

Population and Animals Dynamic Effects on Leishmaniasis in Sudan

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Abstract: In Sudan, the visceral leishmaniasis hyper-endemic foci are mainly in east and south of the country. Cutaneous leishmaniasis is mainly found in northern and western regions beside scattered foci in central part of the country. The Green Valley villages are situated in Nuba Mountain region, South Kordofan province; 600 km west from the capital Khartoum. These villages have been deserted for 20 years (from seventies to nineties) due to a killing disease which was called by the villagers (Marrad Alsayed) and described as a disease brought by the dust created by the movement of cattle and camels. Lately as the villagers resettled back during late nineties a similar disease reappeared among them affecting all age groups. Leishmanin Skin Test (LST) was conducted to all villagers as a screening test on three consecutive field visits August, October and February which resemble pre-raining season, raining and post raining seasons. Which reflect the transmission load according to the breeding of sandflies, the leishmaniasis vector.

Key words: Leishmaniasis, LST (Leishmanin Skin Test), PCR (Polymerase Chain Reactions), animals, population

INTRODUCTION

Leishmaniasis is a parasitic disease transmitted to human by an infected female sandfly bite of the genus *Phlebotomus* (Killick-Kendrick, 1990). *Leishmania donovani* is the main parasite species causing visceral leishmaniasis in Sudan (El-Hassan *et al.*, 1990) while *Leishmania major* is responsible for most infection leading to cutaneous leishmaniasis known as Zoonotic Cutaneous Leishmaniasis (ZCL) that confere a zoonotic reservoir (El-Safi *et al.*, 1991). Leishmaniasis is a worldwide distributed and endemic in 82 countries. The annual incidence is estimated at some 600,000 new clinical cases, officially reported of 12 million cases and a population at risk about 350 million (WHO, 1990).

The leishmania belt in Sudan crossing the country from east to west at the sub-savanna region, in this area several nomadic tribes are living. They composed of variable ethnic population, they are farmers and shepherds. Annually they migrate north and south within this region following the rains and grass for better condition of living.

These villages were deserted for 20 years due to a killing disease that resemble leishmaniasis clinically as described by the villagers. Resettling in these villages have lead to re-appearance of similar illness. Leishmaniasis is an important parasitic disease with a great public health problem to many countries. The

clinical presentation of the disease depends mainly on parasite genotype versus host immunogenetic profile. In old world transmission to human via a bite of female sandfly mainly due to *Phlebotomus* sp. (*papatasi*, *orientalis*). *L. donovani* complex (*donovani*, *infantum* and *chagasi*) is causing visceral leishmaniasis while *L. tropica* (arthroponotic) and *L. major* (zoonotic) are responsible for cutaneous leishmaniasis.

Leishmania donovani is the main causative species for visceral leishmaniasis while *Leishmania major* needs an animal reservoir and it the main causative parasite for cutaneous leishmaniasis in Sudan (Kirk and Sati, 1940; Neave, 1904). In Sudan the leishmania belt extends from Gadarif region from east where visceral leishmaniasis is a predominant form to Darfor region to the west where the cutaneous form has been reported (El-Hassan and Zijlstra, 2001). The Nuba mountain is situated in Kordofan region which lies in the mid way in the leishmania belt this area has rarely been studied (Abdalla *et al.*, 2001; Abdalla, 2010) while several studies in the field of leishmaniasis have been conducted mainly in the eastern and southern Sudan (Siddig *et al.*, 1990; Zijlstra *et al.*, 1994).

Many mobile, nomadic tribes are settled in this area from various ethnicity such as Massaleet, Arabs and Nuba they work as shepherds and farmers. Variable types of domestic and wild animals are found such as camels, cows, sheep, dogs and rodents. The ecology is suitable for the vector (sandflies) existence and breeding, this is

shown by the presence of sub-savna climate and the plants forests belonging to *Acacia Balanitis* (Kirk and Lewis, 1955). The cracked dry muddy sand offer sandflies optimum breeding sites. Wild range of investigations are available for detection of leishmania cases but still the most reliable and easy test used in screening and epidemiological tool to be used in field studies is the skin test LST (Montenegro test) (Ho *et al.*, 1983). This study aimed to identify the leishmania cases among the inhabitants of The Green Valley villages (three adjacent villages each one consist of around 24 cottages, each cottage represent a family). Detection of the leishmania parasite DNA was performed to detect the illness rate among the villagers.

MATERIALS AND METHODS

The work site: A small village lies in Nuba mountain, west of Sudan (12N-9S and 32E-29W). The total population was around 332. The area was selected due to it's unique geographical site being in the middle of Sudan leishmania belt.

Sampling: Numbering of houses (cottages) in each village and identification numbers for all villagers were performed. Three consecutive field intervention were performed in months August, October and February as they reflect the summer, winter and autumn, respectively or pre-raining, raining and post-raining seasons, as these periods affect greatly the breeding of the disease vector (sandflies) so the variation of the vector population will lead to variation in transmission rate.

Leishmanin Skin Test (LST) was done to all villagers in three consecutive occasions August (summer), February (winter), October (autumn). The 0.1 mL leishmanin was injected subcutaneously in the upper extensor part of the left arm. The ball pin technique was applied after 48-72 h indurations, redness and swelling >5 mm was considered positive. Actually the LST grades was applied to blot the test results.

PCR

Molecular biology: A finger prick blood spotted filter papers were collected from all the villagers. All the samples were tested for leishmania parasite detection using specific donovani primers AJS3 (5'CCAGT TTTTCCCGCCCCT3') and DB8 (5'GGGTTG GTGTAAAATAGGGC3') (Barker, Cambridge). Donovani complex probe B4 Ras (85 bp) and Infantum specific probe EH [version: S49390.1 G1:28802 Pubmed 1331940] were used for parasite identification.

RESULTS AND DISCUSSION

The total population under study was 332 which included all the inhabitants of the three villages (Raheima, Radina and Green Valley villages). All of them were relatives they were descendant from three brothers who settled in 77 cottages (Table 1). Children <16 years old comprises around 50% of the total population under study (155/332) as shown in Table 2. The positive LST estimated during the three consecutive field intervention was 43.6% in August, 45.7% February and 51.2% in October. The distribution of the final LST results (after October field intervention) among children 53 positives (34.2%), 102 negatives (65.8%) and among adults 117 positives (66.2%), 60 negatives (33.8%) among the total population; 170 positives (51.2%).

The screening for leishmaniasis by hypersensitivity skin test (LST-Leishmanin Skin Test) and by detection of parasite DNA using the PCR (Polymerase Chain Reaction) have detected a total of 51.2% positive LST (exposed/ carrier) and 32 PCR (ill/cases) as shown in Table 3. The exposed and ill members of some families are shown in Fig. 1 the pedigree (family tree) (12 families).

The prevalence of Leishmaniasis was estimated to be 0.3% for the population with annual incidence of 1-10 cases per 1000, this was difficult to be estimated exactly as most of human and animals showed subclinical manifestation and the fluctuation in number estimated varies with population animal dynamic migration inside and outside these villages (Raheima, Radina and Green Valley villages) as a northe triangle endemic disease focus beside the southern focus of (Allery, Kayoga and Kalogi). The location of houses (cottages) within these foci influenced the progression of the disease, so the indoors transmission is the main mode this have been reflected in the all age groups illness. But some families have not encountered single illness or case among the family member as shown by the LST and PCR results. The example for these families are family (members sharing

Table 1: Population of each village under study and number of cottage

The study area	Village 1	Village 2	Village 3	Total
Number of population	120	140	72	332
Number of cottages	24	36	17	77

Table 2: LST-distribution among children (<16 years) and adults (≥16 years)

Age group	Positive LST (%)	Negative LST (%)
Children <16 years	53 (34.2)	102 (65.8)
Adults ≥16 years	117 (66.2)	60 (33.8)
Total population	170 (51.2)	162 (48.8)

Table 3: The LST (Leishmanin Skin Test) and PCR final results obtained during the study

Tests/Time	August	February	October	Final
LST +ve results (%)	43.6	45.7	51.2	51.2
PCR +ve results	1.0	2.0	29.0	32.0

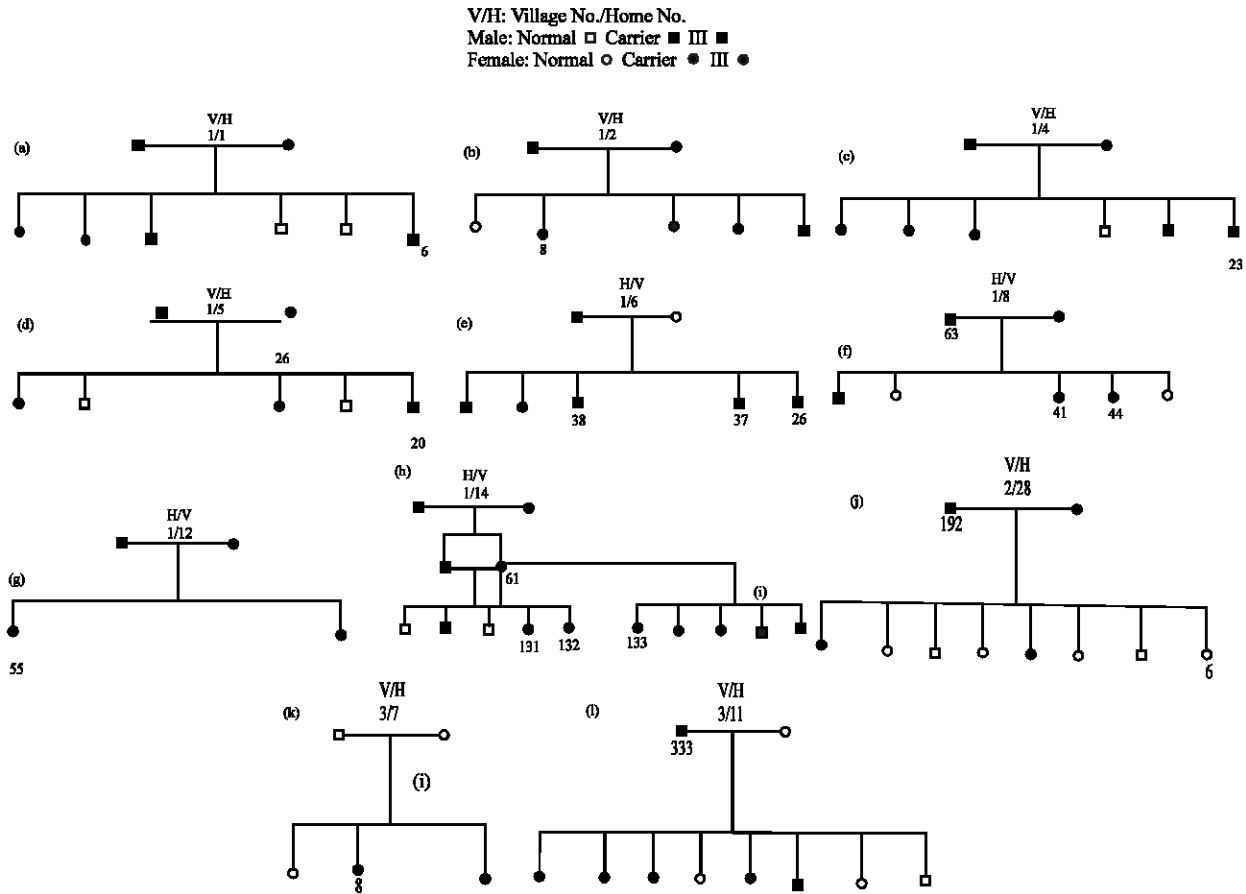


Fig. 1: Pedigree of families

one cottage) were all negative by LST and PCR. In the other hand, ten positive cases of leishmaniasis proved to have leishmania DNA by PCR technique were from one family in fact they were sibs while three cases were also sibs belonging to another family sharing same housing, sleeping, dressing habits. So a genetic factor can not be excluded.

The domestic animals available within the village inhibitors such as dogs, goats, cows and camels have an important role in disease spread as shown in studies of reservoir held in this area (Abdulghani *et al.*, 2003). Which showed the presence of positive animals reservoirs by variable means of investigations that include microscopy, direct agglutination test and PCR.

Epidemiological studies from endemic regions worldwide showed the predominance of cases among children in indoors infections (Badaro *et al.*, 1986; Jahn *et al.*, 1986), this is explained by the low immunity in children in comparison to adults. Kala-azar is a potential fatal disease characterized by long term fever, spleen enlargement, immunosuppression and weight loss.

The parasites inhabit the macrophages of the spleen, liver and bone marrow in the aflagellated amastigotes. During the course of the disease, there is a marked depression of cellular immunity to leishmania antigens (Carvalho *et al.*, 1981) and a poly-clonal B cell activation with high titer of both specific and non-specific antibodies. After successful treatment both T cell proliferation to leishmania antigens *in vitro* and delayed type hypersensitivity to killed *Leishmania promastigotes in vivo* develop (Sacks *et al.*, 1987). Leishmaniasis is mainly T cell-mediated disease (Liew, 1989) so selection of an efficient clinic-epidemiological tool has lead to chose the leishmanin skin test (Montenegro test; LST) which proved to be a good screening and epidemiological tool for identification of leishmania cases.

LST (Leishmanin Skin Test) results have shown the continuous conversion of this test among negatives after consecutive testing, this denotes the continuous transmission of the disease in the area. Estimation of positive rates reflect the attack rate within the study group, the progressive rising means that some of

negative LST cases have changed to positive and this seroconversion was noticed among all age groups.

This study have screened these villages and showed that the capability of leishmaniasis to exist in deserted areas can be explained by the disease capability to maintain internal circulation within the vectors and animal reservoirs and this can last as long as 20 years.

CONCLUSION

The results showed the sequential increase in attacks rates among the villagers as reflected by positive LST; 43.6% in August, 45.7% in February and 51.2% in October. All age groups were involved indicating indoors transmission.

The final positive cases detected by PCR and hybridization tests were 32 out of 332 belong to donovani species. The presence of many types of animals (Camels, sheep, cows, dogs and rodents) might suggest maintenance of leishmania parasites in animal cycles.

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