995–1996		
Diagnosis	Number	M/F	Mean age (range)	Number	M/F	Mean age (range)
Clinical						
VL confirmed	2	1/1	14 (10–17)	4	2/2	8.8 (5–14)
VL retrospect	1	1/0	15	0	0/1	12
VL suspect	0			1	0/1	3
VL deaths	0			1		
Subclinical						
LST-negative persons						
DAT conversion	7	6/1	8.7 (0.6–23)	0		
LST conversion	24	5/19	27.5 (95–85)	9	5/4	13.3 (4–25)
DAT and LST conversion	1	1/0	7	1	1/0	18
LST-positive persons with DAT conversion	4	2/2	32.3 (5–75)	1	0/1	40
Ratio clinical/subclinical*	1/11			1/2.5		
Incidence rates/1000 person-years						
VL confirmed and retrospect		3.3			4.6	
VL all		3.3			6.9	
Subclinical (in LST negative)		35.3			11.5	
Subclinical all		39.7			12.7	
VL all and subclinical all		43.0			19.6	

* Clinical includes visceral leishmaniasis (VL) confirmed and in retrospect; subclinical in leishmanian skin test (LST)-negative individuals only.

Cure of VL was achieved in 25, giving a cure rate of 89%. Those failing to respond were referred to Soba University Hospital in Khartoum for further management.

Post-kala-azar dermal leishmaniasis

PKDL developed in 58% (11/19) of successfully treated cases of confirmed VL compared with 80% (36/45) of cases diagnosed in retrospect in Um-Salala village. The prevalence of PKDL in the village was 5.5, 10.7, 12.3 and 12.9 per 1000 in the November 1994, April 1995, November 1995 and April 1996 surveys, respectively. In Mushrau Koka, prevalence of PKDL was 0, 0, 1.2 and 2.4 per 1000 in the November 1994, April 1995, November 1995 and April 1996 surveys, respectively. The mean age of PKDL patients was similar to that of VL patients. All treated PKDL patients were eventually cured with 1 or 2 months treatment of sodium stibogluconate.

Discussion

The incidence of VL and PKDL is much higher in Um-Salala than in Mushrau Koka despite the higher numbers of susceptible individuals (leishmanin-negative) in the latter village. The differences in the incidence rates are most likely due to difference in exposure. Unlike the Hausa of Mushrau Koka, the people of Um-Salala engage in various activities in the neighbouring Dinder National Park, where transmission of VL is high among soldiers and game wardens stationed in the Park and infected sand flies were more commonly found than in the two villages (Ibrahim *et al.* 1999; ME Ibrahim, B Lanbson, AO Yousif, NS Deifalla, DA Elnaiem *et al.* unpublished data). Another activity causing high exposure is fishing at night in the Rahad River, practiced by women and children of Um-Salala from January to June. Women spend the night with their children on the banks of the river, which is only a few hundred metres from the Park. The vector *P. orientalis* was only found in scanty numbers in the two villages but collected in abundance in the *Acacia seyal* woodland around the villages during a 1-year survey (*Leishmaniasis* Research Group/Sudan, unpublished data). There are more PKDL cases in Um-Salala than in Mushrau Koka. It is probable, but as yet not proved, that PKDL may be an important reservoir of infection. Although these factors leading to high exposure are not found in Mushrau Koka, its inhabitants are exposed to *Leishmania,* as evidenced by the high leishmanin positivity rate that increases with age. Whether this is due to *L. donovani* and hence a subclinical infection or to an infection by a non-pathogenic *Leishmania* species is unknown. Other factors to be considered are the better standard of living and

nutritional status of the Hausa; the latter factor has been reported to be of importance in development of clinical disease in VL (Harrison *et al.* 1986; Cerf *et al.* 1987). Ethnically the populations are quite different and therefore the role of genetic factors in susceptibility or resistance to the parasite cannot be ruled out. The presence of an animal reservoir should be taken into account; rodents such as *Arvicanthis niloticus* and *Acomys albigena* as well as genets and several cats have been found infected with *Leishmania* parasites and identified as possible animal reservoirs in the Malakal area (Hoogstraal & Heyneman 1969). Reservoir studies in the study villages have been limited; Dereure *et al.* (2000) found *Leishmania* parasites in dogs with similar zymodemes as previously isolated from VL and PKDL cases in the area.

The epidemiology of *L. donovani* infection in Um-Salala village is complicated by the fact that many of the Masaleet inhabitants of this village migrated recently from El-Geneina in western Sudan where cutaneous leishmaniasis caused by *L. major* is endemic but VL virtually unknown. Most individuals are aware of cutaneous sores occurring during childhood and many have scars. In April 1994 the LST positivity rate was 56%; mostly because of immigrants from western Sudan (Zijlstra *et al.* 1994). Although having a scar was significantly correlated with a positive LST result, this correlation was not maintained when controlling for age (data not shown). None of the individuals born in El Geneina (50% of the village population in April 1994) who were leishmanin positive developed VL after migration to Um-Salala (Zijlstra *et al.* 1994). Only leishmanin-negative migrants or those born in the village developed VL. The situation in Mushrau Koka is different, as the village was established 50 years ago and most of the inhabitants were born there. Only few had scars suggestive of CL and it is unclear whether they had travelled to an area endemic for CL. By and large they were therefore exposed to *L. donovani* only, but exposure to other *Leishmania* species non-pathogenic to man cannot be ruled out. It remains to be studied if age-related variation in susceptibility plays a role, as observed in Iran where *L. infantum*, not *L. donovani,* is the causative organism (Davies & Gavgani 1999).

The higher ratio of clinical to subclinical infection in Um-Salala is in contrast to what is found in Mushrau Koka and also to findings in other endemic areas, e.g. Brazil where subclinical infection is at least six times as frequent as clinical disease (Badaro *et al.* 1986). Reasons for these discrepancies are not known with certainty, but in addition to differences in definitions used, these may include differences in species and virulence of the parasite, ethnic or genetic differences and differences in nutritional status. The higher mean age in the group of subclinical infections in both villages may reflect pre-existing partial immunity built up in previous years. While a difference in parasite species exists between Brazil on one hand and Um-Salala and Mushrau Koka on the other, the parasites in the latter two villages are the same. Hence other factors determining susceptibility or resistance apart from differences in parasite species are a more likely explanation for differences in clinical manifestations between the two villages.

This study also shows that the contribution of confirmed VL cases to the total incidence rate of infection is limited and that the true incidence of infection is much higher when all categories of clinical VL and subclinical VL are taken into account. We have also shown that infection takes place in presumed immune individuals (LST positive) as reflected by seroconversion in the DAT. Apparently these individuals have transient parasitaemia resulting in a humoral immune response as measured by the DAT. None of these individuals develop clinical VL, which supports our observation that primary VL only occurs in LST-negative individuals; we have seen few relapse cases of VL in patients with a positive LST.

This study showed the value of the DAT as an epidemiological tool and useful to diagnose clinically suspected cases in which parasites were not detected in lymph node or bone marrow aspirates. Its sensitivity of 80% is rather limited and a negative result in a clinically suspect patient should be interpreted with caution. The zymodemes that were identified in our *Leishmania* isolates were also reported from southern Sudan (Ashford *et al.* 1992) and among the Misairiya tribes in western Sudan (El Hassan *et al.* 1993).

PKDL occurred with high frequency in Um-Salala. One contributing factor could be the often erratic and underdosed treatment with stibogluconate that patients received when they were treated by medical assistants between our visits. In those patients PKDL rates were 69% as opposed to 35% in patients treated by us (Zijlstra *et al.* 2000). Similar PKDL rates were reported by Gasim *et al.* (1998) who worked in the same area; their treatment regimen was stibogluconate 10 and 20 mg/kg for 30 days, for adults and children, respectively. In the Médecins sans Frontières (MSF)-Holland kala-azar treatment centres in southern Sudan, where treatment was with stibogluconate 20 mg/kg or 30 days, PKDL rates exceeded 50% (Veeken 1999). This may suggest that effective but short duration of treatment does not increase the chance of developing PKDL. Almost 20% of patients develop PKDL during treatment (para kala-azar dermal leishmaniasis). No conclusions can be drawn on the PKDL rate in Mushrau Koka as the incidence of VL was low.

The incidence of VL and PKDL in both villages increased in 1995/1996 as compared with 1994/1995 and reached

the levels that were reported from 1991/1992 and 1992/1993. These fluctuations were most probably because of the fluctuations in annual rainfall in 1995/1996. An entomological survey in Dinder Park over 1 year showed that by June, as the rain started and the relative humidity began to rise and the temperature began to fall, there was a remarkable increase in the number of *P. orientalis* collected (Elnaiem *et al.* 1998).

In conclusion, there is a significant difference in the incidence of VL and PKDL in the two villages studied in the endemic area of eastern Sudan. The villages have the same ecology and are 35 km apart. This difference in incidence may be due to difference in exposure, nutritional, genetic or other unidentified factors.

Acknowledgements

This work was funded by EC project TS3-CT93-0245. We would also like to acknowledge the assistance offered by MSF-Holland, Khartoum, that was vital for the execution of the project. Our special thanks to Ms Nashwa El Hassan for typing the manuscript.

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