Risk Factors for Kala-Azar In Fangak County, Jonglei State, Southern Sudan

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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DEDICATION

I dedicate this work to my parents Leonardo Tongun Nyungura and Mary Ossa Nyungura and to my daughter Kaka Lagu.

ACKNOWLEDGEMENTS

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LIST OF ACRONYMS AND ABBREVIATIONS

AIDS	Acquired immune deficiency syndromes
CDC	U. S. Centers for Disease Control and Prevention
CI	Confidence interval
CL	Cutaneous leishmaniasis
COSV	Coordinating Committee of the Organization for Voluntary Service
CSA	Crude soluble antigen
DALY	disability adjusted life years
DAT	Direct agglutination test
DCL	Diffuse cutaneous leishmaniasis
ELISA	Enzyme linked immunosorbent assay
FELTP	Field Epidemiology and Laboratory Training Program
GoSS	Government of South Sudan
HIV	Human immuno-deficiency virus
IDSR	Integrated disease surveillance and response
ITROMID	Institute of Tropical Medicine and Infectious Diseases
JKUAT	Jomo Kenyatta University of Agriculture and Technology
Kg	Kilogram
Ltd	Limited
MCH	Mother and child health
MCL	Mucocutaneous leishmaniasis
mg	Miligram
OP	Outpatient

OR	Odds ratio
P	Probability that an event occurs by chance alone
PCR	Polymerase chain reaction
PHCC	Primary health care center
PKDL	Post kala-azar dermal leishmaniasis
rK39	A recombinant antigen for kala-azar
SAG	Sodium antimonite gluconate
TDR	Tropical disease research
UNAIDS	United Nations Acquires Immune deficiency Syndromes
UNSIG	United Nation Sudan Information Gateway
USD	United States dollars
VL	Visceral leishmaniasis
WHO	World Health Organization

ABSTRACT

Leishmaniases have been considered a tropical affliction that constitute one of the six entities on the World Health Organization tropical disease research list of most important diseases. The disease is one of the most important parasitic tropical

diseases in Sudan and other parts of the world. Leishmaniases rank only second to malaria among human protozoan diseases. It is estimated that about 500,000 persons are affected by visceral leishmaniasis annually; often people living in poor rural areas with limited health care resources. Unmatched case control study was conducted in Fangak County in Jonglei State, Southern Sudan from October 2007 to December 2007. Subjects were interviewed using a structured questionnaire to assess behavioral and environmental variables presumed to be risk factors for kala-azar transmission. The questionnaires which were originally in English were translated into Nuer for study recruits and their responses translated back into English for the principal researcher to complete the forms. A total of 144 of participants were recruited for the study with (33%) cases and (67%) controls. Of the total study participants, (44%) were males and 56% were female. The mean age for cases was eight years with a range from nine months to forty five years, while for the controls it was ten years for the mean age with a range from five months to sixty two years. The types of bed nets in use were: long lasting insecticide treated, cotton cloth- untreated ("Dhamoria"), and, 'silk cloth' untreated ("Smooking"). On the use of a bed net, significantly more kala-azar cases were using the "Smooking" type of bed net compared to the controls. Sometimes smearing of cow dung ashes on the body was associated with kala-azar. People who occasionally

engage in traditional dances at night or children playing around the houses during the night were also found to be significantly associated with kala-azar. During the rainy season, the consistent use of different types of bed nets namely; "Polyethylene", "Dhamoria" and "Smooking", were protective. The study found out that more than half (56%) of the participants affected by the kala-azar were children less than five years. Accessibility to treatment is delayed and most people use wrong types of bed nets. Irregular use of cow dung ashes is associated with kala-azar and is not protective contrary to the local believes and practice. Playing/dancing outside in the dark may lead to an increased risk for kala-azar among children. The study recommends the establishment of kala-azar treatment units in existing health facilities in the counties with high prevalence.

CHAPTER ONE

LITERATURE REVIEW

1   INTRODUCTION

Leishmaniases are group of diseases with broad range of clinical manifestations caused by several species of obligate intracellular parasites belonging to the genus *Leishmania* and Family, Trypanosomatidae (Osman *et al.,* 2000).

Leishmaniases have been considered a tropical affliction that constitute one of the six entities on the World Health Organization tropical disease research (WHO TDR) list of most important diseases (Desjeux, 2001).

Leishmaniases rank only second to malaria among human protozoan diseases (Chang *et al.,* 1985). Visceral leishmaniasis (VL)/kala-azar was first described in 1824, in Jessore district, Bengal (now Bangladesh) (Sengupta, 1947; Sanyal, 1985). The disease remained undetected until 1903, when Charles Donovan in Madras and Leishman in London independently demonstrated the causative parasite in splenic tissue in autopsies from kala-azar patients infected in India (Leishman, 1903; Donovan, 1903a). In the same year Donovan performed a splenic aspirate in an Indian patient in Madras and demonstrated the parasite for the first time in a living patient (Donovan, 1903b). Within a few months, Ronald Ross proposed the name *Leishmania donovani (L. donovani)* (Ross, 1903) for the newly discovered parasite.

1.1   Taxanomy of *Leishmania* (Kudo, 1966)

Phylum: Protozoa

Subphylum: Sacromastigophora

Superclass: Mastigophora

Class: Zoomastigophora

Suborder: Kinetolastidae

Family: Trypanosomatidae

Genus: *Leishmania*

Several species and subspecies of *Leishmania* infect human in the Old and New Worlds

(Molyneux and Ashford, 1983; Barker, 1989; Appendix 1)

The genus *Leishmania* has been divided into two subgenera on the basis of development in the sandflies (Wenyon, 1926; Lainson and Shaw, 1979). In the subgenus *Leishmania*, the development of the parasite, takes place at the anterior portion of the alimentary tract of the *Phlebotomus* sandflies and this process is known as suprapylarian development. This *Leishmania* subgenus includes the *L. donovani* complex (*infantum, donovani,* and *chagasi), L. major, L. tropica* and *L. aethiopica.*   On the other hand, the organisms belonging to the subgenus *Viannia* develop in the midgut and hindgut of the *Lutzomyia (Lu)* species of sandflies and this is known as peripylarian development. *Leishmania viannia* complex includes; *L.   braziliensis (braziliensis,   guyanensis,   panamensis), mexicana, amazonensis and peruviana.*

1.2   Epidemiology of Leishmaniases

Leishmaniases are considered endemic in 88 countries (16 developed countries, 72 developing countries) on 5 continents: Africa, Asia, Europe, North America, and South America (Conjivaram *et al.,* 2007).

The epidemiology of leishmaniasis in a given area is directly dependent on the behavior of the human and/or animal population in relation to the cycle of transmission. There are variety of factors that influence the transmission of the disease for instance: proximity of residence to sandfly breeding and resting sites, type of housing, occupation, extent of exposure to sandfly bites, natural resistance, genetic or acquired, virulence of the parasite species, zoonotic or anthroponotic reservoirs, the vectorial capacity, which is defined as the number of density, seasonality, longevity and flight range of sandfly populations (Kettle, 1995; Lane, 1993) or the infective bites delivered per human per annum (Dye, 1992).

Leishmania donovani causes anthroponotic kala-azar in the Indian subcontinent and in some parts of China (Ashford and Bettini 1987). Humans are the only known reservoirs especially in areas where the presence of Post kala-azar dermal leishmaniasis (PKDL) is common. The vector *P. argentipes* rests in cattle sheds that are often closely attached to houses and breeds in organic debris on the ground. A subpopulation of *P. argentipes* is anthropophilic (Thakur *et al.,* 1981).

Leishmania donovani are also found in Sub-Saharan Africa, especially in southern Sudan, Ethiopia, Somalia and northern Kenya. In Sudan *P. orientalis* is the vector for kala-azar and certain rodents transmit the infection while in Kenya *P. martini* is

responsible for transmitting the parasite. Dogs are rarely infected and are not considered to be reservoirs in Kenya (Mansour *et al.,* 1970; Mutinga and Ngoka*,* 1980).

Leishmania infantum causes VL in the Mediterranean basin, Western Asia and Eastern China. The infection is zoonotic in dogs, especially in domestic dogs in South Europe, but feral dogs also may serve as a reservoir in the Middle East and foxes in South Europe and North Africa (Rioux *et al.,* 1968).

Leishmania chagasi causes kala-azar in the New World (Shaw and Lainson*,* 1987) and the main endemic area is Northeast Brazil where the vector is *Lutzomyia longipalpis* and the reservoirs are foxes, dogs and opossum.

Ninety percent of kala-azar cases occur in 5 countries in the world; Bangladesh, India, Nepal, Sudan and Brazil (WHO, 2002). There are about 30-100 sub-clinical infections for every overt case of VL (Ho *et al.,* 1982).

Leishmania major is responsible for most zoonotic cutaneous leishmaniasis (CL) of the Old World. It is endemic in the hot semi deserts and dry silt valleys of North Africa, Middle East, the Arabian Peninsula, India, Turkmenia, Uzbekistan, Tadjikistan, Kazakhstan, central Sudan and Northern Kenya. The reservoirs are gerbils, girds and fat rats *Arvicanthus, Tatera*. *Phlebotomus papatasi* is the main vector but also *P. sergenti* plays some role.

Leishmania tropica causes anthroponotic CL in the Old World. It has been reported round the Mediterranean basin from Greece, Northern Serbia, Romania, Turkey, Middle East, Afghanistan, Pakistan, India and on the whole of the northern African littoral. There have been a few cases of VL by *L. tropica* in India, Kenya and Saudi Arabia

(Mebrahtu *et al.,* 1989). Humans are the principal reservoir although the parasite has been isolated from Nile rat *Rattus rattus (R. ratus)* and dogs. *Phlebotomus sergenti* is the main vector and the *P. papatasi* secondary vector.

Leishmania aethiopica is responsible for cutaneous leishmaniasis in the highlands of Ethiopia, western Kenya and eastern Uganda. The vectors are *P. longipes* and *P. pedifer* and the reservoirs are the hyraxes *Procavia habessinica* and *Heterohyrax brucei* (Ashford *et al.,* 1973).

Leishmania braziliensis is the most common agent that causes CL and muco-cutaneous leishmaniasis (MCL) or espundia, in Central and South America. It is found in Guatemala, Honduras, Costa Rica, Panama, Peru, Argentina, Bolivia, Paraguay, Colombia, Venezuela and throughout the Amazonian forest and Central America. Incidental infections have been found in dogs, equines in the suburban areas (Aguilar *et al.,* 1987). The natural reservoir hosts have not been identified but the disease is transmitted by the sandfly *Psychodopygus welcomei, Lutzomyia (Lu) whitmani* and *Lu. intermedia,* all of which are anthropophilic(Aguilar *et al.,* 1987).

Leishmania panamensis is responsible for leishmaniasis in Costa Rica, Honduras, Nicaragua, Panama, Colombia and the pacific coast of Ecuador. Its natural host is the sloth *Cleopus hoffmanni* (Herrer and Christensen*.,* 1980). Accidental infections were reported in wild animal species and in dogs. Infection rates in humans are high (Sanchez *et al.,* 1992).

Leishmania guyanensis is restricted to the Amazonian forests of Brazil, Colombia, French Guyana, Guyana and Surinam. The main vectors are *Lu. umbratilis* and *Lu. Anduzei* (Dedet *et al.,* 1989).

Leishmania peruviana is responsible for CL in the high valleys of the Peruvian Andes and the Argentinean highlands. The dog is considered to be the urban reservoir. The vectors are *Lu. peruenis* and *Lu. verrucarum* (Llanos-Cuentas *et al.,* 1999).

Leishmania mexicana is prevalent in the Yucatan peninsula of Mexico, Guatemala, Honduras, Panama and Colombia. It causes CL and diffuse cutaneous leishmaniasis (DCL). Various forest rodents are the reservoir hosts.

Leishmania amazonensis infections are found in the Amazon forests of Brazil, Bolivia, Colombia, Ecuador, Peru, French Guyana and Venezuela. Although human infections are rare, there is a high rate of (DCL) and some of the infections cause VL (Barral *et al.,* 1991).

There are two unnamed species of *Leishmania* which have been found in Namibia, Angola, Zaire and Tanzania (Grove, 1989). Humans and rock hyraxes *Procavia capeses* are the reservoirs of the species.

A rarer species *L. donovani archibaldi* has infrequently been reported to cause leishmaniasis in the Horn of Africa (Lainson and Shaw, 1987).

1.3   Socio-economic Impact of Leishmaniases

An estimated 350 million people are at risk of leishmaniasis and another 12 million are affected worldwide. The disability adjusted life years (DALY) burden due to leishmaniasis is 860,000 for men and 1.2 million for women (WHO, 2002). It is also

estimated that about 500,000 persons are affected by VL annually; often people living in poor rural areas with limited health care resources (Desjeux, 1996).

Poverty and malnutrition play a major role as risks for the increased susceptibility to the disease. Another risk factor is the movement of susceptible populations into endemic areas, including large-scale migration of populations for economic reasons.

1.4   The Life Cycle of *Leishmania*

Leishmania parasite exists in two forms, amastigote and promastigote forms. The amastigote form occurs in humans, whereas the promastigote form occurs in the sandfly and in artificial culture. Only the female sandfly transmits the protozoan. It becomes infected with the *Leishmania* parasite when it sucks blood from the human or mammalian host. Four to five days after feeding, the parasite begins to develop in the mid-gut of the sandfly, where it undergoes a major transformation into the promastigote form. During this period, a large number of flagellate forms (promastigotes) are produced by binary fission in the mid gut. The flagellates migrate to the pharynx and buccal cavity of the sandfly between the sixth and ninth day of infected blood meal. A sandfly bite of a host during this period results in the spread of leishmaniasis. Following the bite, some of the flagellates that enter the circulation are destroyed, whereas others enter the cells of the reticuloendothelial system, where they change into the amastigote form. The amastigote forms also multiply by binary fission, with multiplication continuing until the host cell is packed with the parasites and ruptures, liberating the amastigotes into the circulation. The free amastigotes then invade fresh cells, thus repeating the cycle and, in the process, infecting the entire reticuloendothelial system.

Some of the free amastigotes are drawn by the sandfly during its blood meal, thus completing the cycle (Figure 1).

The usual incubation period for visceral leishmaniasis is typically 2-6 months (Rees and Kager, 1987). In addition, the extremes of the incubation period are highly variable, with a reported range from 10 days to 2 years (Rees and Kager, 1987; Pearson *et al*, 1999). *Leishmania* parasites may however, remain dormant and not present themselves until one has a compromised immune system (Pampiglione *et al.,* 1974).
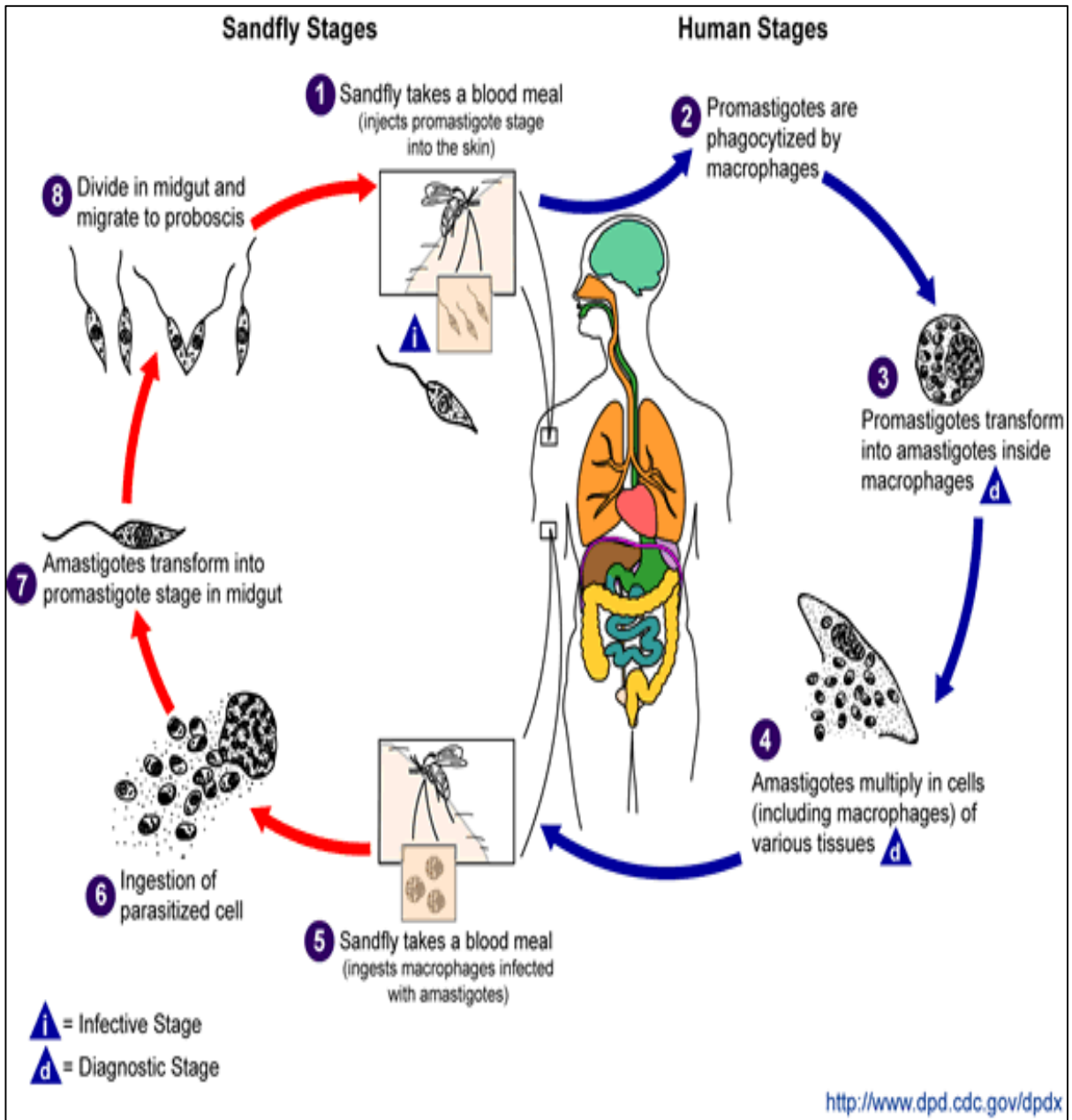
Figure 1: Life Cycle of *Leishmania* (www.dpd.cdc.gov/dpdx)

1.5   Transmission Cycle of *Leishmania*

There are two main epidemiological entities: zoonotic, where animal reservoir hosts are involved in the transmission cycle; and anthroponotic, where humans are the sole reservoirs and sole sources of infection for the vectors (WHO, 2002). In southern Asia, infected humans are the only known reservoir, and the parasite is transmitted from one person to another via the bite of the female sandfly. The sandfly rests inside mud-walled houses during the day, and is active from dusk to dawn, when transmission is presumed to occur (Indu *et al.,* 2003).

Visceral leishmaniasis is rarely transmitted by blood transfusion, sharing of needles by intravenous drug users (le Fichoux *et al.,* 1999), sexual intercourse (Symmers, 1960), accidental or deliberate inoculation in the laboratory (Manson-Bahr *et al.,* 1963), or congenitally (Nyakundi *et al.,* 1988).

Cutaneous leishmaniasis has been reported to be transmitted by deliberate scarification as a form of immunisation (Gunders, 1987) and through suckling (Marsden *et al.,* 1985). In Sudan, transmission of the disease takes place both in *Acacia seyal* and *Balanites aegyptiaca* woodland with the black cotton soil (Elnaiem *et al.,* 1998a, b) and inside villages (Elnaiem *et al.,* 1998c). It is probable that both anthroponotic and zoonotic transmission of *L. donovani* take place in eastern Sudan. There is evidence that the Egyptian mongoose *Herpestes ichneumon* (Elnaiem *et al.,* 2001) is a probable sylvatic reservoir host of *L. donovani* in woodland habitat. The female sandfly of genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World are the only proven vectors responsible for transmission of VL (Berman, 1997).

1.6   Clinical Forms of Leishmaniasis

There are three clinical forms of leishmaniasis, namely, VL including post kala-azar dermal leishmaniasis (PKDL), cutaneous leishmaniasis (CL) and Mucocutaneous leishmaniasis (MCL; Osman *et al.,* 2000).

1.6.1   Visceral Leishmaniasis

Visceral leishmaniasis /kala-azar is the systemic and disseminated form of the disease, in which the primary target of infection is the bone marrow, the spleen and the liver. The disease is characterized by prolonged irregular fever, splenomegaly, hepatomegaly, progressive anemia and pancytopenia along with hypergamma-aglobulinemia (Argwal *et al.,* 2005). In addition to the classic features of kala-azar, unusual features such as neuropathy with foot drop and nerve deafness were seen in kala-azar epidemic in western Sudan. (Hashim *et al.,* 1994).

Various terms have been used to describe VL including Dum-dum fever, Sikari disease, Burdwan fever, Shahib's disease and tropical splenomegaly. However, the most commonly used term is kala azar, which in Hindi means black sickness or black fever. The terms originally referred to Indian VL due to its characteristic symptoms, blackening or darkening of the skin of the hands, feet, face and the abdomen. VL is typically caused by *L. donovani* complex, which includes three species, *L. donovani* (Indian subcontinent and East Africa), *L. infantum* (Mediterranean basin) and *L. chagasi* (Latin America). The disease is a silent killer, invariably killing almost all untreated patients (Boelaert *et al.,* 2000). Even with treatment, case-fatality rates often exceed 10% in VL-endemic areas of Asia and Africa (Berman, 1997).

1.6.1.1 Post Kala-azar Dermal Leishmaniasis

Post kala azar dermal leishmaniasis is a recognized complication of VL of unknown cause. However, PKDL can develop during the treatment of kala azar, and in this case the term Para kala azar dermal leishmaniasis would seem more appropriate (Hashim *et al.,* 1995; Zijlstra *et al.,* 1995). Although PKDL usually follows recovery from a kala azar infection, it has been known to occur in patients who have not suffered previously from kala azar (Hashim *et al.,* 1995). It occurs in India and mainly in Sudan and Kenya in Africa (Hashim *et al.,* 1995). The disease begins with small measles-like lesions (hypopigmented macules, papules or nodules) appearing on the face, and gradually increase in size (rarely greater than 1cm in diameter). Eventually the lesions spread to the upper trunk, arms, forearms, thighs, legs, abdomen, the neck and the back. The multiple lesions can coalesce to form larger lesions and can lead to the gross enlargement of facial features such as the nose and lips, giving an appearance similar to leprosy. The disease is particularly severe if the lesions spread to the mucosal surfaces of the nasal septum, hard and soft palate, oropharynx, larynx or the eye lids and the cornea leading to blindness (Hashim *et al.,* 1995; Ramesh and Mukherjee, 1995). The lesions are usually self limiting; however those that do not heal spontaneously within six months have to be treated (Hashim *et al.,* 1995). Indian PKDL appears between 1-7 years after apparent cure of kala azar, although longer periods of up to 20-30 years have been reported (Zijlstra *et al.,* 1995). The African form of the disease usually appears within a few months after cure, in most cases within 6 months, on average within 56 days (Zijlstra *et al.,* 1995).

1.6.2 Cutaneous Leishmaniasis

It is known, as 'little sister' in some countries where the disease is so common that it is part of the family (WHO, 1998). In the Old World it is known as oriental sore (WHO, 1998). It produces skin lesions, sometimes as many as 200 on the face, arms and legs, causing serious disability and permanent scars (WHO, 1998). In the Old World CL is caused by *L. major* (WHO, 1996) *L. tropica* (WHO, 1996) and *L. aethiopica* (WHO, 1996). In the New World CL is caused by *L. mexicana* (WHO, 1996) and *L. braziliensis* (WHO, 1996) complexes. Some *L. infantum* and *L. donovani* strains can also cause lesions. Ninety percent of all cases of CL occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria, with 1-1.5 million new cases reported annually world-wide (WHO, 1996). *Leishmania major* usually produces self-healing lesions, while *L. tropica* is usually more chronic, and its most severe form, recidivans leishmaniasis, is very difficult to treat. In the New World, *L. mexicana* usually produces relatively benign lesions but some locations such as the pinna of the ears are very difficult to treat in general (Desjeux, 1996).

There are three types of CL; nodular or nodular ulcerative; ulcerative; and diffuse infiltrative. The nodular form (localized CL) has limited nodule and does not spread as the other forms. They are usually chronic sores. The ulcerative form (recurrent CL) is rare but does significant tissue damage and the parasites are very difficult to detect. The diffuse infiltrative form (diffuse cutaneous leishmaniasis – DCL) which is due to *L. aethiopica* and *L. amazonensis* (Desjeux, 1996) is less common, chronic in evolution and especially difficult to treat. It produces lesions resembling leprosy, which do not

heal spontaneously. There is systematic relapse after treatment due to deficiency of the immune response (WHO, 1998).

1.6.3 Mucocutaneous Leishmaniasis

This form is also called "espudia" in South America, produces disfiguring lesions on the face, destroying the mucous membranes of the nose, mouth and throat. Most cases of this type (90%) are found in Bolivia, Brazil and Peru. MCL is mainly caused by *Leishmania* species of the New World such as *L. braziliensis, L. Panamensis* and *L. guyanensis,* but mucosal lesions have also been reported in the Old World due to *L. donovani, L. major* and *L. infantum* in immunosuppresed patients (Desjeux, 1996).

1.7   Vectors and Reservoirs of Visceral Leishmaniasis

Leishmaniases are caused by 20 species of *Leishmania* and transmitted by 30 species of sandfly (Desjeux, 1992; Killick-Kendrick, 1990; Ashford, 1997). The insects are 2-3 mm long and are found throughout the tropical and temperate parts of the world. The sandfly larvae require organic matter, heat and humidity for development and so are commonly found in house-hold rubbish, bark of old trees, burrows of old trees and in cracks in house walls. It readily bites humans at night, primarily during twilight, while the host is resting. Most of the leishmaniasis infections are zoonotic (dogs, foxes, jackals); rodents and canids are reservoir hosts (Singh *et al*., 2006).

The distribution and incidence of leishmaniasis in various endemic areas is closely related to the distribution of specific sandfly (Kirk and Lewis, 1955).

Vectors of *L. donovani* in which infection in the sandfly gut has been confirmed are: *Phlebotomus argentipes* (*P. argentipes*) in India, *P. chinensis* in China, *P. perniciosis* in

North Africa, Italy, France and Portugal, *P. perfiliewi* in Greece, *P. orientalis* in Sudan and Ethiopia, *P. martini* in Kenya (Le Blancq and Peters, 1986). *Leishmania infantum* is transmitted by *P. perniciosus*, *P. ariasi, P. perfiliewi* and *P. neglectus. Leishmania chagasi* is transmitted by the *P. longipalpis* (WHO, 1990).

In the past a number of studies had been devoted to the identification of the reservoir host of *Leishmania* in Sudan and no parasite was found in birds, dogs, cats, fowl, rats, mice, sheep, goats, squirrels, bats, lizards and geckos (Archibald and Mansur, 1937). However, *Leishmania* parasites were later isolated from the East African monkey *Cercopithecus aethiops* (Kirk, 1956), Black rat *R. rattus,* Nile rat *Acomys albigena,* spiny black mice *Arvicanthis niloticus,* common genet *Genetta senegalensis* and African wild cat *Felis serval* (Adler *et al.,* 1966; Hoogstral and Hyneman, 1969). Both Kirk (1956) and El-Hassan *et al.,* (1992) also suggested that PKDL patients may serve as a reservoir for the *L. donovani* (Appendix 1).

1.8   Visceral Leishmaniasis in Sudan

Visceral leishmaniasis is a major health problem in the endemic areas in eastern and Southern Sudan where several outbreaks due to *L. donovani* have been reported since the early 1904 (Zijlstra and El-Hassan, 2001). The disease is one of the most important parasitic tropical diseases in Sudan, and is one of the most important foci in the world (Osman *et al.,* 2000). Visceral leishmaniasis in Sudan was first described by Naeve in 1904 (El-Hassan *et al.,* 1995) and at the turn of 20th century it was recognized as a serious health problem in Sudan. A Kala-azar Commission was then formed and operated from 1909 to 1913 (Zeese and Frank, 1987).

The endemic belt stretches from Atbara river in the north-east along Sudanese-Ethiopian border to south of the Sobat River and Nasir and Malakal and extending west across the White Nile and including other foci like the Kapoeta area, the Nuba Mountains and scattered areas in the Darfur region (Osman *et al.,* 2000)

Occasionally, severe epidemics of visceral leishmaniasis have claimed the lives of thousands of people. In recent years, visceral leishmaniasis has spread outside the established endemic areas and resurgence of cases has become apparent in regions with a previously low incidence (Siddig *et al.,* 1990).

The only proven vector for VL in Sudan is *P. orientalis* which is associated with *Acacia seyal* and *Balanites aegyptiaca* vegetation, and black cotton soils (Quate, 1964; Hoogstraal and Heyneman, 1969; Ashford and Thomson, 1991; Elnaiem *et al.,* 1997; Elnaiem *et al.,* 1998a; Elnaiem *et al.,* 1998b). However, in Kapoeta area of Southern Sudan, *P. martini* may, as in Kenya, be the main vector of VL (Minter *et al.,* 1962).
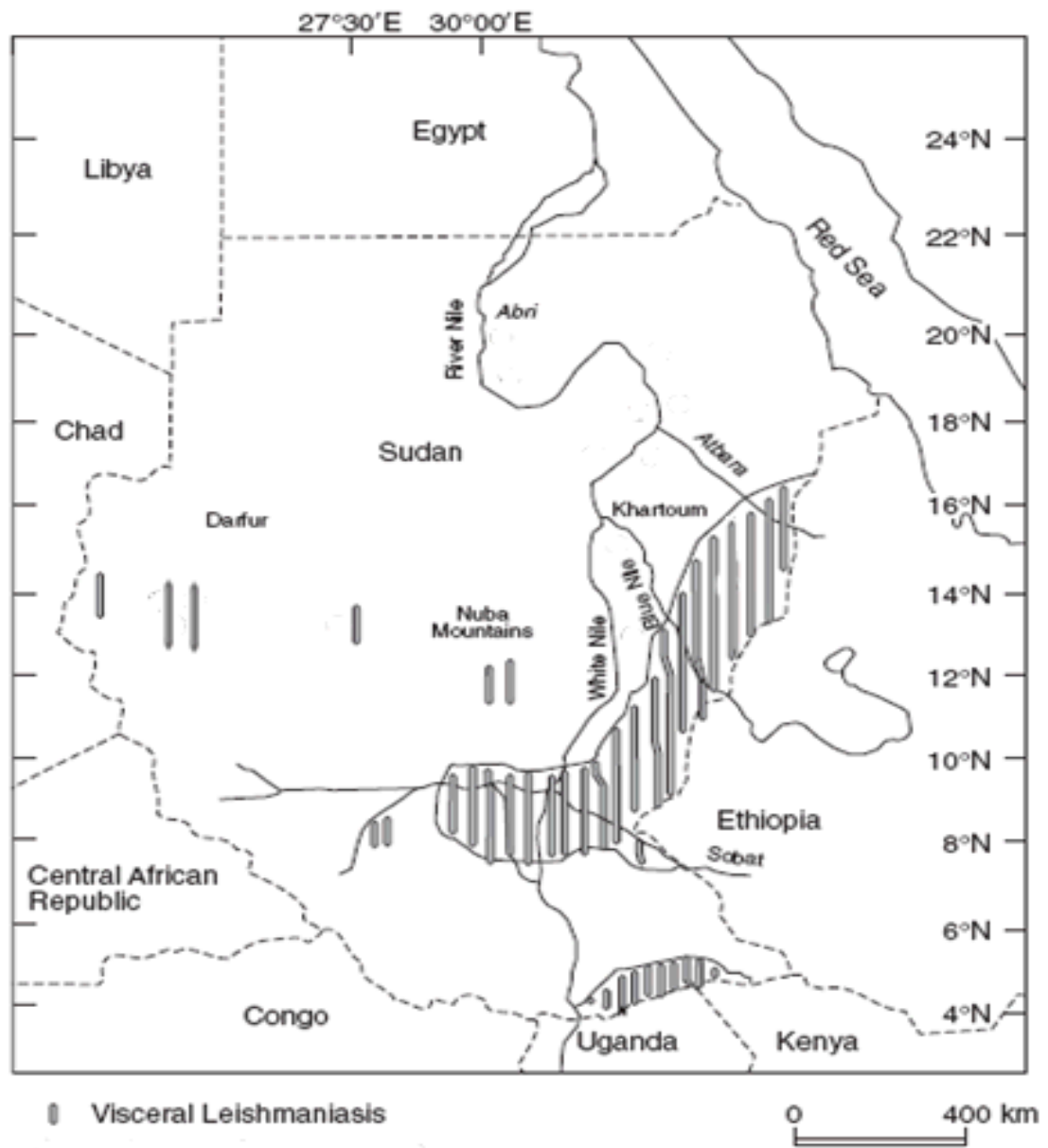
Figure 2: Distribution of visceral leishmanaiasis in Sudan (Osman *et al.,* 1998)

1.9   Visceral Leishmaniasis in Southern Sudan

Until the late 1980s, VL had been thought to be endemic only in areas ranging from Malakal and the Sobat River in the South (Upper Nile) to Kassala in eastern Sudan (Zijlstra and El-Hassan, 2001). However, in 1988 an increasing number of VL cases were found in Khartoum, among internally displaced persons that had come from the western Upper Nile region of Southern Sudan which was not previously known to be endemic for VL (de Beer *et al.,* 1991). A retrospective mortality survey suggested that from the start of the epidemic in 1984 to 1994 around 100,000 deaths among a total population of 280,000 might be attributed to VL. In the most affected areas, up to 70% of the population had died from the disease (Seaman *et al.,* 1996). A combination of factors such as introduction of the new parasite into the community, population movements in response to war, food shortages and poor nutritional status probably has increased the susceptibility to clinical disease after infection in Southern Sudan. It is now known that kala-azar is endemic in four of the ten states in Southern Sudan namely, Unity, Upper Nile, Jonglei and Eastern Equatoria (WHO, Southern Sudan-unpublished data; Figure 3). *Phlebotomus orientalis* has exceptionally high infection rate (10%) in Southern Sudan (Schorscher and Goris, 1992).
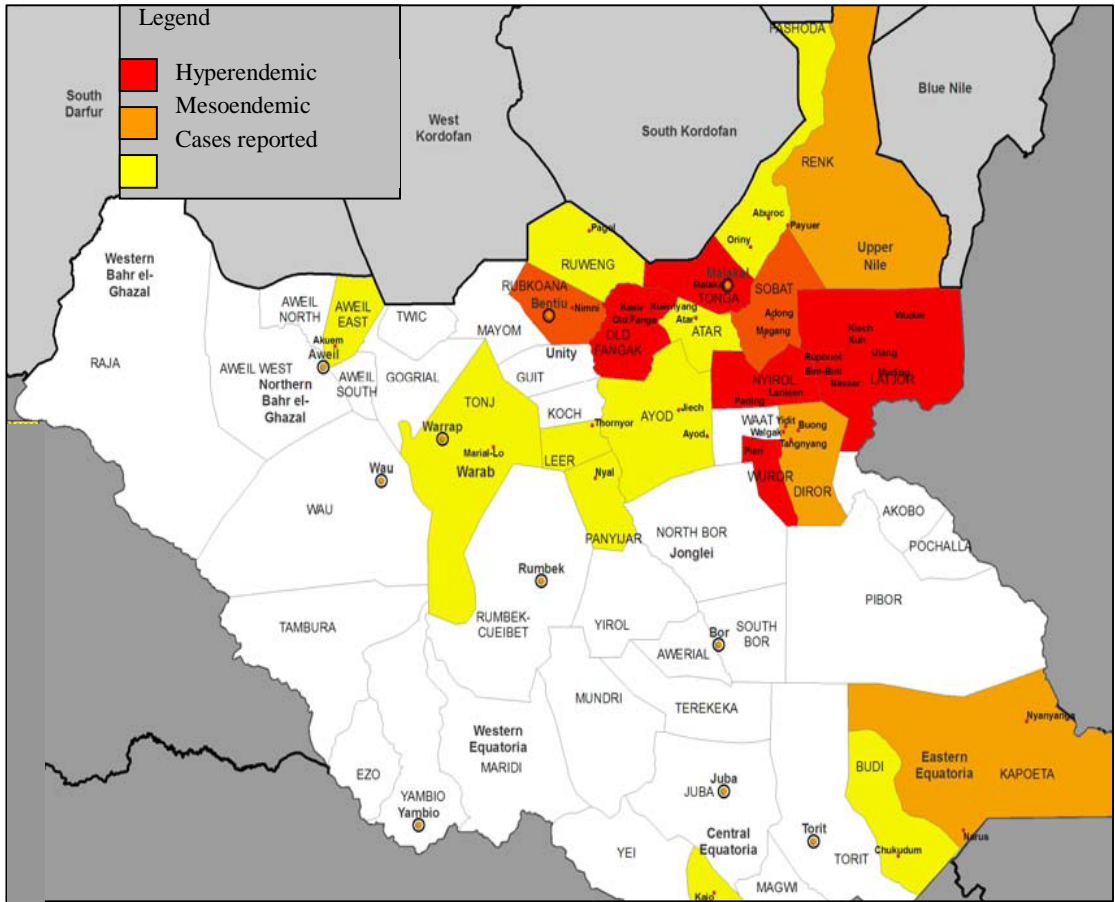
Figure 3: Kala-azar endemic areas of Southern Sudan (WHO, 2007 Southern Sudan, unpublished)

1.10 Risk Factors for Kala-azar

Studies have demonstrated that environmental factors that affect sandfly ecology (Walsh, 1993), human activities that increase exposure to sandflies (Dedet *et al.,* 1989; Alcais *et al.,* 1997; Desjeux, 2001) and the presence of other animals permissive to the *Leishmania* life cycle (Costa *et al.,* 1999; Ashford, 2000) play critical roles in the development of the human disease. Poor economic conditions (Desjeux, 1996; Thakur, 2000a), malnutrition (Badaro *et al.,* 1986; Dye and Williams, 1993) and impaired reactivity of the immune system (Alvar *et al.,* 1997; Wolday *et al.,* 1999) have all been shown to increase the risk of VL. Laboratory mice infected with *L. donovani* have shown that their genetic make-up plays a role and that the *Nramp1* gene controls *L. donovani* multiplication in the liver at an early stage following intravenous delivery of the parasites (Bradley *et al.,* 1979; Vidal *et al.,* 1995; Leclerq *et al.,* 1996).

Previous work in Sudan has shown that *P. orientalis* (the vector of *L. donovani* in Sudan) was present in the huts of the village and that many dogs were infected by the same *Leishmania* strains as those isolated from patients with VL (Dereure *et al.,* 2000; Pratlong *et al.,* 2001). Environmental studies have also shown that neem trees were protective while the presence of cows near the living quarters was associated with kala-azar (Bruno *et al.* (2002).

Studies in Bangladesh have established that young children and people who were sleeping in the same room with a kala-azar patient are at an increased risk of kala-azar infection (Caryn *et al.,* 2005). In India occupations such as Agriculture is also known to be associated with kala-azar (Kumar *et al.,* 1999). A case-control study in Nepal has

shown that owning cattle or buffalos have conferred a strong protective effect (Bern *et al.,* 2000a) and continuous use of mosquito nets in summer was shown to be protective (Caryn *et al.,* 2005) against kala-azar. Studies conducted in Bangladesh (cross-sectional) and Nepal (case-control) confirms the protective effect of untreated nets in the control of kala-azar (Bern *et al.,* 2000a; Caryn *et al.,* 2005). The study in Bangladesh observed no difference in terms of income, education or occupation, housing materials, keeping goats and chickens inside bedrooms among study participants (Caryn *et al.,* 2005).

1.11   HIV/*Leishmania* co-infection

Leishmaniasis is one of the opportunistic infections associated with human immuno-deficiency virus (HIV)-infected individuals. Most of the co-infections are due to the visceral leishmaniasis (Desjeux and UNAIDS, 1998). Acquired immune deficiency syndromes (AIDS) and VL are locked in a vicious circle of mutual reinforcement. The gridlock produce cumulative deficiency of the immune response, as *Leishmania* parasites and HIV destroy the same cells (WHO, 1998).

The greatest prevalence of *Leishmania*/HIV co-infection has been in the Mediterranean basin. So far, more than 2,000 cases of co-infection have been notified to the WHO and ninety per cent of them were reported from Spain, Italy, France and Portugal (Desjeux and Alvar, 2003).

The majority of the cases of co-infection registered in the South Americas were reported from Brazil, where the incidence of AIDS has increased from 0.8/100,000 inhabitants in 1986 to 12.3/100,000 in 2001 (Sampaio *et al.,* 2002 and Rabello *et al.,* 2003).

In Asia cases of co-infection are being registered in India, Bangladesh and Nepal. The situation in those countries is being aggravated by the increasing resistance to antimonials (Sundar, 2001a).

In Africa, the number of co- infection has started increasing and is being exacerbated by social phenomena such as mass migration and wars (Guiguemde *et al.,* 2003).

1.12 Diagnosis of *Leishmania*

The diagnosis of VL is complex because of the commonly occurring diseases such as malaria, typhoid, and tuberculosis which share its clinical features. Many of these diseases can be present along with VL as co-infection. Further, sequestration of the parasite in the spleen, bone marrow or lymph node complicates the issue. Some of the diagnostic methods employed include microscopy, serology, culture and molecular method.

1.12.1 Microscopy

This is the most commonly used method for diagnosing VL in patients. Samples of infected tissues are obtained from aspirate of spleen or bone marrow or lymph nodes and the amastigotes are either seen in Giesma or Leishman stained smears of the tissues or cultures. Microscopic finding of the parasite is considered the golden standard for diagnosis; although the method is relatively simple and cheap, it has low sensitivity (Weiss, 1995; Osman *et al*., 1997). It has been estimated that the sensitivity of microscopy may only be 50-85% when a single specimen is examined by a competent microscopist (Hommel, 1999). The splenic aspirate also carries a small but definite risk of serious hemorrhage (Sundar *et al.,* 2002 and Lightner *et al*., 1983).

1.12.2 Serology

Serological methods are highly sensitive and non-invasive. They are comparatively more suited for diagnosing VL in endemic regions. These methods are either based on detection of antibodies or antigens. Many conventional methods for antibodies detection, for instance, gel diffusion, complement fixation test, indirect haemagglutination test, indirect fluorescent antibody detection test (IFAT) and counter current electrophoresis have been evaluated with varying sensitivities and specificities (Haldar *et al.,* 1981, Hockmeyer *et al.,* 1984, Sinha and Sehgel, 1994).

Currently, the most used methods for diagnosis of VL are direct agglutination test (DAT) and enzyme linked immunosorbent assay (ELISA). DAT was introduced about two decades ago and was rapidly followed by its improved version for field use (Badaro *et al.,* 1983).

DAT has been found to be 91-100 per cent sensitive and 72-100 per cent specific in various studies (Vinayak *et al.,* 1994; Sundar *et al.,* 1996; Zijlstra *et al.,* 2001).

However, in spite of its excellent diagnostic accuracy, its use is limited due to non-availability of standardized antigen (freeze-dried and liquid antigen) and its adaptability at the rural centers (Singh *et al.,* 2006).

ELISA is now being used as potential serodiagnostic tool for VL. Although this technique is highly sensitive, its specificity depends upon the antigen used. There are two types of ELISA antigens, namely, crude soluble antigen (CSA) and a recombinant antigen (rk39). CSA is obtained by freezing and thawing of live promastigotes while

(rk39) is a conserved part in kinesin region (Choudhry *et al.,* 1990; Burns *et al.,* 1993; Bern *et al.,* 2000 b).

Recombinant antigen (rK39) using the cloned antigen of 39 amino acid repeats of a kinesin like gene found in *L. chagasi* instead of whole *Leishmania* parasites has been developed. Studies using the rK39 antigen, either in an ELISA or dipstick form (Sundar *et al*., 1998), performed well in Brazil, India and Europe (Medrano *et al*., 1998; Sundar *et al*., 1998). An rK39-based ELISA was found 93% sensitive and 80% specific in patients of kala-azar in Sudan (Zijlstra *et al*., 1998).

1.12.3 Culture

Culture of parasite can improve the sensitivity of detection but is often done only when other methods fail (Weigle *et al.,* 1987 and Navin *et al.,* 1990).

Parasite culture is mainly required for obtaining sufficient number of promastigotes to be used as antigen for immunological diagnosis, inoculation of experimental animals and *in-vitro* screening of drugs.

1.12.4 Molecular Diagnosis

The test based upon antibodies detection may remain positive for several years due to high and persistent antibodies titers in patients and hence fails to detect past and present infection and in immuno-compromised patients (Singh *et al.,* 1995). Inspite of significant development of diagnostic tools, none of the immunological methods have proved to be useful as a test of cure (Singh *et al.,* 1995).

The *Leishmania* (Polymerase chain reaction) PCR assays using peripheral blood as clinical specimen was shown to be highly efficient and non invasive alternative with sensitivity varying from 80-100 per cent (Fisa *et al.,* 2002). PCR can be more useful in the prognosis of VL since none of the available methods can be used as test of cure. However, in prognosis, PCR is still far from standardization and has been done only for few workers and mostly in HIV/VL co-infected patients (Fisa *et al.,* 2002).

1.13   Treatment of Leishmaniasis

The treatment options for VL are limited and far from satisfactory. All the drugs available need to be given parenterally except Miltefosine which is potentially toxic.

1.13.1 Pentostam (sodium stibogluconate) and Glucantime (Meglumine antimoniate)

The pentavalent antimonials compounds have been the mainstay of antileishmanial therapy for more than sixty years (Singh *et al.,* 2006). Due to high cost of the branded sodium stibogluconate (approx 200 USD/ patient), a generic sodium antimony gluconate (SAG, Albert David Ltd, India, 13 USD/ patient) is being used with satisfactory cure rate (Peters *et al.,* 1981).

Unfortunately, the parasite has become resistant to Sbv in India (Singh *et al.,* 2006). However, Pentostam continued to be used in Sudan as a medicine of choice although it is expensive (Desjeux, 1992). It is administered at WHO recommended dosage of 20mg i/m daily for 30 days (WHO, 1990).

1.13.2 Amphotericin B

This is the most effective antileishmanial drug which induces high cure rates. Use of formulation of amphotericin B, a pollen antibiotic, for treatment of leishmaniasis is biochemically rational because the target of amphotericin B is ergosterol, which are the major membrane sterols of *Leishmania* species (Berman *et al.,* 1986).

Due to high affinity of amphotericin B for sterols, aqueous pores are formed in the membrane leading to increased membrane permeability and killing of *Leishmania* (Croft and Yardley, 2002).

Amphotericin B is more widely used for treatment of VL and has constituted a major development in antileishmanial chemotherapy during the last 10 years. At a dose of 0.75-1.0 mg/kg for 15 infusions on alternate days, Amphotericin B cures more than 97 per cent of patients (Mishra *et al.,* 1992 and Thakur *et al.,* 1999). Occasional relapse (1%) might occur with amphotericin B, which can be treated successfully with the same drug (Singh *et al.,* 2006). It has been recommended as first line drug in India. However, the cost of the treatment is expensive.

1.13.3 Petamidine

This was the first drug to be used for VL patients who were refractory to Pentavalent antimonials in India. This drug is associated with serious adverse events like insulin dependent diabetes mellitus, shock, and hypoglycemia and death in significant proportion of patients. The declining efficacy, resistance and serious toxicity associated

with the drugs have made it unsuitable as a viable alternative to Pentavalent antimonials for kala-azar patients (Thakur *et al.,* 1991, Mishra *et al.,* 1992 and Sundar, 2001b).

1.13.4 Paromomycin (Aminosidine)

This drug is an aminoglycoside antibiotic with unique antileishmanial activity. It acts synergistically with antimonials *in vitro* and the combination has been used effectively in India (Thakur *et al.,* 1995 and Thakur *et al.,* 2000b).

1.13.5 Miltefosine

Miltefosine is the only antileishmanial drug that is administered orally, at a dosage of (2–3) mg/kg per day (100 mg/day for patients weighing more than 25 kg) for 28 days. Increasing the daily dosage to 150 mg has been suggested by some experts for HIV positive adult patients. In patients co-infected with HIV and *L. donovani*, miltefosine is less effective than antimonials but the mortality rate is lower as a result of better tolerance and fewer adverse effects. In refractory patients previously treated with miltefosine, repeated courses increase the cure rate. The principal side-effects are gastrointestinal toxicity and teratogenicity; thus miltefosine is contraindicated during pregnancy and in lactating women and women of childbearing age must use effective contraception during and for 3 months after treatment. There is serious concern that unsupervised use of miltefosine might lead rapidly to high relapse rates.

1.13.6 Combination therapy for kala-azar

A potent combination should have a short half life which would rapidly bring down the parasite load below which new mutants are less likely to emerge. The second combination drug should have a long half life which will kill the remainder of the parasites. This combination therapy helps in shortening the duration of treatment. Unfortunately, there are only few drugs available for combination. The only feasible combination therapy would be miltefosine and paromomycin, due to less toxicity (Singh *et al.,* 2006). Antimonials will be less suitable in combination with miltefosine because of toxicity and variation of parasite sensitivity.

1.14 Control of Leishmaniasis

Currently, it is not possible to devise a single strategy for leishmaniasis control program. A strategy combining the following three approaches can help in eradication of the disease:

1.14.1 Vector control strategy at national level

The classical example of efficacy of this strategy is the almost disappearance of VL cases in India in 1960s when residual insecticides were used extensively as a part of the National Malaria Eradication Programme (now National Vector Borne Disease Control Programme). Commercial production of pyrethroids impregnated fabrics (bed nets and curtains) or insecticides paints in a slow release emulsifiable solution should be encouraged to prevent the transmission of the disease.

1.14.2 Serological diagnosis at the infection stage

A prompt treatment will prevent *Leishmania* infection evolution to overt disease and reduce morbidity and mortality. It will also reduce the parasite load and transmission rate in anthroponotic type as in India.

1.14.3 Health education to the population of the endemic areas

Physicians serving in the endemic area need to incorporate health education strategy in order to improve the awareness regarding transmission, clinical features of the disease and importance of complete treatment.

1.15   Problem Statement

Kala-azar is endemic in several areas of Southern Sudan. The factors causing these epidemics have not been documented due to civil war and lack of resources dedicated to the control of the disease. This is the first case control study to be conducted in Southern Sudan to find out the risk factors for kala-azar.

1.16   Justification of the Study

Visceral leishmaniasis is a major health problem in the endemic areas in eastern and Southern Sudan where several outbreaks due to *L. donovani* have been reported since the early 1904 (Zijlstra and El-Hassan, 2001).

A retrospective mortality survey suggested that from the start of the epidemic in 1984 to 1994 around 100,000 deaths among a total population of 280,000 might be attributed to VL.

Between 2000 and 2005, it is estimated that at least 2,000 cases of VL occurred in Southern Sudan (Moses Chol-personal communication). With the current geographical distribution of VL, it is endemic in four of the ten states in Southern Sudan namely, Unity, Upper Nile, Jonglei and Eastern Equatoria (WHO, Southern Sudan-unpublished data). Despite the importance of the disease, very little is known about the ecology of the vector and the transmission dynamics of the disease (Thomson *et al.,* 1999) in Southern Sudan. In addition, the risk factors associated with kala-azar transmission in Southern Sudan remains largely undocumented. This study aimed to examine the risk factors associated with kala-azar transmission in Southern Sudan. The findings and recommendations arising from this study will help in prioritizing kala-azar control efforts in Southern Sudan.

1.16   Hypotheses

1.16.1 Null Hypothesis

Behavioral characteristics and environmental factors are not risk factors for kala-azar transmission in Fangak County Jonglei State, Southern Sudan.

1.16.2 Alternate Hypothesis

Behavioral characteristics and environmental factors are risk factors for kala-azar transmission in Fangak County Jonglei State, Southern Sudan.

1.17   Objectives of the Study

1.17.1 General Objective

To determine the risk factors for kala-azar transmission that can be targeted to reduce the related morbidity and mortality in Fangak County, Jonglei State.

1.17.2 Specific Objectives

1. To determine behavioral characteristics as risk factors for kala-azar

2. To determine environmental factors as risk factors for kala-azar

CHAPTER TWO

2 MATERIALS AND METHODS

2.1 Study Design

Unmatched case control study was conducted in Fangak County in Jonglei State, Southern Sudan from October 2007 to December 2007 because there was an ongoing epidemic in the area and other neighboring counties. Kala-azar cases were also readily available within a very short time. Subjects were interviewed using a structured questionnaire to assess behavioral and environmental variables presumed to be risk factors for kala-azar transmission. The questionnaires which were originally in English were translated into Nuer for study recruits and their responses translated back into English for the principal researcher to complete the forms. The research assistant on the other hand, completed the questionnaires forms directly as the respondent answered (Appendix 3). During the study period, the respondents' exposure histories had been elicited retrospectively for the past one year. In order to establish the typical community way of life on the uses of bed nets and cow dung ashes, about twenty five homes within Fangak town and one other near by village (Wangshot) were visited during the study period for an observational data collection.

2.2 Study area

The present Old Fangak County was part of Phou state which comprises of Ayod, Atar/Khorfulus and Old Fangak/Zeraf. The County now borders Shilluk Kingdom and Ruweng County to the north, Atar County to the east, Ayod County to the south and

Quit and Rubkona to the west. Fangak County lies in the eastern flood plains zone that is characterized by seasonal flooding of the low lying areas (during the rainy season). The rains start in May and end in October. The soil is of clay type "black cotton" with *A. seyal* forests and numerous *B. aegytiaca* trees. The livelihood of the people is mainly agricultural activities which include cattle rearing and fishing.
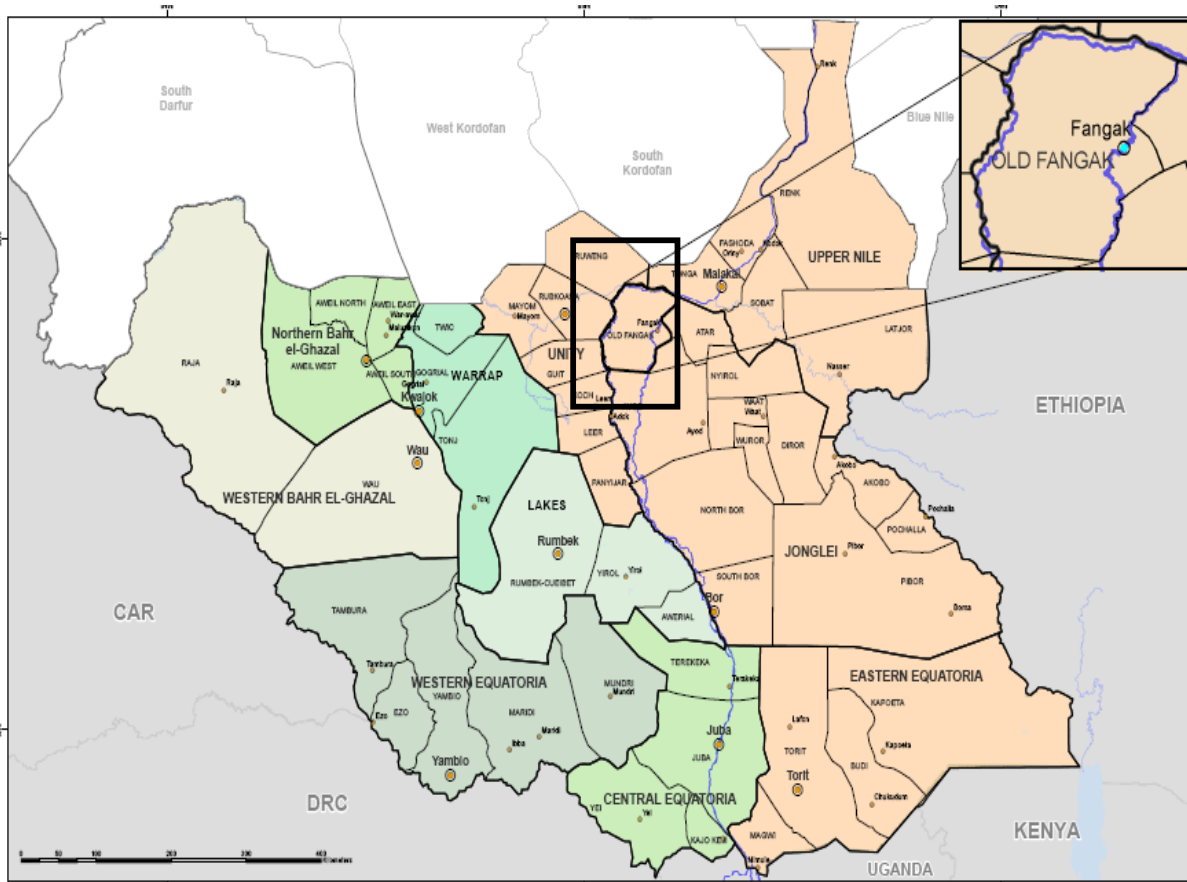
Figure 4: Study site (Fangak County) (WHO, 2007 Southern Sudan, unpublished)

2.3   Study Site

This study was carried out at a Coordinating Committee of the Organization for Voluntary Service (COSV) primary health care center (PHCC) in Old Fangak town (Figure 4). The PHCC with a 20 bed capacity has three clinics, namely, the general out patient (OP), maternal and child health (MCH) and the kala-azar clinic. The OP and MCH operate on Mondays to Fridays while the kala-azar clinic operates daily in the evenings including weekends. The patients seen at the kala-azar clinic were not exclusively the kala-azar patients. Fangak PHCC was the only health facility providing kala-azar treatment in the county; other health facilities in endemic states were recording few cases of kala-azar and the patients were coming from distances as far as 50 - 75 km away. There was no way of getting comparative controls from such patients.

2.4   Study Population

The target population was patients above 2 months old who lived in Fangak County for at least 2 months and had attended any of the three clinics during the study period.

2.5   Study variables

The study variables are described in details in Appendix 3 include demographic variables, clinical information, behavioral characteristics and environmental factors.

2.6 Sampling Approach

2.6.1 Sample Size

The sample size was calculated using the formula for unmatched case-control studies using statcalc in Epi Info 3.3.2 software. The following was employed: ratio of controls to cases, 2:1; power of 80%; confidence interval level (1-of alpha) 95%, percentage of exposure in cases is 10%; and an odds ratio of 4 for lack of use of bed net (as a risk for kala-azar transmission). Using these parameters, a sample size of 144 was obtained; 96 controls and 48 cases.

2.6.2 Definition of a case

2.6.2.1 Suspected case

A suspected case of kala-zar was defined as any person who had lived in Fangak County for 2 or more months and had developed a condition or a disease characterized by fever for at least 2 weeks and had either palpable spleen (splenomegaly) or wasting and enlarged lymph nodes (lymphadenopathy).

2.6.2.2 Confirmed case

A patient meeting the suspected case definition of kala-azar as defined above, plus a laboratory confirmation of the presence of kala-azar antibodies using a dipstick.

2.6.3 Selection of Cases and Controls

2.6.3.1 Cases

Cases selected for the study were individuals aged 2 months and above that met the confirmed case definition as described in the section (2.6.2.1) Patients visited the clinic for various illnesses. The attending clinician sent all those suspected to have kala-azar to

the laboratory for both dipstick and DAT tests. Results for dipstick were obtained within 15 to 20 minutes. If the dipstick was positive, the patient was enrolled for kala-zar treatment and follow-up at the PHCC. Those enrolled were purposely consecutively selected for the study until the sample size was attained.

2.6.3.2 Controls

Two hospital controls were randomly selected for each case. Individuals selected as controls were those who had attended the clinic for other ailments other than kala-azar and were not clinically suspected to have kala-azar. The selected controls were those attending the clinic at the same time as the case or at least within a few hours or the following day. The controls were tested with a dipstick to ensure they did not have kala-azar. Only those who tested negative were enrolled in the study.

The patients who had tested negative by dipstick were potential cases, if the DAT test results, which took 24 hours were used. However, for the purpose of this study, the DAT results were not followed due to the long time taken to obtain the results and the potential for losing the opportunity to select other controls and case.

2.6.4   Inclusion Criteria

1.   Patients attending health services at Fangak PHCC

2.   Patient must live in Fangk County for at least 2 months

2.6.5　Exclusion Criteria

1. Relatives of kala-azar patients were excluded as control.

2. Cases and controls not from Fangak County were excluded.

3. Children not accompanied by their mothers. Only mothers would have been able to accurately respond to the questionnaire. The other reason was to ensure consistency in data quality by questioning a similar child caretaker, who was the mother in this case.

2.7　Dipstick Method

The Dia Med-IT LEISH/Dipstick™ was used to confirm suspected cases and for screening the controls. The test is an immuno-chromatographic rapid test that uses the recombinant antigen rK39, to detect the presence of antibodies against *Leishmania* species. The manufacture's instructions were followed in testing blood and interpreting the results.

Blood for testing was collected from the patient using standard clinical procedures and universal precautions, ensuring no infection was introduced to the study participant, protection of the person collecting the sample, and to avoid any external contaminants. For each test, about 10µl of blood were collected.

2.8　Data Management

The completed questionnaires were rechecked for completion and accuracy before being transported to the central location for data entry. Data were kept confidential and safe to protect individual identity. Data were then entered into computer using Epi Info version 3.3.2 and checked for outliers and missing values. Data was cleaned and univariate, bivariate and unconditional logistic regression analysis was performed.

CHAPTER THREE

3   RESULTS

3.1   Demographic Factors

A total of 144 of participants were recruited for the study with (33%) cases and (67%) controls. Of the total study participants, 44% were males and 56% were female. Fifty six percent of the kala-azar patients were under five years old (Table1 and Figure 5). Among the children of less than five years of age who had kala-azar, 89% were under three years of age (Table 2). The age distribution of the study population is almost uniformly distributed between cases and controls (Figure 5). The numbers of kala-azar patients categorized in an age interval of five years decreases sharply from children under five years to ten years of age and remained steady there after. (Figure 5).

There were 6% of the cases in primary school and none of the control went to school (Table 1). Over all both cases and control did not attain secondary school level of education. Only two percents of the study population were employed (Table 1).

Table 1: Demographics Factors

Variables	Cases n (%)	Controls n (%)
Gender		
Male	22 (46)	42 (44)
Female	26 (54)	54 (56)
Age in years		
> 0-5	27 (56)	60 (63)
> 5-10	10 (21)	8 (8)
> 10-15	2 (4)	4 (4)
> 15-20	3 (6)	4 (4)
> 20-25	3 (6)	4 (4)
> 25	3 (6)	16 (17)
Mean age	8yrs	10 yrs
Age range	9 mo-45 yrs	5 mon-62 yrs
Level of education		
Primary school	3 (6)	0 (0)
Secondary school	0 (0)	0 (0)
Employment status		
Employed	1 (2)	2 (2)
Unemployed	47 (98)	94 (98)

Table 2: Age groups of under five years old

Age groups	Cases		Controls	
	Frequency	Percent	Frequency	Percent
> 0-1	8	30	15	16
> 1-2	7	26	25	26
> 2-3	9	33	10	10
> 3-4	2	7	6	6
> 4-5	1	4	4	4
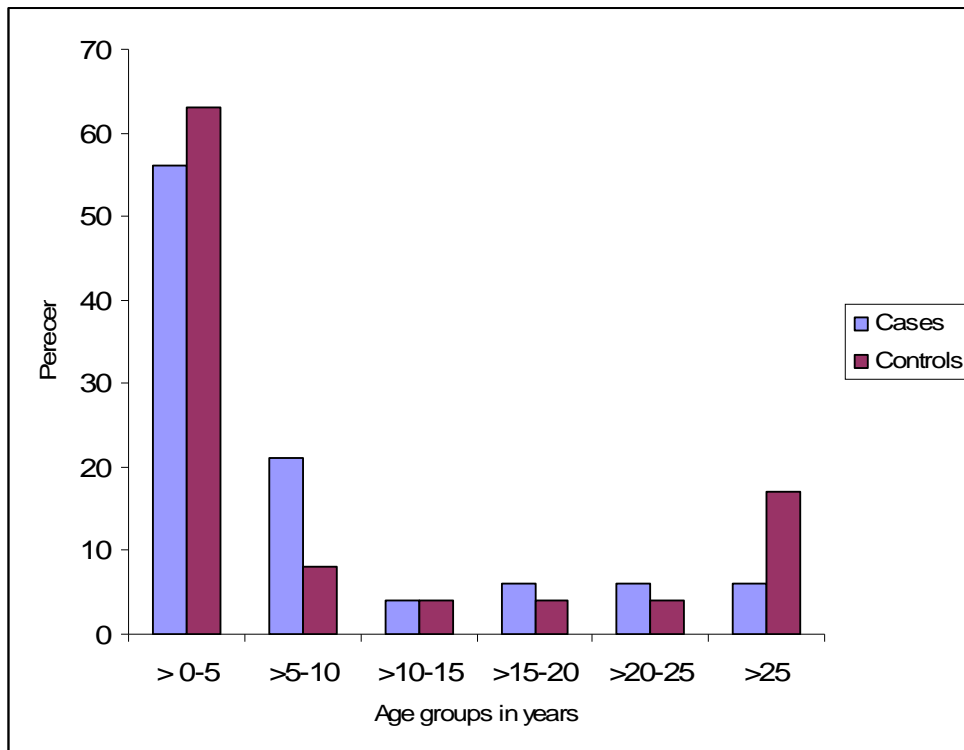
Figure 5: Percent Age group distribution

3.2   Clinical Factors

Forty one percent of kala-azar patient presented for treatment in about two months after the on set of symptoms of kala-azar, while 33% presented for treatment in less than two months. Twenty five percent started kala-azar treatment after two months (Figure 6 and Table 3).

A hundred percent of the cases had fever and enlarged lymph nodes. Ninety two percent of the cases reported having lost weight. Kala-azar patients with an enlarged spleen and liver were 46% and 42% respectively. Most of the cases had loss of appetite, with only 19% reporting as having good appetite (Table 4).
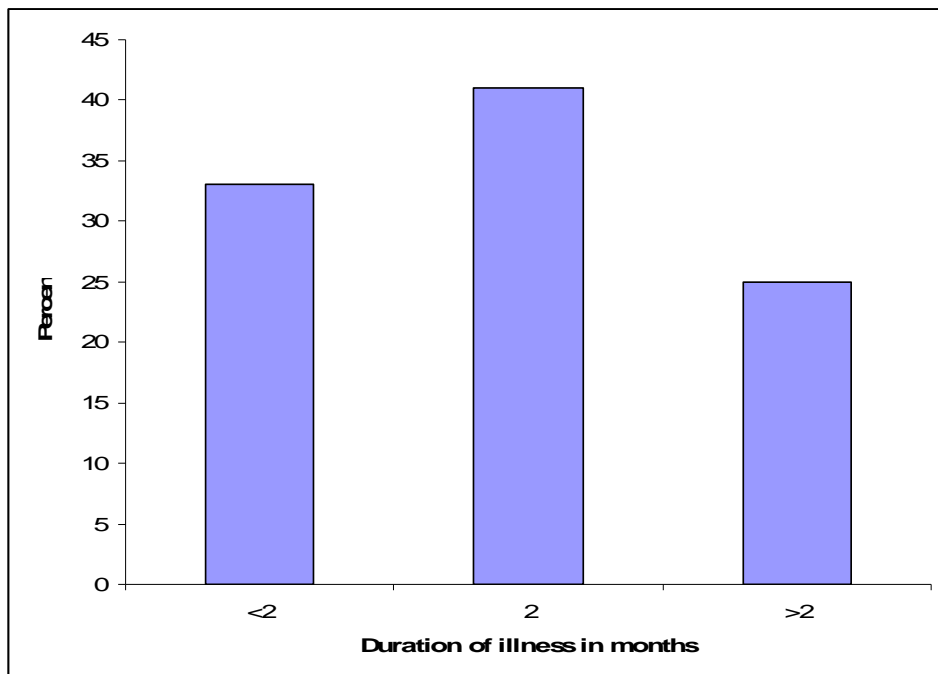
Figure 6: Duration of illness before initiation of treatment by kala-azar patients

Table 3: Duration of illness before initiation of treatment by kala-azar patients

Periods of illness in weeks	Frequency	Percent
1	2	4
2	4	8
4	10	21
8	20	41
12	7	15
16	2	4
20	2	4
28	1	2

Table 4: Clinical symptoms and signs of kala-azar patients

Clinical signs and symptoms	No of patients	Percent
Fever	48	100
Lymphadenopathy	48	100
Splenomegaly	22	46
Hepatomegaly	20	42

3.3 Behavioral Factors

There were different types of bed nets in use: long lasting insecticide treated ("Polyethylene"), cotton cloth- untreated ("Dhamoria"), and 'silk cloth' untreated ("Smooking") (Table 5).

Ninety two percent of the cases and 98% of the controls slept under a bed net respectively. Eight percent of kala-azar patients did not use any type of bed nets while those who sleep under bed nets, 69% bought their own bed nets (Table 6). Two percent of the controls did not use bed nets and 69% of those who use bed nets also bought their own bed nets.

Among the cases of kala-azar nobody was using the treated type of bed net, 50% of them used the "Dhamoria" and 42% used the "Smooking" type of bed nets. Twenty five percent of controls used the "Smooking" and "Polyethylene" type of bed nets respectively, while 48% used the "Dhamoria" type (Table 6).

Table 5: Behavioral characteristics/ Total number of persons using bed nets

Variables	Cases n (%)	Controls n (%)
Uses "Polyethylene" insecticide treated bed nets	0 (0)	24 (25)
Uses "Dhamoria" non insecticide treated bed net	24 (50)	46 (48)
Uses "Smooking" non insecticide treated bed nets	20 (42)	24 (25)
Total number of persons using any type of bed nets	44 (92)	94 (98)

Table 6: Behavioral characteristics/Types of bed nets

Variables	Cases n (%)	Controls n (%)
Do not use bed net	4 (8)	2 (2)
Bought own bed net	<u>33 (69)</u>	<u>66 (69)</u>
Types of bed nets		
Uses "Polyethylene" insecticide treated bed nets	<u>0 (0)</u>	<u>24 (25)</u>
Uses "Dhamoria" non insecticide treated bed net	24 (50)	46 (48)
Uses "Smooking" non insecticide treated bed nets	20 (42)	24 (25)

Table 7: Behavioral characteristics/Host

Variables	Cases n (%)	Controls n (%)
Occasionally play in the dark in forest or around houses	26 (54)	32 (33)
Sleeps in the same room with kala-azar patient	7 (15)	11 (12)

3.4 Environmental Factors (animals)

There were 10% of cases who kept goats, 9% kept chicken and 10% kept dogs in the sleeping houses while 17% of the controls kept goats, 26 % kept chicken and 12 % kept dogs in the sleeping houses respectively (Table 8). Twenty one percent of kala-azar cases always smear cow dung ashes on the body and 33% of them sometimes use cow dung ashes. There were 19 % and 17% of the controls who always and sometimes smear cow dung ashes on the body respectively (Table 8).

Fifty six percent of the cases and 49% of the controls have homes situated less than a hundred meters from the stagnant waters or a river (Table 9). On the other hand, 54% of the cases and 39% of the controls reported to have ant hills near by their compounds respectively.

There were 35% cases of kala-azar who had *A.seyal*, 65% *B.aegyptiaca* and 31% had *A. indica* in their compounds while among the controls 40% had *A. seyal*, 49% *B. aegyptiaca* and 39% had *A.indica* vegetations in their compounds (Table 9).

Table 8: Environmental factors /Animals

Variables	Cases n (%)	Controls n (%)
Always smear ashes on the body	10 (21)	18 (19)
Sometimes smear ashes on the body	16 (33)	16 (17)
Sleeps in the same room with goats	10 (21)	17 (18)
Sleeps in the same room with chicken	9 (19)	12 (13)
Sleep in the same room with dogs	8 (17)	12 (13)
Grass thatched houses	48 (100)	95 (99)

Table 9: Environmental factors (vegetation/natural)

Variables	Cases n (%)	Controls n (%)
Sleeping house less than 100m from the river/stagnant water source	27 (56)	47 (49)
Is your house near by an ant hill	26 (54)	37 (39)
Acacia seyal	17 (35)	38 (40)
Balanites aegyptiaca (Thaou)	31 (65)	47 (49)
Azadiracta indica (neem)	15 (31)	37 (39)

3.5   Observational Home Visit

On the first the day of visit, one child was seen being bathed and on the second day two children were being bathed among twenty five homes visited on the two occasions. On another day, a visit was made to establish the type of bed nets in use by the community.

3.6   Demographics Factors

In the bivariate analysis, age and gender variables were not associated with kala-azar (Tables 10 and 11).

Table 10: Risk assessment for demographic variables/Gender

Demographic variables	Cases	Controls	OR	(95% C.I.)	P-value
Male	22	42	1.09	0.54-2.18	0.81
Female	26	54			

Table 11: Risk assessment for demographic variables/Age

Demographic variables (Age)	Cases	Controls	OR	(95% C.I.)	P-value
≤5 years	27	60	0.77	0.38-1.56	0.47
≥ 5 years	21	36			

The status of education could not be verified because the controls did not attend any formal education. On the other hand whether a person was employed or not, there was no statistical association with kala-azar (Tables 12 and 13).

Table 12: Risk assessment for demographic variables/Education

Demographic variables	Cases	Controls	P-value
Education	3	0	Undefined
No education	45	96	

Table 13: Risk assessment for demographic variables/Employment

Demographic variables	Cases	Controls	OR	(95% C.I.)	P-value
Employed	1	2	1	0.09-11.31	0.71
Unemployed	47	94			

3.7   Environmental Factors (on bivariate analysis)

Among the environmental factors subjected to bivariate analysis, none of the factors were associated with kala-azar except outdoor night activities (Table 14). Persons who reported to have ant hills near their homes and *B. aegytiaca* in their compounds were borderline factors associated with kala-azar (Table 14).

The two border line environmental factors (having ant hills near their homes and *B. aegytiaca* in their compounds) and outdoor night activities were subjected to multivariate analysis. All the borderline factors remained insignificant, while the outdoor night activities retained its statistical significance (Table 15).

Table 14: Environmental factors

Variables	OR	(95%C.I.)	P-value
Keep sleeping floor damp (wet)	1.30	(0.64-2.64)	0.47
Cracks inside or outside your sleeping house	1.20	(0.61-2.51)	0.55
Sleeping house less than 100m from the river/stagnant water source	1.34	(0.67-2.69)	0.41
House near by an ant hill	1.89	(0.94-3.80)	0.07
Acacia seyal	0.84	(0.41-1.72)	0.63
Balanites aegyptiaca (Thaou)	1.90	(0.93-3.88)	0.08
Azadiracta indica (neem)	0.72	(0.35-1.52)	0.39
Occasionally play in the dark in forest or around houses	2.36	(1.16-4.80)	0.02

Table 15: Multivariate analysis for border line environmental factors

Environmental variables	OR	(95% C.I.)	P-value
B. aegyptiaca in the compound	1.90	0.93-3.88	0.08
Ant hills near home	1.88	0.94-3.80	3.80
Spending more nighttime outside (traditional dances and children games)	2.59	(1.20-5.57)	0.01

The presence of the following animals such as goats, dogs, cows and chicken in the compound or in the sleeping house had no statistical association with kala-azar (Table 16).

Table 16: Bivariate analysis for environmental factors

Variables	OR	(95% C.I.)	P-value
Goats kept in the sleeping room	1.22	(0.51-2.92)	0.65
Chicken kept in the sleeping room	0.62	(0.26-1.46)	0.27
Keeps dogs at home	1.42	(0.67-3.02)	0.36
Sleep with a dog in the same room	1.40	(0.53-3.70)	0.50
Keep cattle less than 100m from sleeping house	1.16	(0.69-3.78)	0.27

3.8   Behavioral Factors

On the risk factor analysis for other behavioral characteristics, "Smooking" type of bed net was associated with kala-azar (Table 17). Sometimes smearing of cow dung ashes on the body either at home or at the cattle camp was also associated with kala-azar (Table 17). People who occasionally engaged in traditional dances at night or children playing around the houses during the night were also found to be associated with kala-azar (Table 17). The consistent use of a bed net, during the rainy season (May to October, 2007) was found to be positively associated with kala-azar (Table 17).

Multivariate analysis to control for confounding was done on these significant factors on bivariate analysis and all the factors remained statistically significant (Table 18).

Table 17: Bivariate analysis for behavioral factors

Variable	OR	(95% C.I.)	P- value
Always uses bed net in rainy season	0.38	(0.19-0.78)	0.01
Using "Smooking" type of bed nets	2.14	(1.03-4.48)	0.04
Sometimes smear ashes on the body	2.50	(1.12-5.59)	0.02

Table 18: Multivariate analysis behavioral characteristics

Variables	Odds ratio	(95% C.I.)	P-value
Smear cow dung ashes on the body sometimes	2.91	(1.20-7.01)	0.02
Uses "Smooking" type of bed nets	2.55	(1.14-5.71)	0.02
Consistent use of bed during rainy season	0.33	(0.15-0.72)	0.01

Bed nets which had been in use for less than 6 months were border line protective against kala-azar (Table 19). Sleeping on the bed and on the floor were also boarder line protective associated with kala-azar (Table 19). On the other hand, bed nets in use for more than six months had no statistical association with kala-azar (Table 19).

Table 19: Bivariate analysis for behavioral characteristics

Variable	OR	(95% C.I.)	P- value
Period of bed net in use (less than 6 months)	0.28	(0.06-1.28)	0.07
Period of bed net in use (from than 6 to 12 months)	0.71	(0.31-1.62)	0.41
Period of bed net in use (more than 12 months)	1.64	(0.79-3.41)	0.18
Sleeps on the floor at home	3.29	(0.70-15.32)	0.09
Sleep on the bed at home	0.30	(0.07-1.42)	0.09

There were three border line behavioral characteristics: period of bed net in use less than six months, the modes of sleeping at home whether on the floor or bed were also subjected to multivariate analysis. The factors remained insignificant even with multivariate analysis (Table 20).

Table 20: Multivariate analysis for border line behavioral characteristics

Behavioral characteristic variables	OR	(95% C.I.)	P-value
Period of bed net in use less than six months	0.28	0.06-1.28	0.08
Sleep on the ground at home	3.29	0.70-15.32	0.13
Sleep on the bed at home	0.30	0.07-1.42	0.13

CHAPTER FOUR

4   DISCUSSION

This is the first case control study conducted in Southern Sudan to examine the effect of environmental factors and human behavior as risk factors for kala-azar transmission. Although kala-azar is endemic in several areas of Southern Sudan, certain years (1990 and 2003-WHO South Sudan unpublished data) seemed to have favored the occurrence of kala-azar epidemics. The factors causing these epidemics have not been documented in Southern Sudan due to civil war and lack of resources dedicated to the control of the disease. However, previous reports during the civil war in Southern Sudan indicated that there may be various factors which have caused the outbreaks of kala-azar; these include famine, non immune individuals moving into endemic areas and vice versa (Seaman *et al.,* 1996). It has also been documented in India that poor economic conditions (Desjeux, 1996; Thakur, 2000b) and malnutrition (Dye and Williams, 1993; Badaro *et al.,* 1986) help to fuel the development of kala-azar.

This study was conducted immediately after heavy rainfall within the year and there was already an ongoing outbreak of kala-azar in Fangak and the neighboring Counties (Fangak PHCC kala-azar records unpublished data). Since heavy rains usually destroy agricultural crops, it is possible that this contributes to famine in the population which in turn may lead to malnutrition. Therefore this may help to fuel the development of kala-azar.

A total of 144 of participants were recruited for the study with (33%) cases and (67%) controls. The female participants were (56%) slightly more than the males. There were 6% of the cases in primary school and none of the control went to school (Table 1). Over all both cases and control did not attain secondary school level of education. Only two percents of the study population were employed (Table 1). Studies in Bangladesh had observed no association between education or occupation and the risk of developing kala-azar (Caryn *et al.,* 2005), the data from Fangak could not be analyzed for the same risk factors due to inadequate information.

This study has demonstrated that more than half (56%) of the participants affected by kala-azar were children less than five years; and among this group, 89% were under three years of age (Table 2). Globally, leishmaniasis is considered to be principally a childhood disease (Heisch, 1954; Aggarwal and Wali, 1991; Belazzoug; 1992). In the Mediterranean basin, the majority of the kala-azar patients are the three years olds (Kafetzis, 2003). In contrast, a study done in eastern Sudan showed that children who are less than five years old were 3.2% less likely to develop kala-azar than children of five to ten years (32.6%; Bruno *et al.,* 2002). The greater susceptibility of children less than five years to kala-azar could be due to being prone to malnutrition (Dye and Williams, 1993; Badaro *et al.,* 1986) and immature immune system (Alvar *et al.,* 1997; Wolday *et al.,* 1999). The other contributing factors may be the outdoor night activities which could have contributed immensely to the exposure status of the children due to high infective bites delivered per person per annum (Dye, 1992).

This study has also demonstrated the occurrence of kala-azar in a baby of nine months. This is also in contrast to the assertion that children are less affected by kala-azar because they are being covered by their mothers at night (Bruno *et al.,* 2002). However, since the studies were done in different Sudanese communities, it is possible to observe such a difference. In the age category of five to ten years there was a significant reduction in kala-azar cases (Figure 5). Although this was followed by further decrease of cases after ten years, the number of cases within the following age categories remained constant up to twenty five years and above (Figure 5). There was no significant difference between cases and controls in terms of age (Table 5).

The commonest symptoms of kala-azar presentation at the Fangak PHCC were fever and enlarged lymph nodes (100%), loss of weight (92%), loss of appetite (81%), enlarged spleen and liver (46%) and (42%) respectively (Table 4). Sixty six percent of the kala-azar patients started treatment at two months (Figure 6).

The delayed initiation of treatment by the kala-azar patients could mean that a lot of patients may be reporting with complications of kala-azar thus reducing the prognosis of better treatment outcome. The late health seeking behavior could also mean lack of knowledge about the disease. This study did not seek to measure the community attitude of modern medical care. However, Fangak Community is a rural setting and the possibility of intact traditions and bond to the traditional healers' advice for treatment is high. In such circumstances, the traditional healers' treatment is a first choice and the modern medical attention is considered as an alternative. It was observed during the

study period that children who came for kala-azar treatment had tattoos meaning that some sort of traditional treatment was being provided at home. Further studies need to be carried out to establish whether the late health seeking behavior among the respondents was related with traditional healers or issues of accessibility to the treatment center. Fangak County is water logged during the rainy season and people in the villages along River Phom use local canoes for transport. It is therefore possible that relatives of the patients who live far away from the river tend to wait to allow water to recede rather than wade for several kilometers to the treatment center and this further contributes to the deterioration of the patient condition.

There were different types of bed nets in use: long lasting insecticide treated ("Polyethylene"), cotton cloth-untreated ("Dhamoria"), and silk cloth-untreated ("Smooking") (Table 5). Ninety two percent of the cases and 98% of the controls slept under one of the three types of bed nets. The number of study participants who reported using bed nets were 96% of which more than half (69%) bought their own bed nets.

Fangak County is seasonally flooded and has plenty of water pools. Therefore, the availability of the stagnant water provides a breeding place for several species of night biting insects in the County. Insects bite humans from dusk to dawn during the rainy season and this could explain why the rate of bed net use in this county is very high. Although there is a high rate of bed nets use in the county, unfortunately most people use non insecticide treated bed nets (Table 6). There were only 25% of the study participants who were using the treated type of bed nets "polyethylene". Among the

kala-azar cases nobody was found using the "polyethylene" bed nets; 50% of them used the "Dhamoria" and 42% used the "Smooking" type of bed nets. On the other hand, 25% of controls used both the "Smooking" and "Polyethylene" type of bed nets while 48% used the "Dhamoria" type (Table 6). The preference of bed nets use among different study participants was evaluated for statistical association with kala-azar as modifiable behavioral factors. On bivariate analysis, the status of "polyethylene" could not be evaluated because none of the cases used this type of bed nets. The use of "Dhamoria" was not associated with kala-azar. The "Smooking" types of bed nets on the other hand, were significantly associated with kala-azar on both bivariate and multivariate analysis OR=2.55, (95% C.I. =1.14-5.71), P= 0.02. The reasons for the association of "Smooking" with kala-azar could not be established either. However, assumptions were made that the "Smooking" type of bed nets cannot easily be tucked to keep out the vectors and that the nets are made of silk fiber which is light and could easily be blown away by wind while the host is sleeping. Equipped with these possibilities, it is plausible that infected sandfly could get access and the host becomes exposed and infected.

 Rural children tend to play during the night and traditional dances are performed at night away from homes. In this study, both bivariate and multivariate analysis has established association between outdoor night activities and development of kala-azar OR=2.59, (95% C.I. =1.20-5.57), P=0.01. Outdoor activities at night could possibly expose children and adults to the bite of infected sandfly that transmits kala-azar. It would be interesting to carry out a comparative study to verify the relationship between playing outside at night in the compounds by children and traditional dances which are

held away from homes in near by bushes. This would confirm the results obtained which showed that most of the kala-azar cases were children less than 5 years who could have been bitten while playing outside at night.

The study have shown that consistent use of bed nets during rainy season was statistically protective against kala-azar (Table 18) OR= 0.33, (95%C.I. = 0.15-0.72), P=0.01. This finding agrees with the result of the study conducted in Bangladesh (Caryn *et al.,* 2005).

Sleeping on the floor or on the bed was not associated with kala-azar.

The Nuer people are pastoralist and their livelihood depends much on animal raring. The study found that 57% of study participants share their sleeping houses with their own animals for instance among kala-azar cases, 10% of goats and dogs and 9% of chicken were sharing the same house with the owners. On the other hand, 44% of controls were also sharing accommodations with their animals (Table 8). The close relationship between humans and domestic animals was tested epidemiologically for any statistical association with kala-azar. The results obtained were all insignificant on bivariate analysis (Table 16).

In addition, the Nuers have a traditional culture where they burn cow dung in the evening causing extensive smoke. This is done in order to repel night biting insects. The ashes are then used by youth of twenty years of age and below to smear on the body with the view to repel night flies, other day flies and above all as a prestige to conform to traditional culture. Although, this culture has degenerated considerably amongst the community, 54% of the cases and 36 % of the controls admit to smearing of the dung on

the body either always or sometimes (Table 8). The mothers who were interviewed on behalf of their children said that children usually roll in the ashes to play and are bathed before going to bed but this need further verification because of the sensitivity of the issue. Consequently during the study, it was observed that only one child on the first day and two children on the second day were being bathed from 25 homes visited. This indicated that the majority of the children in Fangak urban centers were not bathed before they sleep. Thus children after rolling in the ashes, during the day, could be sleeping with the ashes on the body till the next day. Therefore, it is possible that the parents in the villages might not as well be bathing their children before sleeping. Despite using the ashes so as to keep away the biting insects, the study found that sometimes smearing ashes was associated with kala-azar on both bivariate and multivariate analysis OR=2.91, (95%C.I.=1.20-7.01), P=0.02. The result of this association could have been confounded by unknown factors because persons who always smear cow dung ashes should have been protected from kala-azar to conform with the common belief held by the community. There is a need to conduct further studies to ascertain the dilemma of this practice. All of the participants in the study sleep in a grass thatched houses except only one control that sleep in a house with corrugated roof. The relationship of housing materials with kala-azar could not be tested statistically because of inadequate data.

The other environmental factors (presence of ant hills, stagnant water/river, types of roof, types of wall, wetness of the sleeping floor and the presence of the local vegetations: *A. seyal, B. aegyptiaca and A. indica*) presumed to be risk factors were

tested using a bivariate analysis. Two of these factors; ant hills near homes and presence of *B. aegyptiaca* in the compound were found to be border line significant. On multivariate analysis, none of the factors were statistically significant.

The high density of the local vegetations *A. seyal and B. aegyptiaca* around the villages may explain why these trees are not important risk factors for VL (Bruno *et al.,* 2002). Although dogs were found infected with *Leishmania* zymodemes that infected humans (*Dereure et al.,* 2000), they probably do not play a major role in the distribution of cases in the villages in Fangak.

4.1   Study Limitations

1. The study was done in only one clinic and may not be representative. This was because in other two centers visited there were few kala-azar cases and they were living over 50 km from the health facilities. Therefore, it would have been difficult to find comparative controls. The clinic was chosen because of an ongoing outbreak of kala-azar in the county in Fangak. The required number of cases were recruited within three months of the planned period of the study.

2. DAT test was not used as a screening test. There was a likelihood of mis-classification of controls leading to bias.

3. There is no road transport system between Juba and Malakal. There is only one ferry between Juba and Malakal which takes about seven days. However, this ferry is very irregular. Travelling from Malakal to Fangak takes two days by boat.

4. There is no power in Fangak. Communication, data processing, photocopying and printing facilities were not available.

5. Recall bias: Wrong estimate of date of onset of disease, seasonality in the use of bed net

6. Selection bias: interview refusal

4.2 Conclusions

1. Kala-azar affect younger children more than the adults

2. Uses of cow dung ashes and night time activities could be contributory factors for kala-azar

3. Inappropriate bed net use ("smooking") could be a risk factor for kala-azar

4. Consistent use of bed net during rainy season is proctective against kala-azar

4.3 Recommendations

1. Distribute long lasting insecticide treated bed nets (LLITN) in kala-azar endemic areas

2. Establish kala-azar treatment units in existing health facilities in the counties with high prevalence

3. Improve nutrition for children of 5 years of age and under

4. Further studies need to be carried out on the following:

Use of cow dung ashes, use of "Smooking" type of bed nets, and comparative study to establish the transmission behavior and its relationship to playing and dancing at night is recommended.

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APPENDICES

Appendix 1: The major *Leishmania* species of Public Health interest (WHO, 1996)

Leishmania species	Vector	Reservoir	Geographical distribution
L. infantum	*Phlebotomus perniciosus, P. ariasi*	Dogs, foxes, jackals	Mediterranean basin, Middle east, China, Central Asia
L. donovani	*P. argentipes*	Humans	North east India, Bangladesh, Burma
L. donovani	*P. orientalis, P. martini*	Rodents in Sudan, Canines, humans, gerbils	Sudan, Kenya , Horn of Africa
L. major	*P. papatasi, P. duboscqi*	Gerbils, rodents (Arvicanthis, Tatera)	Middle east. North India, Pakistan, North Africa, Central Africa, Sub-Saharan savanna, Sudan
L. tropica	*P. sergenti*	Humans	Middle East, Mediterranean basin, Central Asia
L. aethiopica	*P. longipes, P. pedifer*	Hyraxes	Highlands of Kenya, Ethiopia
L. chagasi	*Lutzomyia longipalpis*	Foxes, dogs, opossums	Central America, Northern South America (Brazil, Venezuela, Yucatan, Belize, Guatemala)
L.brasiliesis	*Lutzomyia spp; Psychodopygus wellcomei*	Forest rodents, peridomestic animals	Tropical forest of South and Central America
L. guyanensis	*Lu.umbratilis*	Sloths (Choleopus), arboreal anteaters (Tamandua)	Guyana, Surinam, Brazil
L. panamensis	*Lu. trapidoi*	Sloths (Choleopus)	Panama, Costa Rica, Colombia
L. mexicana	*Lu. omelca*	Forest rodents	Yucatan, Belize, Guatamala
L. amazonensis	*Lu. flaviscutellata*	Forest rodents	Tropical forsests of South America
L. peruviana	*Lutzomyia spp*	Dogs	West Andes of Peru, Argentine highlands

Appendix 2: Consent Form

Consent Form

Title of Study: Risk factors for Kala-azar in Fangak County, Jonglei State, South Sudan, 2007.

IFORMATION FOR CONSENT

Invitation:
You are invited to participate in a study whose goal is to study risk factors for kala-azar

in Fangak County, Jonglei State, South Sudan, 2007.

Introduction:

The Leishmaniasis are group of diseases with broad range of clinical manifestations

caused by several species of parasites belonging to the genus *Leishmania* (Family:

Trypanosomatidae). It occurs in 88 countries in tropical and temperate regions, 72 of

them developing and 13 among the least developed. An estimated 350 millions people

are at risk and 12 million people are affected by the disease worldwide. The DALY

burden is 860,000 for men and 1.2 million for women. Annual incidence for kala-azar is

estimated at 500 000 cases.

Purpose of Study:

Leishmaniasis has been considered a tropical affliction that constitutes one of the six

entities on the World Health Organization tropical disease research (WHO TDR) list of

most important diseases. In the south Sudan, it is estimated that over 2000 cases of VL

occur each year from 2000 to 2005. At the moment there are no established risk factors for Kala-azar transmission in Southern Sudan. Else where in India it has been established that environmental, behavioral and economic factors could be responsible for kala-azar transmission.

This study hope to examine the risk factors associated with Kala-azar transmission in Southern Sudan by recruiting a total of 48 cases and 96 unmatched controls. The result of the study will be used to control kala-azar in the endemic regions of Southern Sudan.

Procedures

If you agree to participate in this study, you will be assigned into one of two groups (Case or Control). A case is defined as any person who lives in Fangak County for two or more months and develops a condition or a disease which is characterized by prolonged fever, splenomegaly, and hepatomegaly, weight loss in combination with a healthy appetite, lymphadenopathy and pancytopenia. In addition, the person should test positive for Kala-azar by Dipstick test.

Risks

There is no known literature indicating the risk involved in the needle prick for taking blood for Dipstick test if aseptic procedure was observed and no bleeding tendency.

Benefits

The benefit you will get from participating in the study includes the detailed medical and physical examination by health centre staff. This is not different from rest of the patients

in the clinic should you choose to participate. The expected results of the research may be of great benefit for the people living in endemic regions of kala-azar in Southern Sudan.

Payment

There is no payment for participating in the research.

Alternatives

If you decide not enter this study, you will still receive routine care.

Confidentiality

The clinical and laboratory data and all other information recorded will be kept confidential and used for this research only. The result of this research may be published in scientific journals or presented at medical meeting, but your identity will not be disclosed.

Compensation for illness/injury

It is expected and known that no additional illness or severe injury will result from this non invasive procedure. Should one become sick, treatment will be provided on site at the treatment centre.

Voluntary Participation

Your participation is voluntary and you may choose not to join the study.

Approval of the Study

This study had been approved by:

Jomo Kenyatta University of Agriculture and Technology

Institute of Tropical Medicine,

Field Epidemiology and Laboratory Training Program,

Nairobi, Kenya.

CONSENT

I have been fully informed about the study and the benefits of it. I had the opportunity to ask questions and any questions I asked had been satisfactorily answered. I therefore consent voluntarily to participate in the study.

Name of Participant………………………………………………………………….

Signature or thumb print of participant or parent/legal guardian (if participant's age less than 18 years): ………………………………………………………………

Date…………………………………

Name of Research Assistant………………………………………………………

Signature…………………………………………………………………………

Date…………………………………

QUESTIONNAIRE FOR INVESTIGATION OF RISK FACTORS OF VISCERAL LEISMANIASIS (KALA-AZAR) IN FANGAK COUNTY, JONGLEI STATE, SOUTH SUDAN

DATE: October 2007

QUESTIONNAIRES

Do you agree to participate in this study?   Y   N

<u>Thank you very much for participation.</u>

Assistant researcher's name _____

Date of investigation _____   _____2007

Status: 1. Case

  2. Control   A

               B

DEMOGRAPHIC INFORMATION

1. Name_____
2. Gender (sex)

3. Age_____

4. Village_____

5. Nationality_____

6. Tribe/ ethnicity_____

7. Education [ ]

 a) Primary school

 b) Secondary school [ ]

 c) Post secondary education [ ]

 d) No education [ ]

 8. Place of study_____

 9. Employment

 a) Employed

 b) Unemployed

 10. Place of employment _____

 11. Occupation _____

CLINICAL AND LABORATORY INFORMATION
(This section <u>not applicable</u> for controls except no.Qs.12 and 13)

 12. Patient care facility name _____

 13. Date of admission in the health facility _____

 14. Date of onset of first symptom _____

 15. Symptoms and Signs:

 a) Prolonged fever (more than 3 weeks) [Y] [N]

b) Weight loss

c) Good appetite Y N

d) Splenic enlargement (splenomegaly) Y N

e) Liver enlargement (hepatomegaly) Y N

f) Lymph nodes enlargement (lymphadenopathy) Y N

16. Date of blood specimen collected _____

17. DAT Positive ☐   Negative ☐

18. Dipstick positive ☐   Negative ☐

BEHAVORAL (INDIVIDUAL) RISK FACTORS

19. Do you sleep on the ground at home? Y N

20. Do you sleep on the bed or cot at home? Y N

21. During which season do you sleep inside the mosquito net? ☐ ☐

a) Always at rainy season ☐ ☐

b) Sometimes at rain season ☐ ☐

c) Always at dry season ☐ ☐

d) Sometimes at dry season ☐ ☐

22. Did you buy your mosquito net? ☐ ☐

23. What type of mosquito net do you have?

a) dhamouria ☐ Y ☐ N

b) polyethene type ☐ Y ☐ N

c) Smooking ☐ Y ☐ N

d) None ☐ ☐

24. How old is your mosquito net?

Less than 6 months old ☐ Y ☐ N

☐ Y ☐ N

a) About 6 months to 12 months old

b) More than 12 months | Y | | N |

25. Are there holes in the nets? | Y | | N |

26. Do you sleep in the same room with kala-azar patient? | Y | | N |

27. Do you keep goats or calves (young cows) in your sleeping room? | Y | | N |

28. Do you keep chicken in the sleeping room?

29. Do you have dogs at your home? | Y | | N |

30. Do you sleep with a dog in the same room? | Y | | N |

31. Do you keep cattle less than 100m from your sleeping house? | Y | | N |

32. Do you sleep inside the cattle camp? | Y | | N |

33. Do you always smear ashes on your body at the cattle camp? | Y | | N |

| Y | N |

34. Do you sometimes smear ashes on your body at the cattle camp?

35. Do you occasionally sleep in the forest?

a) During dry season | Y | | N |

b) During rainy | Y | | N |

36. Are you involved in herding (care for animals)? | Y | | N |

37. Do you occasionally play in the dark in forest/houses? | Y | | N |

ENVIRONMENTAL RISK FACTORS

38. Do you keep your sleeping floor damp (wet)? | Y | | N |

39. Is your sleeping house wall made of mud? | Y | | N |

40. Is your sleeping house wall made of tree poles? | Y | | N |

41. Are there cracks inside or outside the wall of your sleeping house? | Y | | N |

42. What is the roof of your sleeping house made of?

a) Tins   Y [ ]   N [ ]

b) Iron sheets   Y [ ]   N [ ]

c) Grass thatched   Y [ ]   N [ ]

43. Do you sleep in the kitchen (house where you cook)?   Y [ ]   N [ ]

44. Is your sleeping house less than 100m from the river/stagnant water source?   Y [ ]   N [ ]

45. Is your house near by an ant hill?   Y [ ]   N [ ]

46. Which of the following trees do you have near your home stead?

a) *Acacia seyal* or (Lor or Seb)   Y [ ]   N [ ]

b) *Balanites aegyptiaca* (Thaou)   Y [ ]   N [ ]

c) *Azadirachta indica* (Neem)   Y [ ]   N [ ]

d) *Acacia polycantha* (Muer)   Y [ ]   N [ ]

Appendix 4:   Ethical Issues

The study was cleared by the Ethical Committee of the Ministry of Health, Government of Southern Sudan. An informed oral consent was obtained prior to enrolling the study participants. For children under the age of 15 years, the mother's consent was obtained.

Appendix 5:   Bivariate analysis for some variables

Variable	OR	(95% C.I.)	P- value
Uses bed net sometimes during rain period	1.64	(0.78-3.48)	0.19
Always uses bed net in dry season	0.80	(0.36-1.77)	0.58
Uses bed net sometimes in dry season	0.94	(0.39-2.26)	0.88
Period of bed net in use (less than 6 months)	0.28	(0.06-1.28)	0.07
Period of bed net in use (from than 6 to 12 months)	0.71	(0.31-1.62)	0.41
Period of bed net in use (more than 12 months)	1.64	(0.79-3.41)	0.18
Holes in the bed net	1.18	(0.58-2.40)	0.65
Sleep inside the cattle camp	2.07	(0.40-0.65)	0.38
Always smear ashes on the body	1.14	(0.48-2.71)	0.77
Keep sleeping floor damp (wet)	1.30	(0.64-2.64)	0.47
Cracks inside or outside your sleeping house	1.20	(0.61-2.51)	0.55
Sleep in the kitchen	1.73	(0.76-3.92)	0.19