

**SITUATION ANALYSIS OF VISCERAL LEISHMANIASIS (KALA-AZAR): KNOWLEDGE ATTITUDE AND PRACTICES (KAP), SYMPTOMS AND RISK FACTORS IN SAPTARI DISTRICT**



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Batch: 063/064

A thesis submitted in partial fulfilment of the requirements for the award of the degree of  
Master of Science in Zoology with special paper Parasitology

Submitted to  
Central Department of Zoology  
Institute of Science and Technology  
Tribhuvan University  
Kirtipur, Kathmandu  
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02, 2015

## DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author or institution.

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## RECOMMENDATIONS

This is to recommend that the thesis entitled "**Situation Analysis of Visceral Leishmaniasis (Kala-azar): Knowledge Attitude and Practices(KAP), Symptoms and Risk Factors in Saptari District**" has been carried out by Dharmendra Kumar Yadav for the partial fulfilment of Master's Degree of Science in Zoology with special paper Parasitology. This is his original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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This thesis work submitted by Dharmendra Kumar Yadav entiteled "**Situation Analysis of Visceral Leishmaniasis (Kala-azar): Knowledge Attitude and Practices (KAP), Symptoms and Risk Factors in Saptari District**" has been accepted as a partial fulfilment for the requirements of Master's Degree of Science in Zoology with special paper Parasitology.

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## ACKNOWLEDGMENTS

It would not have been possible to carry out and present the study in this form without the help of many well wishers who in different way helped me during the course of this duty. It is my right as well as study to express gratitude to them.

For most, I must express my sincere and respectful gratitude and hearty thanks to my honourable supervisor Mr. Pitambar Dhakal. So my first gratitude goes to him. He is an ideal guide and a well skilled lecturer presently working at Central Department of Zoology, T.U., Kirtipur, Kathmandu. I am really indebted to him for noble advice, valuable comments and encouragement from the beginning to the end of this thesis work.

I would like to express my gratitude to Prof. Dr. Ranjana Gupta, Head of the Central Department of Zoology, who gave me sharp advices. I would like to express my gratitude to Prof. Dr.P.N.Mishra, Prof. Dr. T.K. Shrestha, former Head of the Central Department of Zoology. Then, I heartily express my gratitude to Prof. Dr. A.S. Tamrakar former chairman of Central Department of Zoology, T.U. Kirtipur. She always encouraged all the students by her behavior. I would like to express several thanks to Mr. Janak Subedi and Ashok Bahadur Bam for their advices.

I am thankful to Mr. Sunil Kumar Yadav, Entomologist and Mr. Shishir Kumar Pant, Assistant Entomologist in vector Borne disease centre, District Public Health office who classified the collected vectors.

I would like to record my thanks to my friends Mr. Madhav P. Yadav, Mr. Sanjay Kumar Yadav, Mr. Sanjan Bdr. Thapa and Mr. Sunil Tiwari for their encouragements throughout the study.

I wish to extend my sincere gratitude to father Sri Mohan Prasad Yadav, my mother Mrs. Anjani Devi Yadav, elder brother Mr. Bisheshwar P. Yadav and his wife Mrs. Durga Devi Yadav, all of them have not only supported economically but encouraged me with love and affection throughout the student life.

Finally, I would like to express my thanks to all the friends and colleagues who directly and indirectly helped me.

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## **ABBREVIATION AND ACRONYMS**

|           |   |   |
|-----------|---|---|
| AIDS      | - | Acquired Immune Deficiency Syndrome   |
| CFR       | - | Case Fatality Rate  |
| CIR       | - | Case Incidence Rate   |
| CL        | - | Cutaneous Leishmaniasis   |
| CR        | - | Central Region  |
| DDT       | - | Dichlorodiphenyltrichloroethane   |
| DHO       | - | District Health Office  |
| EDCD/DoHS | - | Epidemiology and Disease Control Division/ Department of<br>Health Services |
| ELISA     | - | Enzyme-linked Immunosorbent   |
| ER        | - | Eastern Region  |
| HIV       | - | Human Immunodeficiency Virus  |
| HPs       | - | Health Posts  |
| IgG       | - | Immunoglobulin G  |
| IgM       | - | Immunoglobulin M  |
| IM        | - | Intramuscular   |
| IRS       | - | Indoor Residual Spraying  |
| IV        | - | Intravenous   |
| KA        | - | Kala-azar   |
| KAP       | - | Knowledge, Attitude and Practice  |

|          |   |  |
|----------|---|--|
| MoH      | - | Ministry of Health                     |
| NNN      | - | Novy, MacNeal and Nicolle              |
| PHC/ORCs | - | Primary Health Centres/ Health Centres |
| PKDL     | - | Post Kala-azar Dermal Leishmaniasis    |
| RE       | - | Reticuloendothelial                    |
| SAG      | - | Sodium Antimony Gluconate              |
| SHPs     | - | Sub-Health Posts                       |
| SZH      | - | Sagarmatha Zonal Hospital              |
| VDC      | - | Village Development Committee          |
| VL       | - | Visceral Leishmaniasis                 |
| WHO      | - | World Health Organization              |

## ABSTRACT

Visceral Leishmaniasis(VL) Kala-azar(KA)is a chronic infection of reticulo-endothelial system and is nearly always fatal, if left untreated. The present research was conducted in Sagarmatha Zonal Hospital Saptari district with an aim to analyze the situation of VL, VL related knowledge , attitude and practice, and risk factors of the disease. A total of 250 suspected cases were subjected to aldehyde test, rk39 test and bone marrow examination according to the laboratory diagnostic facilities available in District Hospital Saptari. The finding revealed that total 39 +ve KA cases out out from 88 examined sample 44.31% cases were found to be positive for Kala-azar. It has been found that males were affected more than females with the ratio of 5.75:4 in male and female. The age group most affected to be was found to be 15-19 years which constitutes 11.36% of total positive cases. Out of total KA cases, the distribution was found to be 16.8% among labour 22.72% among illiterate people, 28.8% among those earning <1000 Rs/month and 53.40% form the "hut" houses. Likewise, questionnaire survey among 263 respondents reveals that maximum cases were found among those respondents who have never used bed nets 69.23% sleep on ground floor (100%) and who keep cattle nearby the houses 61.53%. Regarding the knowledge of VL, none of the respondents was aware of the fact that KA is transmitted by sandfly.

The Present study show that poverty, age, illiteracy, occupation, type of house, lack of knowledge regarding VL, sleeping on the ground floor without using bet nets, malnutrition, keeping cattle nearby the house and sharing the same house with cattle were responsible risk factors for the spread of KA . It was, therefore, very necessary that the people in the endemic areas should be made well conversant about the disease and vector sandfly for the prospective of sustainable management of the disease

## INTRODUCTION

The genus *Leishmania* was coined by Ross in 1903. *Leishmania*, the flagellate protozoa living in the blood and reticulo-endothelial (RE) tissues of the human host, is an important pathogenic protozoan genus closely related to trypanosome. The genus *Leishmania* is responsible for serious diseases of man, cat, dogs, sheep, horses, etc. collectively known as Leishmaniasis.

*Visceral* Leishmaniasis is also known as Kala-azar, Black fever, Dum Dum fever and tropical splenomegaly. This disease is severe systematic disease which is nearly fatal if not treated. It is a zoonotic disease worldwide but in Nepal, due to lack of epidemiological evidence of existence of animal reservoir host, it is not regarded as zoonotic ( Bista, 2006)

Visceral Leishmaniasis (Kala-azar) is a vector-borne zoonotic disease caused by infection of protozoan parasites *Leishmania donovani*. The genus *Leishmania* is characterized by two forms i.e. amastigote form (Leishmanial or aflagellate form) which is found in vertebrate host and promastigote form (leptomonad or flagellate form) that develops in the vector sand flies and artificial culture. Sand flies act as vector which transfer the parasites from one animal to another. hence it is known as carrier or vector. Reproduction of *Leishmania* takes place in both hosts.

According to clinical manifestation, Leishmaniasis is divide into three types i.e. *cutaneous*, *mucocutaneous* and *visceral*. Cutaneous type of Leishmaniasis is caused by infection of *Leishmania tropica* major and *Leishmania tropic minor*. Mucocutaneous Leishmaniasis is caused by infection of *Leishmania braziliensis* and *Leishmania mexicana* and *visceral* Leishmaniasis is caused by infection of *Leishmania donovani* sub-family phlebotomine of the class insect are responsible for all types of Leishmaniasis. Foxes, horses, jackals, cats, rodent and other wild animals act as reservoir host of all types of Leishmaniasis.

Visceral Leishmaniasis (Kala-azar) has been reported in 14 Terai districts. Mention the name of districts (More than 55 million people from 14 endemic districts of central and eastern) Terai regions of Nepal are believed to be at risk of this disease. Since 1980 a total of 14,865 cases and 215 deaths have been reported from this disease. The case fatality rate (CFR) ranged from 0.84% to 1.75% ([www.moh.gov.np](http://www.moh.gov.np))

Since 1980, the central and eastern lowland region of Nepal had experienced a resurgence of VL in parallel with large epidemic in the neighboring India states of Bihar, Uttarpradesh and West Bengal. *Leishmania donovani* is the parasite implicated in South Asia and is transmitted by the bite of the infected female sand flies (*Phlebotomus argentipes*). In India & Nepal, VL has an anthroponotic transmission cycle but the vector feeds on livestock and is attracted by their presence.

VL is present in endemic form in India, Bangladesh, China, Terai, Sudan, Kenya, Somalia, Ethiopia, Morocco, Tunisia, Mediterranean Island and Venezuela to North Argentina in South America. The disease is more or less completely absent from the western hemisphere except the eastern part of Brazil. Leishmaniasis currently threatens 350 million men, women and children in 88 countries around the world, 72 of which are developing and 16 developed countries (WHO, 2004). An estimated 12 million cases of Leishmaniasis exist worldwide with an estimated number of 15.2 million new cases occurring annually and VL alone is reported to affect 500,000 people worldwide each year. Ninety percent of VL cases are found in Bangladesh, Brazil, India, Nepal and Sudan. This represents the tip of iceberg since not all infected individuals develop a disease. Malnutrition, stress and immune defects are severity factors and it is not surprising that epidemics of VL are often associated with poverty, famine, war and immunosuppression. VL can cause large epidemics with case fatality.

In HIV infected patients, Leishmaniasis accelerates the onset of AIDS and shortens the life expectancy of HIV infected people by cumulative immunosuppression and by stimulation of the replication of the virus. Since VL can spread intravenously, sharing of needles by intravenous drug users is a direct way of spreading Leishmaniasis. Unusual findings such as the of hepatosplenomegaly, more involvement of the gastrointestinal and respiration systems, pleural effusion, ulcers and masses in gastrointestinal tract andodynophagia are more frequently demonstrated in KA patient with HIV infection.

Primary treatment failure and relapses are particularly common in KA patient with HIV infection Leishmania/HIV co-infection, therefore, has become great threat to the complete treatment KA in HIV and KA endemic areas.

VL is one of the vector-borne diseases in Nepal. It was known to be endemic in southern Terai of Nepal as postulated by an Indian scientist Raghvan in 1953. During the 1960s and 1970, VL cases to be a public health problem which was mainly due to country wide malaria eradication programme with DDT spraying. After more than a decade of curtailment of insecticide spraying particularly in southern Terai VL cases started coming up and were first recorded in 1980 with the incidence rate of 1.5 per 100,000 populations and case fatality Rate (CFR) of 5.88 percent. More than 5.5 million people from 12 endemic districts of central and eastern Terai regions bordering Bihar state of India are estimated to be at risk of contracting KA (EDCD, DoHS, 2002-2003)

In 2005, the government of Nepal, India and Bangladesh signed an agreement to eliminate Kala-azar from the subcontinent by 2015. Official government statistics from 2007 show Kala-azar district incidence rates of as high as 5.4 cases per 10,000 people per year, well above the elimination target of 1 per 10,000 for each Nepal's district. Moreover, many cases may go unreported to the government; either because they were treated to the government, or they were treated in the private sector in India or the patient did not seek treatment. In India (Bihar), that under reporting factor has been found to be between 5 and 8 (IRC)

## **1.2 OBJECTIVES**

### **General objective:**

To determine the prevalence, symptoms and risk factors of Kala-azar in Saptari district of Eastern Development Region of Nepal.

### **Specific objectives**

- To determine the method used for laboratory diagnosis of Kala-azar.
- To carry out symptomatic analysis of Kala-azar in the study area.
- To determine indigenous and imported cases of KA.



- To identify the treatment failure cases treated with sodium antimony gluconate and (SAG), amphotericin (Fungizone).
- To determine the status of manpower at differed level of health institutions such as sub-health post health post and hospital i.e. training, supply, supervision, monitoring, recording, reporting and treatment.
- To determine where and why do people prefer to visit (public or private health center) for diagnosis and Treatment.
- To conduct public awareness programme regarding KA.

### **1.3 HISTORICAL BACKGROUND ABOUT LEISHMANIASIS**

#### **1.3.1 Global Review**

All things have their limitation on the globe or universe except disease. Disease in any part of the globe is giving warning to another part, because it has many transmissible routes. Therefore, it was Poul Russel, who said, nothing is more international than disease on the globe”

Cunningham was first man, who saw the parasite Leishmania in the histological section of oriental sore in India in the year 1885. The oriental sore is also known as Delhi boil”. He was under impression; however, the organism belonged to Mycetozoa “slime fungi”. After some years, it was Borovsky, who established the protozoal nature of the parasite when he gave his description of oriental sore “Sort sore” in Turkistan in the year 1898.

The disease is caused by infection of *Leishmania donovani* and continues to have significant impact on the life of different communities over the globe especially the tropical and subtropical region. Though it is very difficult to plot accurately the present geographical distribution of the disease and to determine their prevalence in man because of the continuous changing effects of environmental factor on the prevalence of these disease and because of their grossly under-reporting or non reporting due to variety of reasons, yet the information obtained from intensive studies and surveys can be correlated to draw a credible and reliable picture of current situation over the globe.

When we turn our attention on background information about the visceral leishmaniasis, we cannot find the accurate information of morbidity and mortality data due to lack of regular recording system. It was recognized that in developed and developing countries, the standard of health services as the expectation of the public was not being provided. Kala-azar dates back to the 19<sup>th</sup> century but till recently it has been the least know to the village people of Nepal and it is one of the neglected diseases. Medical and diagnostic facilities both in quality and quantity are not adequate in vast tracts of the developing world. The disease is, therefore, vastly under reported. In spite of this, it is clear that KA is an important public health problem in quite a few countries. Monsoon, forest and woodlands with seasonal rains are the most suitable biotopes. Desert, Semi-deserts and rainy forests are unfavorable (WHO, 1984).

### **1.3.2 Kala-azar in Asian Region**

Extensive distribution of Kala-azar or visceral leishmaniasis is found in the North Asia, West Asia, Manchuria, Turkistan, Afghanistan and India. It is also found in Iran, Iraqi, Turkey, Jordan, Syria, Lebanon, Israel, Yemen, Saudi Arabia, Vietnam and Nepal. The visceral leishmaniasis is at present a major problem in India Bangladesh and Nepal, while Sri-Lanka and Burma have shown some cases.

In Asia, KA has an extensive distribution in north-west and west China, Manchuria, Turkistan, Afghanistan and India. It is also found in Iran, Iraq, Turkey, Jordan, Syria, Lebanon, Israel, Yemen, Saudi-Arabia, Vietnam and Nepal. The VL is at present a major problem in India, Bangladesh and Nepal. India, Nepal and Bangladesh are examples of countries with a porous border and frequent migration of population. Migrants are particularly vulnerable populations, and their movement across borders entails for propagation of KA. The Health Ministries of three member states of WHO's South-East Asia Region, India, Nepal and Bangladesh, on 8<sup>th</sup> May, 2005 signed a memorandum of understanding (MoU) pledging to collaborate to eliminate VL from their countries. The disease affects 100,000 people each year in these countries, while 147 million people are at risk. The MoU was signed at an event during the 58<sup>th</sup> session of the World Health Assembly. The signing of MoU demonstrates the spirit of solidarity and cooperation in developing joint action, networking and mobilization through intercountry cooperation. In the endemic countries of the region, most cases are limited to 96 districts, half of them

across the international borders. The estimated 100,000 cases of KA in the Region constitute about 20% of the global cases. This MoU signals greater political will and commitment to collaborate in reducing the annual incidence of KA to less than one per 10,000 population, at the district or district of sub-district level, by 2015 (leishmaniasis Press Release, Geneva/New Delhi, 2005).

### **1.3.3 Kala-azar in India**

India is one of the world's hot beds of visceral leishmaniasis. Along with Brazil, Sudan and Bangladesh, India contributes to 90% of the global burden of VL (TDR news, 2000). KA is known to exist in India since long time, and with the help of available records it can be traced back as far as 1824-25. Fewer cases of "Burdean-Fever and "Jwar-Vikar", a febrile illness in Jessore, reported during 1860's and 1870's in Bengal and "Kala-Dukh" (Black suffering) reported during 1980's in Bihar were most probable cases of Kala-azar. It is said that the epidemic of "Jwar-Vikar" caused death of no less than 750,000 during a period of three years. The disease as such was first reported in Assam, Bengal and Bihar in 1980's. Full scale epidemic broke out between 1890 and 1900. On another sever epidemic broke, it ravaged the Ganges and Brahmaputra. It has been known to occur epidemically, and endemically in well-defined areas in the eastern sector of the country, viz. Assam, West Bengal, Bihar, Eastern Districts of Uttar Pradesh, foothills of Sikkim, and to a lesser extent in Tamil Nadu and Orissa (Park, 2003). Presently the disease is endemic only in Bihar and West Bengal with sporadic cases/incidence in Uttar Pradesh (Raghunath and Nayak, 2005).

Epidemiological situation of Kala-azar in Some Bordering States of India BIHAR: Kala-azar in Bihar was first recorded in Purnea district in 1882 and then labeled as "Kala-dukh" (Black suffering) by Brown (1898). The notable epidemics came in 1891, 1917 and 1933, when the number of cases reached a colossal figure of 91,942. Thereafter, there was a declining trend. There was a virtual disappearance of disease around 1958-1960 as a collateral activity. Consequent to withdrawal of DDT spraying indigenous transmission of KA established initially in 4 districts of Bihar namely Muzaffarpur, Sitamarhi, Vaishali and Samastipur by 1974. Gradually the disease spread to adjoining areas becoming endemic in entire north Bihar. In 1977, a sample survey was conducted by the National Institute of communicable Disease, Delhi that showed an estimated National Institute of

communicable Disease, Delhi that showed an estimated number of 70,000 cases in the state of Bihar during 1991-1998, 5,365 cases succumbed to the disease. Programme for control of KA in this region is presently made by DDT spray. Reports of resistance to DDT are being reported from several parts of Bihar (Memorias Institute Oswaldo, March, 2004)

An organized centrally sponsored control programme launched in endemic areas in 1990-91. The programme strategy included.

- vector control through IRS with DDT up to 6 feet height from the ground twice annually,
- Early diagnosis and complete treatment,
- Information education communication
- Capacity building

Programme intensified in 1991-92 led to improved case registration through primary health care system. Within 3 years of intensification (1995 as compared to 1992), the annual incidence and deaths declined by 70.66% and 80.48% respectively. Likewise, by 2003 as compared to 1992, the incidence decreased by 76.38% while number of deaths by 85.20%

Miltefosine, the first oral drug against leishmaniasis (TDR news, 2000), has now been approved for use in India, which has 50% of the global burden of VL. With this recently introduced drug, the government of India has taken up the challenge to eliminated VL by 2010. (TDR News, 2000).

#### **1.3.4 Status of Kala-azar in Nepal**

##### **Background**

Nepal, the “country of a thousand gods”, presents a sad paradox. Endowed with exquisite beauty, it is at the same time home to a series of infectious diseases that take a heavy toll on its population. Perhaps the less known among them, and the most neglected, is Kala-azar. Ka was known to be endemic in southern Terai of Nepal as postulated by an Indian scientist Raghavan in 1953. It has reemerged from near eradication. Not enough information is available for the past in Nepal. During 1960’s and 1970’s VL ceased to be

a public health problem which was attributed to countrywide malaria eradication activities with DDT spraying. With the advancement of malaria eradication activities and improvement of malaria situation, insecticide spraying was reduced. After more than a decade of curtailment of insecticide spraying particularly in Southern Terai VL cases started coming up and were first recorded in 1980 with the incidence rate of 1.5 per 100,000 populations and case fatality rate of 5.88 percent. Since then VL cases are in steady rising trend and occasionally cause due to high fatality rate. However, the CFR has ups and downs. The highest CFR was in 1982 and the highest case incidence was in 2007.

**Table 1: Profile of visceral Leishmaniasis in Nepal 1980-2008**

| Year  | No. of cases | Incidence/100,000 | No.of Deaths | CFR (%) |
|-------|--------------|-------------------|--------------|---------|
| 1980  | 51           | 1.50              | 3            | 5.88    |
| 1981  | 133          | 3.95              | 1            | 0.75    |
| 1982  | 266          | 7.90              | 35           | 13.16   |
| 1983  | 60           | 1.78              | 4            | 6.67    |
| 1984  | 94           | 2.79              | 5            | 5.32    |
| 1985  | 95           | 2.65              | 0            | 0.00    |
| 1986  | 199          | 9.27              | 6            | 3.02    |
| 1987  | 169          | 6.48              | 8            | 4.73    |
| 1988  | 442          | 17.18             | 1            | 0.23    |
| 1989  | 291          | 9.01              | 5            | 1.72    |
| 1990  | 446          | 12.45             | 34           | 7.62    |
| 1991  | 870          | 17.45             | 56           | 6.44    |
| 1992  | 1395         | 90.96             | 8            | 0.57    |
| 1993  | 1368         | 34.08             | 5            | 0.37    |
| 1994  | 1976         | 49.03             | 9            | 0.46    |
| 1995  | 1787         | 44.60             | 65           | 3.63    |
| 1996  | 1571         | 39.14             | 55           | 3.50    |
| 1997  | 1342         | 33.23             | 36           | 2.68    |
| 1998  | 1409         | 33.88             | 42           | 2.98    |
| 1999  | 1794         | 43.14             | 24           | 1.34    |
| 2000  | 2090         | 50.26             | 50           | 2.39    |
| 2001  | 1736         | 41.75             | 17           | 0.98    |
| 2002  | 2029         | 48.76             | 14           | 0.59    |
| 2003  | 2229         | 53.61             | 32           | 1.44    |
| 2004  | 2117         | 50.76             | 19           | 0.89    |
| 2005  | 2039         | 48.85             | 24           | 1.17    |
| 2006  | 1927         | 40.19             | 43           | 2.23    |
| 2007  | 2358         | 56.73             | 21           | 0.74    |
| 2008  | 2273         | 52.68             | 17           | 0.74    |
| Total | 34557        |                   | 639          |         |

VL is mainly confined to the southern plain of Eastern and Central regions bordering VL endemic districts of Bihar (the most poverty-stricken of Indian states and the home of Kala-azar) State of India. However, a few sporadic cases are occasionally recorded from other parts of the country. Approximately 5.5 million populations are estimated to be at risk of KA. A total of 34557 cases with 639 deaths were reported during 1980-2008 and the CR varied from 0.23 to 13.16. The figures given do not represent the actual VL situation of the country as these VL cases are reported from the hospital records (mainly government) and patients treated elsewhere are not included. Therefore it is believed that VL in Nepal is a grossly under reported disease. It is, thus, most likely that many cases are left untreated in the community and therefore, the actual magnitude of the disease could be much more substantial. The VL cases are diagnosed on the basis of clinical signs & symptoms supported by aldehyde and bone marrow tests in the hospitals.

### **Situation Analysis**

From 1980 to 1989 the incidence rate per 100,000 populations remained below 10 except in 1988 when the incidence rate was 17.18. The minimum incidence rate was 1.50 in 1980. The CFR was 0 in 1985 and is as 13.1% in 1982. After 1989 the incidence rate remained quite high. Up to 1992 it was around 20 or below, even below 20 most of the year. From 1993 onward it was always above 33 and up to 50 in the year 2000. However, CFR remained low up to mid 1990's. In 1995 and 1996 the CFR was higher than 3% up to 2000 it was below 3% and by 2001 it reduced to below 1% higher case incidence rate and lower CFR during the later part of the last decade indicated probably to the prompt and regular reporting of KA cases and to the betterment of treatment of the cases at the hospitals (Table 2).

### **Study of Leishmania / HIV Co-infection**

Since 1986 VL has been recognized as a complication of infection with HIV. The problem is greatest in southern Europe, where HIV and *Leishmania infantum* are both endemic, and has been reported in Brazil (Gardoon, 1995).

## **Indoor Residual Spraying (Vector Control Measures)**

The Ministry of Health (MoH) started a programme of indoor residual insecticide spraying (IRS) in 1992 for KA control with the insecticide lambda-cyhalothrin (ICON). As per national IRS policy those villages are sprayed, where VL cases were recorded in previous years. During 1993, 1994 and 1995 insecticide spraying was conducted with DDT, malathion and lambda-cyhalothrin extending over the KA affected areas covering approximately 1.9 million population annually in 8 districts of Central and Eastern regions. In 1998 approximately 0.6 million population in districts were protected by lambda-cyhalothrin (ICON) indoor residual spraying (MoH, 1999).

### **1.4 THE CAUSATIVE AGENT OF VISCERAL LEISHMANIASIS: LEISHMANIA DONOVANI**

#### **History**

*Leishmania donovani* is an important haemoflagellate protozoan parasite of human beings causing visceral leishmaniasis or Kala-azar. Leishman and Donovan both reported the parasite simultaneously in the same year, 1903. Leishman demonstrated the parasite in the spleen smear of a soldier in England, who died of fever contracted at Dun Dum in Kolkata, while Donovan found the same in the spleen smear of a patient suffering from KA in India. Sir Ronald named genus and species in recognition of their landmark discovery.

#### **Classification**

Phylum-Sarcomastigophora

Class-zoomastigophora

Order-Kinetoplastida

Suborder-Trypanosomatida

Family-Trypanosomatidae

Genus-*Leishmania*

Species-*donovani*



## **Geographical Distribution**

*L. donovani* is endemic in many places in China, Africa, Southern Europe, South America, Russia, India and Nepal, In India; it is especially common in Assam, Bengal along the coasts of the Ganges and the Brahmaputra. It is also endemic in Bihar, Orissa, Madras and the eastern part of Uttar Pradesh as far as Lucknow.

In Nepal, it endemic in Eastern and central Terai region along the border of India and a sporadic case are also reported from Western region.

## **Habitat**

*L. donovani* is an obligate intracellular parasite of man and other mammalian hosts. They are always found as intracellular amastigote in the reticuloendothelial (RE) cells of spleen, bone marrow, liver, intestinal mucosa and mesenteric lymph nodes.

## **Morphology**

The parasite is dimorphic having amastigote and promastigote. Amastigote or Leishmanial form occurs in the RE cells of human and reservoir hosts. The characteristics of amastigote form are as follows:

Shape and size – It is a round or oval body measuring 2-4 mm along the longitudinal axis.

**Cell membrane** is delicate and can be demonstrated in fresh specimens only.

**Nucleus measures** a little less than 1 mm in diameter. It is oval or round and is usually stained in the middle of the cell or along the side of cell-wall. Kinetoplast Lies tangentially or at right angles to the nucleus. It comprises DNA- containing body and a mitochondrial structure.

Axoneme (rhizoplasta) a delicate filament extending from the kinetoplast to the margin of the body. It represents the root of the flagellum.

Vacuole, a clear unstained space lying alongside the axoneme.

Promastigote stage in only encountered in the cultres and in insect vectors (sandflies).

**Shape and size** - The earlier ones are short oval or pear-shaped bodies, measuring 5 to 10 mm in length by 2 to 3 mm in breadth. The fully developed ones are long slender spide-shaped bodies, measuring 15 to 20 mm in length by 1 to 2 mm in breadth. Nucleus is situated centrally.

**Kinetoplast** lies transversely near the anterior end.

**Eosinophilic vacuole**, a slight staining area lying in front of the kinetoplast over which the root of the flagellum does not curve round the body of the parasite and therefore there is an undulating membrane.

### **Life Cycle**

It completes its lifecycle in two different hosts.

- 1) Human and other mammals: The amastigote forms are present in humans and mammals.
- 2) Sand fly of the genus *Phlebotomies* and *Lutzomyia*: The promastigote form occurs in the sand fly.

The parasite is transmitted to Human and other vertebrate hosts by the bite of bloodsucking female sand fly. During the blood meal, the sand fly deposits promastogotes on surface of the skin. The promastigotes are immediately phagocytosed by fixed macrophages of the host in which they are transferred into amastigotes.

Amastigotes multiply by binary fission to produce a large number of amastigotes, till macrophages are filled with parasites. As many as 50 to 200 amastigotes may be present in the cytoplasm of the enlarged cell. The cell ruptures and releases a large number of amastigotes into the circulation. Free amastigotes are subsequently carried by circulation. They invade monocytes of the blood and macrophages of the spleen, liver, bone marrow, lymph nodes and other tissues of the RE cells.

Free amastigotes in the blood as well as intracellular amastigotes in the monocytes are ingested by female sand fly during a blood meal from man. In the mid gut of the sand fly, amastigotes are transferred within 72 hours through a series of flagellated intermediate promastigote form to flagellated promastigotes. The promastigotes multiply by binary

fission and produce a large number of promastigotes completely filling the lumen of the gut. After a period of 6 to 9 day, the promastigotes migrate from the mid gut of the pharynx and buccal cavity of sandfly.

The sand flies that ingest fruit or plant juice after the first blood meal show heavy pharyngeal infection causing blockage of the pharynx. Bite of the blocked sand fly transmits infections to susceptible persons and the lifecycle is repeated.

### **Reservoir and Source**

Canines (e.g. dogs) are the responsible reservoir of infection of the Mediterranean and Chinese Kala-azar where as it is the rodents in African Kala-azar and foxes in Brazil and central Asia In India and Nepal due to lack of epidemiological evidence of existence of animal reservoirs it is not regarded as zoonotic disease (EDCD/DoHS, 2004). Therefore man is the only source and reservoir of infection in India Kala-azar.

### **Transmission**

The infection is transmitted;

- 1) Mainly, by the bite of vector sand fly (*p.argentipes*)
- 2) Less frequently by
  - a) Blood transfusion
  - b) Congenital infection
  - c) Accidental inoculation of cultured promastigotes in the laboratory workers, and
  - d) Sexual intercourse.

### **Immunology**

Leishmaniasis, frequently referred to as the disease of immune system, is a bipolar disease immunologically (Parijal, 2004). Localized cutaneous disease is one pole of the condition, and visceral or diffuse cutaneous disease is another pole of the disease/persons with malnutrition and young people are increasingly/susceptible to VL.

Amastigotes developing from promastigotes excite a cellular reaction comprising histolytic proliferation followed by invasion of lymphocytes and plasma cells. The former gives shelter to Leishmania in side which the parasites multiply. The latter help to eliminate the parasites by a process of cell mediated immunity (CMI) through sensitized lymphocytes, destroying of man. Therefore, VL is frequently referred to as the disease of the immune system. The disease reflects a complex interplay between virulence of the parasite and host immunity.

#### **Host Immunity in VL is characterized by.**

- a) Specific inhibition of cell-mediated immunity: Delayed hypersensitivity reaction, as determined by leishmania skin test and in vitro lymphocyte responses to leishmanial antigen is completely absent during the infection. The delayed hypersensitivity, however, develops again after successful treatment with antileishmanial drugs. The intact CMI confers protection against the infection.
- b) Profound hyperglobulinaemia: Polyclonal lymphocyte activation causes profound hyperglobulinaemia. It is characterized by the production of a large volume of polyclonal non-specific immunoglobulins especially IgG and also specific anti-leishmanial antibodies. The compliment is activated and immune complexes are produced. The circulating antibodies, however, are not protective. Persons who have recovered from KA are immune to reinfection.

#### **Clinical Manifestation**

The incubation period usually is 3-6 months but can be months or years. The onset of disease may be gradual or sudden. This sudden onset occurs in persons coming from non endemic areas to endemic areas. The disease is characterized by the presence of

**Fever:** Fever is the first symptom to appear. Typically, fever is remittent. It is described as a double rise in 24 hours. It may be accompanied by sweating with chills but rigor and malaise are unusual. Period of pyrexia may be followed by a state of apyrexia. Less commonly, fever is continuous.

**Hepatosplenomegaly:** Splenomegaly is characteristic occupying the entire left side of the abdomen; it is soft and non-tender/Liver is enlarged but less conspicuous.

**Haematological anomalies:** Anaemia (normocytic and normochromic), leucopaenia, thrombocytopaenia and hyper gammaglobulinaemia. Other features; As the disease progresses, the skin becomes dry, thin and scaly, the hair becomes dull, thin and are lost, the nail becomes brittle the skin on the hand, feet and abdomen and around the mouth and forehead become grayish and dark coloured. This feature gives the name of disease Kala-azar, which means Black Fever.

Peripheral oedema, epistaxis, gingival bleeding, ecchymoses and petechiae are the late manifestation. Lymphadenopathy is seen in African and Chinese forms of VL but rarely in the Indian Kala-azar.

Death in ka is always due to some secondary bacterial infection as amoebic dysentery, bacillary dysentery pneumonia, pulmonary tuberculosis and other septic infections.

### **Treatment**

- 1) Pentavalent antimonials: it is available in the form of Meglumine antimonite and Sodium stibogluconate solution.

Route: Intravenous (IV) or Intramuscular (IM).

Dose: 20mg/kg body weight daily to a minimum of 850 mg for at least 20 days, for cases of Indian Kala-azar in adults and for 30 days in infants.

In case of relapse or incomplete responses, therapy should be repeated using 20 mg/kg body weight for 40 days to 60 days.

- 2) Pentamidine (Pentamidin isethionate):

Route: Intramuscularly (IM)

Dose: 0.5 mg/kg body weight for at least 14 days.

- 3) Miltefosine: It is a newer drug given orally. It has been recently evaluated with a 95% cure rate in Indian Kala-azar patients. It is under drug efficacy trial phase.

In Nepal, Sodium Antimony Gluconate (SAG) is used as first line of treatment in VL and Fungizone is used as second line of treatment.

## 1.5 SANDFLY, THE CARRIER AGENT OF VISCERAL LEISHMANIASIS

### Introduction

Sand fly is the vector of protozoan parasite of the genus *Leishmania* causing Kala-azar. *Phlebotomus* vector of KA are most predominant under 2000 feet altitude, in rural area of low alluvial plains. They do not fly far from their breeding places not do they often fly above the first floor of a house. Their life span is about few weeks. In Nepal, three species of sand fly namely-*phlebotomus argentipes*, *P. Papatasi* have been reported; *P. argentipes* is the know vector of Indian Kala-azar in Nepal with human as the reservoir host.

### Classification

Phylum-Arthropoda

Class-Insecta

Order-Diptera

Sub-order- Orthoptera

Family-Psychodiadae

Genus-*Phlebotomus*, *Lutzomyia*

### Habits and Habitat

Sand flies are troublesome nocturnal pests. The adult sand flies are weak flies; they fly in a characteristic hopping way, with short flights and landings in which the fly usually covers a distance less than ½ meter. *Phlebotomus argentipes* have been found up to 2.74 meter from the ground. Most biting occurs outdoor but a few species also feed indoor. Most species are active at dawn and dusk and during the night. They usually rest in the daytime in sheltered, dark and humid sites, such as those used for breeding, but also in tree holes, caves, houses and stables; other resting places near houses are crevices in walls, stakes of firewood, bricks and rubbish. Sand flies on plant juices but for the most part the females need a blood meal in order to develop egg. Their bite is irritation and

painful, blood is taken from humans (anthroponotic) and animals (zoonotic) such as foxes, farm livestock, wild rodents, snakes, lizards and birds. Each sand fly species has specific preferences for its source of blood, but the availability of houses is an important factors. Some species of the sand fly refeed only after laying one hatch of eggs while others may refeed several times. *Phlebotomy's argentipes* is well known vector of VL in many countries including Nepal, which commonly dies blood meal every 3<sup>rd</sup> or 4<sup>th</sup> day for oviposition.

### **Structure**

The adult sand fly is a tiny, fuzzy, delicately proportioned fly, light or dark brown in colour with conspicuous black eyes, usually 1/4<sup>th</sup> of the size of the mosquito. The length of sand fly body ranges from 1.5 to 3.5 mm. the males and unfed females can pass through mosquito net easily. The body and wings have many hairs like structure and this type of presentation is main feature of sand fly. Sexual dimorphism is well developed. Male sand flies are identified on the basis of morphological and anatomical characteristics of genitalia, while the females by the morphology of spermatheca and examination of arthatures in the cibarium or pharynx as well. As other insects, the whole body of sand fly is divided into three parts: Head, Thorax and Abdomen.

**Head:** The head bears a pair of long, slender and hairy antennae, palpi and a proboscis longer than head. An antenna is long slender and provided with hairs while the proboscis consists of fleshy labium containing dagger like mandibles and maxillae. The mandibles and maxillae have saw like teeth at tip and a blade like hypopharynx. The hypopharynx has salivary duct and a flat dagger like labrum epipharynx, which is provided with sensory hairs and spines. Mouthparts are modified for piercing and sucking.

**Thorax:** The thorax bears a pair of wings and three pairs of legs. The elongated wings are hairy, lanceolate in shape, held erect on the abdomen and are bigger than the size of the body. The legs are long and slender and out of proportion to the size of the body.

Abdomen: The abdomen is long slender and bears 10 segments each clothed with hairy structures. In the female sand fly, the tip of the abdomen is rounded, while in the male, there are claspers, which are conspicuous and attached to the last abdominal segments.

### **Life Cycle**

The geotropic cycle of sand flies lasts around 4-5 days and is characterized by complete metamorphosis, having four stages: egg, larva, pupa and adult.

The lifecycle patterns among the various species appear to be similar, differing only in habits and preferred hosts. The elongated eggs are oviposited in small batches under stones, in masonry cracks, between the walls of cesspools, in the vicinity of cattle shed and poultry, crevices in rocks or crumbling houses/buildings, in rubbish, under logs, inside hollow trees, in animal burrows and in other similar out-of-the-way places where the temperature is moderate, the environment is dark, and the humidity high.

The hatching period is from 3-5 days. The eggs of *Plebotomus papatasi* incubate for 4-12 days, after which minute whitish larvae with long anal spines and chewing mouthparts emerge. The larvae are free living, feeding on organic debris such as animal excreta. There are four larval stages as follows:

1<sup>st</sup> stage: 2-4 days; 2<sup>nd</sup> stage: 3-5 days; 3<sup>rd</sup> stage: 3-5 days and 4<sup>th</sup> stage: 5-12 days

Figure: Life Cycle of Sand fly (a) Egg, (b) Larva, (c) Pupa, and (d) Adult.

The fourth larva in star metamorphoses into pupa, which is not enclosed in cocoon. The pupa stage lasts for 6-13 days. Sand fly pupa is non-feeding, elongated, comma in shape and the end of which remains attached with fourth stage larva.

The adult emerges from the pupa. The development from egg to adult takes about 20-36 days. The adults are crepuscular in habit and endophilic in nature.



## LITERATURE REVIEW

Bansaland Karan (1995) carried out susceptibility test on the females of two species of sand flies viz *Phlebotomus papatasi* and *Sergentomyia punjabaensis* against six insecticides viz DDT, Dieldrin (organochlorines), malathion, fenitrothion (organophosphates), propoxur (carbamate and permethrin) in district Bikaner and Rajasthan. A concentration and time dependent effect was observed with insecticides for both the species. *P. papatasi* was found resistant to DDT, dieldrin and propoxur while susceptible to malathion, fenitrothion and permethrin.

WHO (1977) alarmed that there is some records of co-infection between HIV and VL that organization termed as “emerging disease”. In southern Europe 25-70% of adult cases of VL are related to HIV infection and 1.5-9% of AIDs patients have new or reactivated VL/In this area 70% of co-infected patients were intravenous drug users. In 1995, there were more than 100 leishmania/HIV co-infections in the Mediterranean area. In the America most of the co-infections are in Brazil. Leishmania/HIV co-infections have also been reported in Africa and Asia (WHO 1997).

Abraham et al. (1997) reported first case of culture-proven VL in Singapore, in a 30 years old Bangladeshi worker who presented with pyrexia of unknown origin (PUO). He had the classical constellation of symptoms and signs as mentioned above. Diagnosis was confirmed by culture in NNN medium. They were successfully treated with 20 days of Pentamidine isethionate daily infusion at a dose of 2 mg/kg day.

Omran et al. (1998) compared microscopy and PCR (Polymerase Chain Reaction) for use in diagnosis of Post Kala-azar Dermal Leishmaniasis (PKDL) in 63 patients. For 11 patients lymph node aspiration could be repeated 6 months after they recovered from PKDL. During active PKDL, PCR was positive for 42-of 52 (80%-lymph node aspirates and 19 of 23 (82.7% skin aspirates. Whereas microscopy was positive for only 9 of 52 (17.3%) lymph node aspirates and 7 of 23 (30.4%) skin aspirates. PCR was always positive when parasites were seen by microscopy.

Kumar et al. (1999) reported from Pundit Ka Purva village in Varnasi district, Uttar Pradesh, India, that of the total of 518 persons surveyed, 67 (39 male and 28 females) showed typical clinical and parasitological features of KA, including 7 who died. The overall prevalence and CFR were 12.9% and 10.5% respectively. Fever occurred mostly in September and October after the monsoon had ended. All 67 patients were treated with Sodium stibogluconate (20mg per kg body weight per day intravenously for 30 days), 60 (89.6%) of them improved and 7 (10.5%) died from concurrent infection or advanced disease.

In France 91 patients were found to be co-infected with Leishmaniasis and HIV during 1986-1997. Eighty seven percent of them showed fever, 74% splenomegaly and 49% showed hepatosplenomegaly (Rasenthal et al. 2000).

Gonzalez-Beato et al. (2000) reported a 40-year old HIV-positive man who had three relapses of VL. In the third he developed nodular skin lesions of three types, some reminiscent of Kaposi's sarcoma. Biopsy of each type disclosed abundant dermal macrophages with a huge number of intracellular and extra cellular Leishman-Donovan (LD) bodies. Leishmania has been detected both in normal and pathological skin of these patients due to dissemination during VL.

Marshall et al. (2000) reported a case of unusual Leishmaniasis in a Sudanese man with a history of progressively enlarging granulomatous mediastinal lymphadenopathy, worsening haemolysis, and an intense mucosal granulomatous inflammatory response in the large bronchi. Leishmania donovani DNA was detected in bronchial biopsy by PCR. This is a novel description of human Leishmania infection in an immunocompetent patient involving the anatomical site. The patient's condition improved clinically, spirometrically, and radiologically after a course of treatment with Amphotericin B. The cell-mediated immune response was analyzed before, during, and after successful anti-Leishmanial chemotherapy.

Pineda et al. (2001) carried out Leishmania skin test for Leishmania infantum among HIV seronegative intravenous drug users in Southern Spain. The Leishmania skin test was positive in 14 intravenous drug users and in 10 non-users and 3 of 11 active intravenous drug users and 3 of 82 former drug injectors ( $p=0.02$ ). Positivity in Leishmania skin test

was associated with intravenous drug users in higher than that among controls. This suggests that *L. infantum* spreads through the sharing of needles.

Palmira et al; (2001) used for the first time green fluorescent protein (GFP) tagged cells of the human parasite *Leishmania donovani* to observe its development in the gut of phlebotomine sand flies. Low numbers of GFP tagged *Leishmania* were more easily detected than non tagged *Leishmania*, suggesting that GFP tagged *Leishmania* could be used to efficiently study the biology of *Leishmania* in their vectors, and often the possibility of using nonaxenic flies.

Clinical resistance to pentavalent antimonial drugs in the form of SAG has become major problem in the treatment of KA in India. An investigation on the molecular aspect of drug resistance in clinically confirmed SAG resistant field isolates found a novel gene amplified in these (*Leishmania donovani*) drug resistant parasites whose locus is on chromosome 9. The significant finding was that this isolated fragment confers antimony resistance to wild-type *Leishmania* species after transfection. It has been speculated that protein phosphorylation may play a role in signal transduction pathway in the parasites after exposure to drug-conferring resistance (Singh et al; 2003)

Sherchand et al; (2003) evaluated KA test for the detection of urinary antigens in VL patients of Nepal. In the study, 276 urine samples from VL patients were collected from different endemic areas of Nepal, sixty-seven patients (24.3%) were found KA test positive. High degree of sensitivity and specificity. Hence the study field and hospital where bone marrow facilities are limited or where there is a lack of trained manpower as well as microscopic diagnosis is not available.

Bandhyopadhyay et al, (2004) reported identification of 9-O acetyl sialohydrates peripheral blood mononuclear cells in Indian visceral Leishmaniasis.

Tewary et.al; (2004) reported BLB/C mice were vaccinated with the soluble antigen with or without GG-ODN as adjuvant and then challenged with *Leishmania donovani* metacyclic promastigotes.

Miltefosine, a phosphocholine analogue originally developed as anti-malignant drug, and at present the first oral anti-Leishmanial drug, has been found to be highly active against

Leishmania in vitro and animal model. Based on these experiences this drug was tried against human VL and found to be highly effective and achieved 97% and 94% cure in phase 2 and phase 3 trials in children.

Artan et al; (2006) introduced liver biopsy can be recommended for diagnosing suspected visceral leishmaniasis in children when serology and bone marrow, aspiration are inconclusive.

Joshi AB et al; (2006) reported that visceral Leishmaniasis in Nepal is mainly confined to the southern plain of the Eastern and central regions, bordering visceral leishmaniasis endemic districts of Bihar state in India. Occasional sporadic cases areas so are classified as "at risk". A total of 25704 cases with 530 deaths were reported between 1980 and 2004 (440) cases per 100,000 in 20 years, average about 20/100000 per year and the case fatality rate in reported cases varied between 0.23 and 13.6%.

Gatti et al; (2008) performed the study, 67 patients suspected to be cases of visceral Leishmaniasis were each checked for Leishmanial infection by the microscopic evaluation of various biological specimens in vitro culture, serology and an assay based in need PCR most (35) of the subjects were immunocompetent (IC) but (32) were immunodeficient (ID) as the result of HIV infection (18 cases) treatment to prevent transplanted organs being rejected (6) or hematological malignancies (8).

## MATERIALS AND METHODS

### 3.1 Study Area:

#### Introduction of Saptari District:

Saptari, one of the endemic districts for Kala-azar, is located in the Eastern Development Region, Nepal. The Balan river act as a physical barrier in separating the western of Siraha district and Saptari district while the Koshi river separates the eastern region of Saptari and Sunsari district. The northern border of district adjoins with Udaypur district similarly; the southern part is extended from east to west and adjoins with Saharsha and Madhubani district 89 m & minimum height 64 m from the sea level. While 457 m from siwalik ranges and the district covers 13,592.8 sq. km (1, 35,928.8 hector) area. The district has the location of latitude 26°.25'-26°.39' and longitude 86°6'-87°.7'.

The district is an old settlement with total population 520,801 (census report 2068) with population density 382/km<sup>2</sup>, Population growth rate 3.5%

(Average temp of winter season 10°C-15°C while in summer season average temp 38°C-40°C.

Climatic condition varies from hot to cold & dry to rainy season throughout the year minimum and maximum temp 38-°40°C in hot and 10°-15°C in cold season are recorded).

Sapatari district has been divided into 6 election region, 17 Ilakas, 1 Municipality and 114 VDCs. There is a district public office in Rajbiraj Municipality. The district has only one Sagarmatha zonal hospital in Rajbiraj The district has diversity in caste, ethnic groups, culture and socio-economic status. Chamar, Mushar, Mallah, Sada, Khatwe etc. are the economically and socially disadvantaged communities. The main occupation of the inhabitations is agriculture. The commonly cultivated crops are paddy, wheat, maize millet, mustard; cereals etc. mixed livestock rearing system seems to be a common practice throughout district.

Most of the people live in the rural areas when majority of houses are constructed with bamboo and have thatched roofs. In the rural communities people prefer mixed dwelling. It is common to find human beings living with their domestic animals under the same roof in the cowshed.

### **Climatic condition**

Climate Condition of Saptari district is tropical and subtropical. The monsoon usually starts in month of June and continues September. The average annual rainfall is about 1100 to 1442mm. The average annual minimum and maximum temperature is about 10° to 15° and 38° to 40°c respectively.

### **Selected Hospital**

There is only one Sagarmatha Zonal Hospital in Rajbiraj Municipality of Saptari district, was selected for KA survey.

### **3.2 Case Study**

The suspected cases (clinically symptomatic cases) of Kala-azar were included in the sample during field survey. Any person from the selected area having fever for more than 2 weeks duration, anemia, headache and not responding malaria with or without splenomegaly and individual with past history of KA positive considered as suspected cases of KA.

### **3.3 Duration of Study**

The study for collection of field data and examination of the sampled population of field data and examination of the sampled population (cases) was carried out from Jestha 2066 to Chaitra 2066, with the help of health workers and laboratory support of Sagarmatha zonal hospital, Nurshin home and private clinics.

### **3.4 Methods of Data Collection**

#### **3.4.1 Morbidity and mortality data collection**

Prevalence of KA was detected by passive case detection. The secondary data regarding morbidity and mortality of VL was collected from the Sagarmatha zonal Hospital Saptari Rajbiraj, District Public office, Saptari, People Nursing Home, Primary Health Centre and Health Post, During the visit the individuals who were currently ill with clinical syndrome consistent with VL were interviewed and referred to Sagarmatha zonal Hospital Saptari Rajbiraj for Kala-azar test. From the hospital records only the data of KA case who had undergone treatment in the hospital were provided. However the data regarding those patients who had undergone treatment in India or died in their own home without having any treatment were not provided in the hospital and health post. These unrecorded cases produce some trouble in having the accurate data of VL. More information on KA cases was gathered by epidemiology. Disease control division/Teku, Kathmandu and District Health Office.

#### **3.4.2 To determine where and why do people prefer to visit for diagnosis and treatment**

The questionnaire was designed for baseline information based regarding status, knowledge, attitude, health believes. During the study period most people were treated at the zonal hospital, with a few going across the border into Bihar state of India for (extremely expensive) treatment as well as in nursing home and private clinics refer all KA patients to the govt. hospital. People gave three reasons for going to treatment in India and private health centre. They have family and relatives in Bihar and they did not trust the zonal Hospital.

#### **3.4.3 To determine indigenous and imported cases of KA.**

Questionnaire was designed for base line information. During study period 300 suspected cases from Sagarmatha Zonal Hospital from their interviewed all these cases are indigenous of Saptari we found no current indigenous KA cases. While, yet not any imported KA cases are record in zonal Hospital Saptari.

To conduct public awareness programme regarding KA in study area with the help of hospital staff, local people, teachers, students and other people so as to introduce the public about KA and its pathogenic effects, symptoms as well as methods of transmission and about their free treatment in zonal hospital and all government health institutions.

#### **3.4.4 Diagnosis and case detection**

A total of 250 suspected cases were tested for VL in Sagarmatha zonal Hospital Saptari. All the laboratory test facilities were provided by Sagarmatha zonal Hospital Saptari and private pathology clinics.

#### **Blood Sample Collection**

A total of 47 blood samples from KA suspected patients were collected in the zonal hospital and private pathology clinic. 32 blood samples were collected from zonal Hospital and 15 blood samples were collected from 2 private pathology clinics.

#### **Bonemarrow Collection**

It is not allowed to collect bone marrow directly by us. So his bone marrow was collected in Sagarmatha zonal Hospital was used for the study. During this period only microscopically examined of bone marrow.

#### **Aldehyde Test**

The aldehyde test is a simple test widely used in the areas where the laboratory or clinics are not adequately facilitated. The test usually becomes positive 2-3 months after onset of the disease and demands the use of venous blood. Further the test is positive in many other chronic infections in which albumin to globulin rate is reversed. Therefore this test is good for surveillance (Park, 2003).

Procedure: - One to two ml of serum sample was taken and a drop or two of 40% formalin is added. A positive test is indicated by jelly fraction to milk white opacity like the white of a hardboiled egg so that in ordinary light news print is invisible through it. If it occurs within 2-20 minutes, it is said to be strongly positive. Reaction after 30 minutes is not significant (Park, 2003).



### **Rk- 39 Test**

The rk 39 test for VL is a rapid immunochromatographic strip assay for the qualitative detection of antibodies to VL in human serum. This test is simply easy to read and requires only a drop of peripheral blood; a village level health worker can also easily perform the test to diagnose the disease. (Park, 2003)

**Principle:** - The rk 39 test for VL is a qualitative membrane base immune assay for the detection of antibodies to VL in human serum. The membrane is percolated with a novel recombinant VL antigen (rk 39) on the test line region and chicken and protein A on the control line region. During testing, the serum sample reacts with, the dye conjugate (protein A-colloidal gold conjugate) which has been per-coated in the test device. The mixture then migrates upwards on the membrane chromatographically by capillary action to react with recombinant VL antigen on the membrane and generates arced line. Presence of this red line indicates a positive result, while its absence indicates a negative result. Regardless of the presence of antibody to VL, as in the mixture continue as to migrate across the membrane to the control line region will always appear. The presence of this red line serves as verification for sufficient sample volume and proper flow and as a control for the reagents. (Park, 2003)

**Procedure:** - A drop of peripheral blood specimen was obtained and applied to nitrocellulose strip, followed by addition of three drops of test buffer (phosphate buffered saline plus bovine serum albumin) to the dried blood. The development of two visible bands indicated the presence of IgG anti-k39. (Park, 2003)

## RESULTS

The whole research results are divided into three main parts.

1. Analysis of laboratory test result among the suspected cases of visceral leishmaniasis.
2. To determine analytical finding survey results of VL related knowledge attitude practices of population and status of human power by questionnaire method.
3. Situation analysis of Kala-azar in Saptari district based on secondary data.

### 4.1 Analysis of laboratory test result of VL.

Epidemiological survey of kala-azar was carried out in Sagarmatha Zonal Hospital, Saptari. During the study period, 250 suspected cases were selected from above mentioned Hospital and were subjected for aldehyde test, rk 39 test and bonemarrow examination according to the laboratory diagnostic facilities provided in Sagarmatha Zonal Hospital. The results obtained from laboratory work have been presented.

#### 4.1.1 Age of sexwise adehyde test results of VL in Sagarmatha Zonal Hospital Saptari

**Table 2: Age and sexwise adehyde test results of VL in Sagarmatha Zonal Hospital Saptari**

| Number of serum sample |                       |                 |        |      |                 |        |       | Total sample examined   |        |       |
|------------------------|-----------------------|-----------------|--------|------|-----------------|--------|-------|-------------------------|--------|-------|
|                        |                       | Male            |        |      | Female          |        |       |                         |        |       |
| Age group              | Total suspected cases | Serum sample no | +ve no | +ve% | Serum sample no | +ve no | +ve % | Total serum samples no. | +ve no | +ve % |
| 0-5                    | 19                    | 2               | -      | 2    | -               | -      | -     | 2                       | -      | -     |
| 5-14                   | 24                    | 4               | 1      | 2.2  | 3               | 1      | 2.12  | 7                       | 2      | 2.27  |
| 15-19                  | 38                    | 13              | 5      | 8.50 | 9               | 4      | 4.25  | 22                      | 9      | 10.22 |
| 20-29                  | 36                    | 11              | 3      | 4.25 | 6               | 2      | 2.12  | 17                      | 5      | 5.68  |
| 30-39                  | 29                    | 5               | 4      | 2.12 | 3               | 2      | 2.12  | 8                       | 6      | 6.81  |
| 40-49                  | 27                    | 8               | 6      | 4.25 | 6               | 4      | 4.25  | 14                      | 10     | 11.36 |
| 50-59                  | 23                    | 6               | 2      | 2.25 | 4               | 2      | 2.12  | 10                      | 4      | 4.54  |
| 60-64                  | 26                    | 3               | 1      | 2.12 | 2               | 1      | 2.12  | 5                       | 2      | 2.27  |
| 64+yrs                 | 28                    | 2               | 1      | 4.25 | 1               | -      | -     | 3                       | 1      | 1.13  |
| Total                  | 250                   | 54              | 23     |      | 34              | 16     |       | 88                      | 39     |       |

It demonstrated that out of 39 KA positive cases(15.6%), out from 88 examined sample cases, the highest percentage(44.31 or 10cases) was found in age group 40-49 and the lowest percentage(0% or 0 cases)is in the age group 0-5 years.Out of 39 KA positive cases,23(26.13%) percentage were male and 16(18.18%) female, thus the male to female ratio being 5.75:4.

#### 4.1.2 Aldehyde and Bone marrow test for Visceral Leishmaniasis

**Table 3 Aldehyde and bone marrow test for VL**

| Age      | No. of suspected cases | Aldehyde test | +ve no | %   | Bone marrow test | + ve no | %   | Both aldehyde and bone marrow test |               |             |
|----------|------------------------|---------------|--------|-----|------------------|---------|-----|------------------------------------|---------------|-------------|
|          |                        |               |        |     |                  |         |     | examined sample                    | Total +ve no. | Total +ve % |
| 0-5      | 17                     | 2             | 1      | 0.4 | -                | -       | -   | 2                                  | 1             | 1.13        |
| 5-14     | 39                     | 9             | 5      | 2   | 7                | 4       | 1.6 | 16                                 | 9             | 10.22       |
| 15-19    | 36                     | 7             | 3      | 1.2 | 5                | 3       | 1.2 | 12                                 | 6             | 6.81        |
| 20-29    | 29                     | 6             | 2      | 0.8 | 5                | 2       | 0.8 | 11                                 | 4             | 4.54        |
| 30-39    | 28                     | 4             | 2      | 0.8 | 3                | 1       | 0.4 | 7                                  | 3             | 3.40        |
| 40-49    | 24                     | 7             | 3      | 1.2 | 4                | 2       | 0.8 | 11                                 | 5             | 5.68        |
| 50-59    | 22                     | 5             | 2      | 0.8 | 5                | 1       | 0.4 | 10                                 | 3             | 3.40        |
| 60-64    | 27                     | 9             | 3      | 1.2 | 3                | 2       | 0.8 | 12                                 | 5             | 5.68        |
| 64-years | 28                     | 5             | 2      | 0.8 | 2                | 1       | 0.4 | 7                                  | 3             | 3.40        |
| Total    | 250                    | 54            | 23     |     | 34               | 16      |     | 88                                 | 39            |             |

A total 39 positive KA cases (44.31%), out from total 88 examined sample cases. Among them 54(21.6%) patients were aldehyde test and 34(13.6%) patients were bonemarrow test, out from total 250 suspected cases. Among them 23(26.13%) were found positive for aldehyde test and 16(18.18%) were found positive cases for bonemarrow test out from total 88 sample cases. Maximum prevalence rate 10.22% was found between age group 5-14 and least prevalence rate 1.13% was found in the 0-5 age group.

#### 4.1.3 Occupation wise prevalence of Kala-azar in Saptari District

**Table 4 Occupation wise prevalence of Kala-azar in Saptari District**

| Occupation     | No. of suspected cases | Aldehyde test | +ve no. | %   | bone marrow test | +ve no. | %   | Examined sample | Total +ve no. | Total +ve% |
|----------------|------------------------|---------------|---------|-----|------------------|---------|-----|-----------------|---------------|------------|
| Farmers        | 133                    | 17            | 8       | 3.2 | 13               | 7       | 2.8 | 30              | 15            | 17.04      |
| Laborer        | 54                     | 15            | 5       | 2   | 11               | 5       | 2   | 26              | 10            | 11.36      |
| Service holder | 6                      | 3             | -       | -   | -                | -       | -   | 3               | -             | -          |
| Students       | 27                     | 8             | 4       | 1.6 | 5                | 2       | 0.8 | 13              | 6             | 6.81       |
| Business       | 4                      | 2             | 1       | 0.4 | -                | -       | -   | 2               | 1             | 1.13       |
| House wife     | 17                     | 5             | 3       | 1.2 | 3                | 1       | 0.4 | 8               | 4             | 4.54       |
| Vendors        | 9                      | 4             | 2       | 0.8 | 2                | 1       | 0.4 | 6               | 3             | 3.40       |
| Total          | 250                    | 54            | 23      |     | 34               | 16      |     | 88              | 39            |            |

Majority of the suspected cases were found to be involved in agricultural occupation. The total suspected KA cases 133 were farmers 54 were labourers and remaining 63 were belonging to service holder, students, businessman, house wife and vendors out from 250 suspected cases. The total 39 positive KA cases(44.31%) out from total 88 aldehyde and bonemarrow examined sample cases. Maximum prevalence rate 17.04% was found in farmers and least prevalence rate 0% found in service holders.

**Table 5: Education wise prevalence of Kala-azar cases**

| Maximum attainment to education | No. of suspected cases | Aldehyde test | +ve no. | +ve% | Bone marrow test | +ve no. | %  | Total examined sample | Total +ve no. | Total % |
|---------------------------------|------------------------|---------------|---------|------|------------------|---------|----|-----------------------|---------------|---------|
| Illiterate                      | 116                    | 25            | 11      | 10   | 17               | 9       | 37 | 42                    | 20            | 22.72   |
| Primary level                   | 98                     | 21            | 9       | 8.4  | 12               | 1.2     | 5  | 33                    | 14            | 15.90   |
| Secondary level                 | 34                     | 8             | 3       | 3.2  | 5                | 0.4     | 2  | 13                    | 5             | 5.68    |
| Higher level                    | 2                      | -             | -       | -    | -                | -       | -  | -                     | -             | -       |
| Total                           | 250                    | 54            | 23      |      | 34               |         | 16 | 88                    | 39            |         |

Classification of suspected cases on the basis of their maximum attainment of education level that overwhelming majority of suspected cases. The total KA suspected cases 116 were under illiterate group, 98 were primary level, 34 were secondary level and 2 were higher level out from total 250 suspected cases. Both aldehyde and bonemarrow KA positive cases 39 were found out from 88 total examined sample.

#### 4.1.5 Prevalence of KA in relation to the monthly income

**Table 6 Prevalence of KA in relation to the monthly income**

| Income (NRS per month) | No. of suspected cases | Aldehyde test | +ve no. | %   | Bone marrow test | +ve no. | %   | Total examined sample | Total +ve no. | Total +ve% |
|------------------------|------------------------|---------------|---------|-----|------------------|---------|-----|-----------------------|---------------|------------|
| <1000                  | 178                    | 30            | 14      | 12  | 22               | 11      | 4.4 | 52                    | 25            | 28.40      |
| 1000-3000              | 57                     | 18            | 7       | 7.2 | 11               | 5       | 2   | 29                    | 12            | 13.63      |
| 3000-9000              | 13                     | 6             | 2       | 2.4 | 1                | -       | 0.4 | 7                     | 2             | 2.27       |
| >9000                  | 2                      | -             | -       | -   | -                | -       | -   | -                     | -             | -          |
| Total                  | 250                    | 54            | 23      |     | 34               | 16      |     | 88                    | 39            |            |

Majority of the suspected cases 178 were under the income group of Rs 1000 per month. 57 people earning monthly Rs 1000-3000, 13 people earning monthly Rs 3000-9000 and no KA positive cases found in earning monthly income more than 9000. Out from total 250 suspected cases. Maximum 39 positive aldehyde and bonemarrow cases were found in out from total 88 examined samples.

#### 4.1.6 Distribution of Kala-azar in house wise system

**Table 7 Distribution of Kala-azar in house wise system**

| Type of house                     | N0. Of suspected cases | Of Aldehyde test | +ve no. | %   | Bone marrow test | +ve no. | %   | Total examined sample | Total +ve no. | total +ve% |
|-----------------------------------|------------------------|------------------|---------|-----|------------------|---------|-----|-----------------------|---------------|------------|
| Hut                               | 162                    | 27               | 11      | 4.4 | 20               | 9       | 3.6 | 47                    | 20            | 53.40      |
| Khaprai with mud wall             | 46                     | 19               | 8       | 3.2 | 11               | 6       | 2.4 | 30                    | 14            | 15.90      |
| Kutchcha house with thatched roof | 24                     | 8                | 4       | 1.6 | 3                | 1       | 0.4 | 11                    | 5             | 5.68       |
| Pucca (cemonto)                   | 17                     | -                | -       | -   | -                | -       | -   | -                     | -             | -          |
| Total                             | 250                    | 54               | 23      |     | 34               | 16      |     | 88                    | 39            |            |

Majority of the people 163, who did not have their own land had been living in 'hut'. The second highest no. of suspected cases 46 was form 'khaprail' house. Some 24 suspected cases were from kutchcha house with thatched roof while the least no. 17 had cemented (pucca) house. Analysis of positive Ka aldehyde and bonemarrow cases were highest no. 20 and lowest no.0 found out from 88 examined sample cases.

#### 4.1.7 Prevalence of KA in relation to sanitary measures in Population

**Table 8 Prevalence of KA in relation to sanitary measures in Population**

| Types of toilet | N0. Of suspected cases | Aldehyde test | +ve no. | %    | Bone marrow test | +ve no. | %   | Totatal examined sample | Total no. | +ve | Total +ve% |
|-----------------|------------------------|---------------|---------|------|------------------|---------|-----|-------------------------|-----------|-----|------------|
| No Toilet       | 198                    | 32            | 14      | 12.8 | 21               | 12      | 8.4 | 53                      | 26        |     | 29.54      |
| Temporary       | 43                     | 22            | 9       | 8.8  | 13               | 4       | 5.2 | 35                      | 13        |     | 14.77      |
| Permanent       | 9                      | -             | -       | -    | -                | -       | -   | -                       | -         |     | -          |
| Total           | 250                    | 54            | 23      |      | 34               | 16      |     | 88                      | 39        |     |            |

In study area questioned about sanitary measures regarding wether KA suspected people had their own toilet, maximum number of 198, people had no toilet of their own. Temporary toilet had suspected cases 43 and 9 suspected cases were provided with permanent toilet. Maximum positive aldehyde and bonemarrow test cases highest cases no. in 26 and least no. in 0 found in out from total 88 examined sample cases.



#### 4.1.8 Animal husbandry practices among the study of KA population Kala positive cases.

**Table 9 Animal husbandry practices among the study of KA population Kala positive cases**

| Animal species | No. of suspected cases | Aldehyde test | +ve no. | %    | Bone marrow test | +ve no. | %   | Total examined sample | Total +ve no. | Total +ve% |
|----------------|------------------------|---------------|---------|------|------------------|---------|-----|-----------------------|---------------|------------|
| Cattle         | 27                     | 6             | 3       | 2.4  | 4                | 1       | 1.6 | 10                    | 4             | 4.54       |
| Goat           | 19                     | 28            | 13      | 11.2 | 21               | 11      | 4.4 | 49                    | 24            | 27.27      |
| Mixed          | 97                     | 18            | 7       | 7.2  | 9                | 4       | 3.5 | 27                    | 11            | 12.5       |
| Pig            | 7                      | 2             | -       | 0.8  | -                | -       | -   | 2                     | -             | -          |
| Total          | 250                    | 54            | 23      |      | 34               | 16      |     | 88                    | 39            |            |

Majority of the suspected people were found to be involved in tame of domestic animals. Out of 250 suspected cases, 27 were cattle, 119 were goat, 97 were mixed and 7 were pig. The maximum positive Ka cases of aldehyde and bonemarrow tests were highest number of 24(27.27%) and least number of 0(nil) out from 88 examined samples.

**4.2 TO DETERMINE ANALYTICAL FINDING SURVEY RESULT OF VL RELATED KNOWLEDGE ATTITUDE PRACTICES OF POPULATION AND STATUS OF MAN POWER BY QUESTIONNAIRE METHOD.**

**4.2.1 Knowledge of Respondents towards Kala -azar**

**Table 10: Knowledge of Respondents towards Kala -azar**

|  | No of respondents, 263(%)   |
|--|---|
| <ul style="list-style-type: none"> <li>• <b>Knowledge of VL</b></li> <li>-Yes</li> <li>-No</li> </ul>  | 256(97.33)<br>7(2.66)   |
| <ul style="list-style-type: none"> <li>• <b>Knowledge of Signs &amp; Symptoms</b></li> <li>- Headache</li> <li>- Fever</li> <li>- Pigmented/ black Skin</li> <li>- Enlarged abdomen</li> <li>- Did not know</li> </ul>   | 5 (1.90)<br>61(23.19)<br>13( 4.49)<br>3(1.14)<br>181(68.82)         |
| <ul style="list-style-type: none"> <li>• <b>Knowledge of VL risk factors</b></li> <li>- Not using mosquito nets</li> <li>- not having good house</li> <li>- Improper personal hygiene</li> <li>- Sleeping on floor</li> <li>- Sleeping with VL patients</li> <li>- Did not know</li> </ul> | 26(9.88)<br>3(1.14)<br>24(9.12)<br>1(0.38)<br>4(1.52)<br>205(77.94) |
| <ul style="list-style-type: none"> <li>• <b>Knowledge of VL transmission</b></li> <li>-Bite of mosquito</li> <li>- Bite of Sandfly (Bhusana)</li> <li>- Sleeping with VL patient</li> <li>- Did not know</li> </ul>  | 17( 6.46)<br>-<br>9( 3.42)<br>237( 90.11)                           |
| <ul style="list-style-type: none"> <li>• <b>Knowledge of Preventive measure</b></li> <li>- Use of mosquito nets</li> <li>- Household spray.</li> <li>- Use of fire</li> <li>- Use of VL drug</li> <li>- Did not know</li> </ul>  | 57( 21.67)<br>26( 9.88)<br>31(11.78)<br>8(3.64)<br>122( 46.38)      |
| <ul style="list-style-type: none"> <li>• <b>Knowledge regarding free treatment of Kala-azar by government</b></li> <li>- Yes</li> <li>- No</li> </ul>  | 186(70.72)<br>77(29.27)   |

Source: Questionnaire Survey. A high proportion (97.33) of the respondents claimed that they had knowledge about KA and 2.66 were unknown of

the disease.

regarding common signs and symptoms 84.(31.93%) respondents were familiar with at least one of the signs of the disease and they replied headache (1.90%) , fever (23.19%) black of pigmented skin (4.94%) and enlarged abdomen (1.14%) . However, majority of them i.e. 181 (68.82%) responded that they knew nothing about signs and symptoms of VL.

Respondents were asked about the risk factors for disease transmission. Of the total, 26.(9.88%) responded "not using mosquito nets", and 24 (9.12%) responded improper personal hygiene". Very few responded with "not having good houses", "Sleeping with VL patients ", and "sleeping on the floor" are possible risk factors. The irony is that majority of 205 (7.94%) did not know any of the risk factors for the disease. Those who claimed that they knew the disease were not aware of the risk factors.

When asked about knowledge of mode of transmission, none of the respondents gave correct answer. Majority of the respondents i.e. 237 (90.11%) replied "did not know," very few i.e. 9 (3.42) responded "sleeping with VL patients" and 17(6.46%) respondents answered "bite of mosquitoes" as a mode of transmission.

Respondents were enquired to list to protective measures for VL. Following were the responses:57(21.67%) used mosquito nets, 26 (9.88%) used household spray, 31 (11.78%) used fire, 8 (3.04%) used VL drugs and a majority of 122 (46.38%) responded "did not know". Respondents were also evaluated for knowledge regarding "KA treatment at free of cost", of them 186 (70.72) replied "Yes" and 77(29.77% ) responded "No".

#### 4.2.2 Attitude of Respondents Towards Kala-Azar

**Table 11: Attitude of Respondents towards Kala-azar**

| Attitude of respondents  | No. of respondents, N= 263 (%)   |
|--|--|
| <ul style="list-style-type: none"> <li>• Types of advice provided               <ul style="list-style-type: none"> <li>- Buying medicine from a nearby medical store</li> <li>- Consulting medical doctors</li> <li>- Consulting traditional healers (quacks, Dhami, Jhankri etc.)</li> <li>- Not a serious disease</li> </ul> </li> </ul> | <ul style="list-style-type: none"> <li>2(0.78)</li> <li>251(95.431)</li> <li>6(2.28)</li> <li>4(1.52)</li> </ul> |

Questionnaire was applied so as to know the attitude of respondents towards KA. When members were questioned "if some one in your family or a neighbour suffers from KA, what kinds of advices will you seek?" most of them (95.43%) answered, they would consult a medical doctor. Very few (2.28%) responded they would "Consult a traditional healer (quacks, dhami, jhankri, etc.)," and (0.78%) responded that they would buy medicine from nearby medical store." A very few respondents (1.52%) responded that "it is not a serious disease and no need to consult anybody else.

### 4.2.3 Practices of Respondents Towards Kala-Azar

**Table 12: Practice of Respondents towards Kala-azar**

| Practices of respondents  | Number of respondents<br>N= 263(%)                     | Positive cases N=13 (%)<br>(% out of total + ve case) |
|---|--|---|
| <ul style="list-style-type: none"> <li>• Use of bed -nets</li> <li>- Never</li> <li>- Always</li> <li>- Occasionally</li> </ul>   | <p>193(73.38%)</p> <p>34(12.92%)</p> <p>36(13.68%)</p> | <p>9(69.23%)</p> <p>1(7.69%)</p> <p>3(23.01%)</p>     |
| <ul style="list-style-type: none"> <li>• Sleeping habits</li> <li>- Ground floor (Indoor and out door)</li> <li>- Upper floor</li> </ul>  | <p>259(98.47%)</p> <p>4(1.52%)</p>                     | <p>13(100)</p>  |
| <ul style="list-style-type: none"> <li>• Animal husbandry Practice (Keeping cattle)</li> <li>- Away from house</li> <li>- Nearby the house</li> <li>- Sharing the same house</li> </ul> | <p>6(2.28%)</p> <p>181(68.82%)</p> <p>76(28.89)</p>    | <p>1(7.69%)</p> <p>8(61.53%)</p> <p>4(30.76%)</p>     |

Source: Questionnaire Survey.

When questioned about the use of bed-net 73.38% (193) respondents answered that they never used it, 12.92% (34) used it frequently and 13.68% (36) used occasionally. The highest number of KA (69.23%) were found in the group 'not using bed-nets'. Two cases (23.07% of KA were reported in the group using bed-net'occasionally.'

The respondents were enquired about their sleeping habits . Majority of the respondents (98.47%) answered they sleep on the ground floor usually indoor and occasionally outdoor especially in the summer months. Cent percent KA cases were found among the people who always sleep on ground floor, thus no case was found in the people sleeping in upper floor.

When interviewed on how they keep their cattle (animal husbandry), 68.82% (181) responded they keep cattle nearby the house, 28.89% (76) share the same house and 2.28% (6) keep them

away from the house. The highest case of KA (61.53% i.e. 11 cases) were found among the respondents keeping cattle nearby their houses followed by 30.76% (4) in the group 'sharing the same house' and 7.69% ( a single case in the group 'keeping them away from the houses.

The KAP of the individuals inhabiting any endemic region are directly or indirectly related to further enhancement of the disease havoc. Keeping his view in mind, to analyze KAP of public towards KA, questionnaire survey was conducted during each field visit among the suspected sampled population of the study area and patients visiting the hospital as well. In total 263 respondents were interviewed among them 142 were males and 121 females, and thus collected data were deliberately analyzed.

#### **4.3 SITUATION ANALYSIS OF KALA-AZAR IN SAPTRARI DISTRICT BASED ON SECONDARY DATA**

Saptari is one of the most KA endemic district situated in the Eastern Terai of Nepal. On the basis of secondary is presented in the following heads.

### 4.3.1 Health institution wise Kala-azar cases Reported form 2001-2008

**Table 13 Health institution wise Kala-azar cases Reported form 2001-2008**

| Case in health | 01  | %    | 02  | %     | 03  | %     | 04  | %     | 05 | %     | 06 | %     | 07 | %    | 08 | %     | total | %     |
|----------------|-----|------|-----|-------|-----|-------|-----|-------|----|-------|----|-------|----|------|----|-------|-------|-------|
| Dumrisibthan   | 24  | 7.18 | 28  | 8.04  | 12  | 4     | 42  | 20    | 34 | 13.54 | 8  | 408   | 4  | 2.63 | 18 | 15.5  | 170   | 8     |
| Brahampur      | 28  | 8.38 | 26  | 7.47  | 22  | 7.33  | 12  | 5.41  | 18 | 7.14  | 26 | 13.26 | 12 | 7.89 | 11 | 4.32  | 155   | 8.11  |
| Pata           | 32  | 9.58 | 28  | 8.04  | 46  | 16.6  | 34  | 16.1  | 22 | 8.73  | 12 | 6.12  | 6  | 3.44 | 14 | 16.10 | 199   | 10.41 |
| Patharagada    | 26  | 7.78 | 18  | 5.17  | 28  | 9.33  | 12  | 5.71  | 16 | 6.34  | 24 | 12.24 | 22 | 14.4 | 13 | 11.01 | 159   | 8.32  |
| Barsain        | 48  | 14.2 | 14  | 4.04  | 42  | 14    | 24  | 11.4  | 24 | 9.52  | 28 | 14.28 | 14 | 9.12 | 15 | 12.71 | 209   | 10.94 |
| Sitapur        | 24  | 7.18 | 32  | 9.19  | 18  | 6     | 6   | 2.85  | 14 | 5.55  | 6  | 3.6   | 18 | 11.8 | 6  | 5.08  | 124   | 6.44  |
| Hanumanagar    | 26  | 7.78 | 28  | 8.04  | 24  | 8     | 18  | 8.77  | 22 | 8.73  | 16 | 8.16  | 24 | 15.1 | 13 | 11.01 | 171   | 8.45  |
| Bathnaha       | 24  | 7.18 | 36  | 10.3  | 18  | 6     | 10  | 4.76  | 26 | 10.31 | 10 | 5.10  | 12 | 7.89 | 10 | 8.47  | 146   | 7.64  |
| Topa PHC       | 28  | 8.38 | 48  | 13.7  | 16  | 5.33  | 24  | 11.42 | 36 | 14.28 | 32 | 16.32 | 26 | 17.1 | 17 | 11.01 | 223   | 11.67 |
| Kadarbana PH   | 32  | 9.58 | 22  | 6.32  | 34  | 11.33 | 10  | 4.76  | 18 | 7.14  | 24 | 12.24 | 14 | 9.21 | *  |       | 154   | 8.016 |
| Kalyanpur PH   | 28  | 8.38 | 30  | 8.36  | 14  | 4.66  | 4   | 1.40  | 12 | 4.76  | 6  | 3.6   | *  |      | I* | 94    | 4.92  |       |
| Kanchanpur PHC | 14  | 4.19 | 38  | 10.01 | 26  | 17.32 | 14  | 6.66  | 10 | 3.46  | 4  | 2.04  | *  |      | *  | 106   | 5.24  |       |
| Total          | 334 |      | 348 |       | 300 |       | 210 |       |    | 252   |    | 196   |    | 152  |    | 118   | 1910  |       |

Source, EDCCD/Ministry of Health

This table shows KA cases reported from different health institutions of Saptari district from the year 2001-2008. The table indicates there is on uniformity of KA cases in the health institutions. In 2008, the highest number of KA cases (out of 118 cases) was reported from post health post followed by 18 cases from Dumrisibthan health post and 15 cases from Barsain health post. Out of 191 cases recorded from 2001-2008, 11.67% (i.e. 223 cases) was reported from Tope HP, 10.44% (i.e.) 209 cases) from Barsain HP and 10.41% (i.e.) 199 cases from Pato Health post.

The monthly KA cases recorded from various health institutions of Saptari district in 2008. In Pato Health post the highest 16.10% (out of 118 cases) of the total KA cases. The second highest KA case 15.25% (i.e. 18 cases) were reported from Dumrisibthan health post and 6 KA cases were reported from Sitapur Health Post in 2008 when month is considered. July April are the months recording more cases of Kala-azaar.

#### 4.3.2 Health institution and month wise KA cases recorded in SZH in Saptari.

**Table no 14 Health institution and month wise KA cases recorded in SZH in Saptari.(2006-2008)**

| Name          | Jan | Feb | Mar | Apr | May | Jun | July | Aug | Sep | Oct | Nov | Dec | Total |
|---------------|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-------|
| Dumristiithan | 1   | 0   | 2   | 1   | 3   | 2   | 6    | 0   | 0   | 1   | 1   | 1   | 18    |
| Barhampur     | 0   | 1   | 1   | 2   | 1   | 0   | 1    | 1   | 1   | 2   | 1   | 0   | 11    |
| Pato          | 1   | 2   | 0   | 3   | 2   | 1   | 4    | 1   | 2   | 2   | 0   | 1   | 19    |
| Pathargada    | 0   | 1   | 1   | 0   | 2   | 1   | 1    | 0   | 0   | 3   | 2   | 2   | 15    |
| Barsain       | 2   | 1   | 0   | 2   | 1   | 3   | 2    | 1   | 1   | 0   | 1   | 1   | 6     |
| Sitapur       | 1   | 0   | 2   | 0   | 1   | 1   | 0    | 0   | 1   | 0   | 0   | 0   | 13    |
| Hanumannagar  | 0   | 0   | 2   | 1   | 3   | 1   | 1    | 1   | 0   | 1   | 1   | 1   | 10    |
| Bathnaha      | 1   | 1   | 0   | 1   | 1   | 0   | 2    | 1   | 1   | 0   | 1   | 1   | 10    |
| Topa PHC      | 0   | 0   | 1   | 0   | 2   | 1   | 0    | 2   | 0   | 1   | 0   | 0   | 7     |
| Kalyanpur     | 1   | 0   | 0   | 1   | 0   | 2   | 1    | 0   | 0   | 1   | 0   | 0   | 6     |
| Total         | 7   | 6   | 9   | 12  | 16  | 12  | 18   | 7   | 6   | 11  | 7   | 7   | 118   |

Source: District Public Health Office Saptari(unpublished data)



#### 4.3.3 Age and Sex Wise KA Cases Recorded in SZH in Sptari in 2065/066

**Table: 15 Age and Sex Wise KA Cases Recorded in SZH in Sptari in 2065/066**

| Sex   | M  | F  | Total |
|-------|----|----|-------|
| Age   |    |    |       |
| 1-4   | 7  | 5  | 12    |
| 5-14  | 20 | 13 | 33    |
| 15-19 | 8  | 6  | 14    |
| 20-29 | 4  | 10 | 14    |
| 30-39 | 5  | 6  | 11    |
| 40-49 | 8  | 11 | 19    |
| 50-59 | 2  | 3  | 5     |
| 60-64 | 1  | -  | 1     |
| 64+   | -  | 1  | 1     |
| Total | 55 | 55 | 110   |

Source: A compilation of Hospital record (Unpublished data)

#### 4.3.4 Month and Sex wise KA cases recorded in SZH Saptari in 064-065

**Table 16 Month and Sex wise KA cases recorded in SZH Saptari in 064-065**

| Sex      | M | F  | Total |
|----------|---|----|-------|
| Month    |   |    |       |
| Shrawan  | 4 | 9  | 13    |
| Bhadra   | 5 | 6  | 11    |
| Aashwin  | 6 | 7  | 13    |
| Kartik   | 2 | 6  | 8     |
| Mansir   | 4 | 5  | 9     |
| Paus     | 1 | 10 | 11    |
| Magh     | 7 | 6  | 13    |
| Falgun   | 7 | 4  | 13    |
| Chaitra  | 1 | 4  | 5     |
| Baishakh | 9 | 1  | 10    |

|        |    |    |     |
|--------|----|----|-----|
| Jestha | 8  | 7  | 15  |
| Asar   | 4  | 3  | 7   |
| Total  | 58 | 68 | 126 |

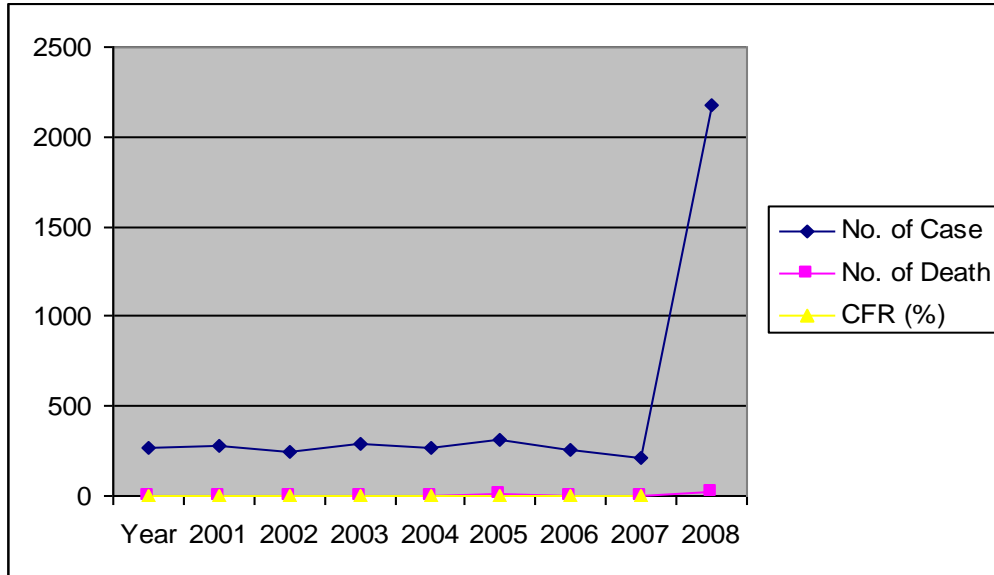
Situation Analysis of Kala-azar Saptari District based on Secondary Data Year wise KA case, incidence, death case fatality rate (CFR %) in Saptari district 2064-065

#### **4.3.5 Year wise KA cases, incidence, death and case fatality rate (CFR %) in Saptari district 2001-2008**

**Table 17: Year wise KA cases, incidence, death and case fatality rate (CFR %) in Saptari district 2001-2008**

| Year  | No. of Case | No. of Death | CFR (%) |
|-------|-------------|--------------|---------|
| 2001  | 263         | 2            | 0.76    |
| 2002  | 279         | 1            | 0.35    |
| 2003  | 243         | 1            | 0.41    |
| 2004  | 289         | 2            | 0.69    |
| 2005  | 263         | 3            | 0.76    |
| 2006  | 307         | 6            | 1.65    |
| 2007  | 256         | 4            | 1.56    |
| 2008  | 217         | 3            | 1.09    |
| Total | 2173        | 22           |         |

The result revealed that the higher KA cases were found among the population of Saptari district for the period 2001-2008 is presented in the table.



#### 4.3.6 Month wise KA cases recorded in Sagarmatha Zonal Hospital

**Table 18: Month wise KA cases recorded in Sagarmatha Zonal Hospital**

| Month   | Sha | Bha | Aas | Kar | Ma | Pu | Ma | Fal | Ch | Ba | Jes | As | Total |
|---------|-----|-----|-----|-----|----|----|----|-----|----|----|-----|----|-------|
| 064/065 | 13  | 11  | 13  | 8   | 9  | 11 | 13 | 11  | 5  | 10 | 15  | 17 | 136   |
| 065/066 | 8   | 8   | 12  | 6   | 11 | 7  | 10 | 10  | 15 | 11 | 4   | 8  | 110   |
| 066/067 | 8   | 5   | 10  | 9   | 8  | 4  | 7  | 13  | 6  | 10 | *   | *  | 80    |
| Total   | 29  | 24  | 35  | 23  | 28 | 22 | 30 | 34  | 26 | 31 | 19  | 25 | 326   |

Source: A compilation of Hospital record (Unpublished data)

Explain: This table shows month wise KA cases recorded in Sagarmatha Zonal Hospital Saptari in fiscal year 064/065, 065/066, and 066/067. In total the maximum KA cases were recorded in 136, 110 and 80 KA cases respectively. However more cases are recorded during raining and summer season.

#### Age Sex Distribution of KA Cases

Age Group Wise distribution of 1655 KA cases showed 9 among infants 3.3% among 1-4 years, 11.4% among 5-9 years, 13.9% among 10-14 years and 70.9% among 15 years, and above years.

Sex distribution showed predominance of male in total, the male to female ratio being 1:0.73 among 1-5 years, 1:0.88 among 5-9 years, 1:0.47 among 10-14 years 1:0.62 among 15 years and above age group. Region wise sex distribution showed similar pattern as of total.

### 4.3.7 Age and Sex wise kala-azar Cases in 2008

**Table 19: Age and Sex wise kala-azar Cases in 2008**

| District  | 0-11 Mb. |   | 1-54 Yr |    | 5-9 Yr |    | 10-14 Yr |    | 15+ Yr |     | Total |     | Grand Total |
|-----------|----------|---|---------|----|--------|----|----------|----|--------|-----|-------|-----|-------------|
|           | M        | F | M       | F  | M      | F  | M        | F  | M      | F   | M     | F   |             |
| Jhapa     |          |   | 1       | 1  | 1      |    | 2        | 3  | 11     | 4   | 15    | 8   | 23          |
| Morang    |          |   | 1       | 2  | 9      | 4  | 10       |    | 43     | 13  | 63    | 19  | 82          |
| Sunsari   |          |   | 5       | 4  | 16     | 8  | 21       | 14 | 9      | 63  | 133   | 89  | 222         |
| Saptari   |          |   | 6       | 6  | 13     | 14 | 33       | 13 | 123    | 71  | 175   | 104 | 279         |
| Siraha    |          |   | 4       | 1  | 16     | 13 | 22       | 12 | 93     | 59  | 137   | 85  | 222         |
| Udayapur  |          |   | 1       |    | 0      |    | 1        | 1  | 19     | 8   | 21    | 9   | 30          |
| Dhanusha  |          |   |         |    | 1      | 1  | 3        | 2  | 26     | 19  | 30    | 22  | 52          |
| Mahottari |          |   |         |    | 0      | 2  | 1        |    | 13     | 12  | 14    | 144 | 29          |
| Sarlahi   |          |   | 2       | 1  | 21     | 24 | 22       | 6  | 132    | 91  | 17    | 122 | 299         |
| Rautahat  |          |   |         | 2  | 17     | 21 | 33       | 9  | 112    | 98  | 162   | 130 | 229         |
| Bara      |          |   |         | 1  | 1      | 2  | 5        | 9  | 41     | 24  | 48    | 33  | 81          |
| Parsa     |          |   |         | 1  |        | 2  | 5        | 8  | 4      | 1   | 9     | 12  | 21          |
| Makwanpur |          |   |         |    | 1      | 3  | 2        | 1  | 3      | 6   | 6     | 10  | 16          |
| Chitwan   |          |   |         |    |        | 1  | 3        |    | 3      | 1   | 6     | 2   | 8           |
| Total     |          |   | 20      | 19 | 96     | 95 | 163      | 79 | 715    | 467 | 827   | 519 | 1655        |

Source EDCC/MoH

## DISCUSSION

From the Kala-azar prevalence data it can be said that Kala-azar is prevalent in Nepal from a long time. But its first reporting was done in 1980 only. Since then some workers have studied on the Kala-azar in Nepal. EDCD/DOHS has been keeping the records of the Kala-azar cases reported in the country. A total of 34557 cases of Kala-azar have been reported in Nepal from the years 1980 to 2008 according to EDCD/DOHS. Vector Borne Disease Research Training Center (VBDRTC) is established in Hetauda which also conducts Kala-azar case control in Nepal.

In the year 2001 only a total of 2020 cases of kala-azar were recorded in 12 different districts of Nepal. The highest number of cases was reported in Saptari district with 345 numbers of cases followed by Siraha district with 294 cases. Morang district was on 8<sup>th</sup> position with 119 cases of Kala-azar.

Saptari district lies in Terai region of Nepal with hot climate and heavy rainfall. The district has suffered from several epidemics of Kala-azar earlier. This district comprises of 114 VDCs. Sagarmatha zonal Hospital is adjacent to these VDCs and Indian state of Bihar from south. Patients including Kala-azar cases from different VDCs visit the hospital. A total Kala-azar cases were reported in 2006 hospital. The highest number of cases (10) was reported from Saurabhaug VDC while 6 cases were from Rangeli VDC. (year 2006)

Since free medicines and treatment were providing for Kala-azar by the World Health Organization (WHO) in year 2002, the number of cases reported in the hospital was high.

In the Sagarmatha Zonal Hospital no previous work in the Kala-azar has been done, so the early records of Kala-azar could not be found. Sagarmatha Zonal Hospital is largest hospital of Saptari district but it has not good facilities of physical infrastructure, manpower, medicine supply and several types of service delivery found out from field survey.

In the present study when the sex wise prevalence of Kala-azar was seen, it was found that male to female ratio for the disease is 5.75:4.

The higher incidence in the males compared to the females shown by S.Z.H. record and Devkota may be attributed to the fact that males have to work outdoor in fields and farms. Some males work as labours and they have to move to distant regions including endemic region of Nepal and India and work from early morning to late evening. Males also remain out door for longer periods in comparison to females. Females are covered with better clothing giving a slight protection against bite of sand fly. These may be the reasons due to which males are found to be more prone to the disease. Thus, sex can be regarded as minor risk factor for the disease.

When age-wise distribution of Kala-azar was studied the higher prevalence 10(11.36%) of Kala-azar was found in between the age group between 40 to 49 years. In a total of 39 +ve cases of Kala-azar reported in S.Z.H. 9 or 10.22% belonged to age group of 15 to 19 years and, 0 or 0% if positive cases belonged to age group 0-5 years.

In studies KA cases of S.Z.H. 3 or 5.68% positive cases belonged to age group 20 to 29 years of age. Similar type of result was obtained in a study at eastern village. About 60.34% of Kala-azar cases were in age group. Also similar observation was published by whom (1994). The highest attack rate of 10 or 11.36% was found in the age group of 40-49 years followed by 9 or 10.22% age group of 15-19 years. Thus, most of Kala-azar cases was found among adults who are supposed to work outdoors and susceptible to bite of sand fly.

But age group of 5 to 14 years which mainly comprises of students is also at higher risk of Kala-azar. This may be due to the fact that though children go to school they do not get proper education due to the lack of proper teaching from the teachers. They are not getting the information regarding the vector born diseases and its preventive measure. Also these children are not getting balanced diet, proper hygienic houses, sanitary measures, bed nets and other hygienic lifestyles. According to Devkota (1993) a sharp rise in Kala-azar cases among males after 15 years of age coincides with the common practice of boys helping their father in farming.

Occupation also seems to play a major role in the infection of the disease. The highest case of 17.04.% of the total positive cases of Kala-azar was reported among farmers and 11.36% were among labourer while 6.81% of the total positive cases were reported among students. Yadav

(1998) in Siraha reported a total of 11 positive cases out of 223 populations of which 27% were vendors, 15% were farmer, 45% were laborers and 18% were students. The disease difference in occupation group can be attributed to many factors. Not all of the farmers possess their own land. The socioeconomic status is not so high. The farmers keep cattle for their purpose. Cattle sheds are one of the favoured breeding sites of the vector. The house is surrounded by the damp and decaying organic agriculture products. They have the habits of moving at dawn and dusk which is the perfect time of biting of sand fly. These all may be the reasons for higher infection among the farmers.

Laborers are those who work for others at daily wages. They have to move in other endemic areas of India and Nepal for the pursuit of livelihood. Laborers are economically poor, illiterate, are not conscious about the norms of hygiene. They are nutritionally devoid. These factors are major risk factor for acquiring the disease. Comparatively businessman and service holders have a better life and possess healthy lifestyles. The study showed that they are conscious about their health and have knowledge of the disease. Hence, only case was reported from these groups.

Illiteracy was found to be major risk factor for high prevalence of the disease. About 22.72% of the total positive cases were illiterate while only 15.30% of cases having primary level of education had the disease. None of the case was reported from people having higher level of education. Due to the lack of education people do not adopt the hygienic habits and simple measures of avoiding the bites of flies. On the other hand people who are educated have a hygienic habits and are aware of the disease and its preventive measures. Ignorance about the disease, its vector and mode of transmission is also the result of illiteracy.

The study on the surveillance of Kala-azar was done in SZH for the first time. Though the Kala-azar is present in the area from many years the proper study of Kala-azar and its risk factor is not done. The study of risk factors reveals that sex is not a major risk factor of the disease as the infection ratio in the study reported the ratio of male to female to be 5.75:4 as compared to the ratio of 1.56:1 by Devkota (1993) and 1:5:1 by Yadav (1998). This may be due to the fact that in the VDC females are also active in outdoor works like selling vegetable, working in fields and as labors like males. Thus males and females are equally at risk of getting the disease.



## CONCLUSION AND RECOMMENDATIONS

According to the results, discussions and findings it can be assigned from the present study, it becomes clear that visceral leishmaniasis is one of the major health problems in Sagarmatha Zonal Hospital Saptari district. Following suggestions are made for the better and effective control of the disease. These suggestions have been made after a close study of the actual problems faced by the patients of this endemic area and the study of risk factor of Kala-azar.

- a) It was found that ignorance of the disease and its preventive measure is one of the main risk factor of the disease. So awareness about the disease and mode of infection and transmission should be done at community level by the health personals of the health post office. People should be explained the simple and effective means of protection from the disease.
- b) The vector of Kala-azar, sand fly is prevalent in this area and is a great risk factor for the disease. Regular anti-vector activities should be practiced under Primary Health Structure. For this insecticidal spraying in the endemic areas at regular intervals should be done. Studies on vector aspect are very limited. Intensive studies should be undertaken under a view to incriminate the vectors. Its biology, bionomics and insecticide resistance status should be studied.
- c) Vector residing places should be destroyed. For this cracked walls removed from the surroundings. Awareness about environmental modification is helpful.
- d) People should be made aware of the different risk factors, like sleeping on grounds, sleeping without nets, placing cattle sheds near their house and sleeping places.
- e) Benefits of proper housing and sanitary measures should be explained.
- f) Districts should develop their own simple and usable indicators for the supervision, monitoring and evaluation of Kala-azar control programme from the local level.

- g) Laboratory facilities should be available at Primary Health Centers. The diagnostic procedure should be cost effective.
- h) SAG is a first line drug of VL and is commonly used in Nepal. But other drugs Amphotericin B; a second line drug should be made available from the zonal hospital level and upwards. New oral Drug Miltefosine is licensed in India. In Nepal also the drug is under 1<sup>st</sup> phase of pilot survey in Sagarmatha zonal Hospital Saptari.
- i) Studies on the possible reservoir host and its role in the human infection should be carried out.

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## Annex

### Leishmaniasis (Kala-azar) survey form

Zone: - District: - VDC: -

Ward number: - Village: - House number: -

Age: - Sex: - Marital Status: -

Number of the members in the family: -

#### A. Socio-Economic Factors

1. Occupation: (a) Farmer (b) Laborer (c) Service holder (d) Student (e) Business

(F) Vendor

2. Average income of the family:

a) <1,000 Rs. / month b) 1,001-900 Rs. / month c) >9,000 Rs. month

3. Properties owned:

a) House b) Land c) Others, specify .....

4. Educational attainment:

a) Primary b) Secondary c) Higher d) illiterate

5. Do you have your own house?

6. Do you have employees under you?

7. Have you heard about Kala-azar?

8. What kinds of advices will you seek, if someone in your family or a neighbour suffers from Kala-azar?

9. How does this disease occur?
10. Do you know about sand fly (Bhusuna)?
11. When does the sand fly bite?
12. What are the symptoms of Kala-azar?
13. Do you know about the preventive measures of Kala-azar?
14. Have you any information regarding free treatment of Kala-azar in government hospitals?
15. If health volunteers offer to spray your house, will you agree?
16. Do you use a mosquito net?
17. Where do you usually sleep, outdoor or indoor, or on the ground floor or upper floor?
18. Have you been to India or other district in Nepal?

c. Environmental factors

19. Type of house:

- a) Hut b) Khaprail c) Kutchcha d) Cemented e) Other .....

20. How many domestic animals do you own?

21. Location of cattle:

- a) Nearby the house b) away from the house

22. Are the following located near your house?

- a) Ponds b) Gardens c) Water pump

23. Do you own toilet?