CHAPTER 1

INTRODUCTION
1. Introduction

1.1 Leishmania and Leishmaniasis

Leishmaniasis is a parasitic infection caused by the obligate, intracellular protozoan of the genus Leishmania (Schuster & Sullivan, 2002) and accounts for approximately 40,000 deaths per year (Guerin et al., 2002). Over 15 species of Leishmania are capable of infecting man classified into two main groups: Old World: *L. major, L. tropica, L. aethiopica* and the donovani complex (*L. donovani, L. infantum*) and New World: *L. mexicana, L. amazonensis* and Viannia complex (e.g. *L. brasiliensis, L. guyanensis*). It is a poverty-related disease affecting the poorest of the poor and is associated with malnutrition, displacement, poor housing, illiteracy, gender discrimination, weakness of the immune system and lack of resources. Leishmaniasis is an ancient disease that may have been historically portrayed in figures, papyrus, statues and ceramics, and has been discussed from analysis of mummified human remains and archaeological findings (Altamirano-Enciso et al., 2003). The discovery of a chronic ulcer that heals over time has been cited under several names; however, the description of Visceral Leishmaniasis (VL) from historical papers is absent. Nevertheless, the identification of New World leishmaniasis was facilitated by descriptions of a typical mucosa lesion, which was common among pre-Colombian inhabitants. Leishmania belongs to the phylum Euglenozoa and class Kinetoplastida, which lacks a fossil record. Molecular studies have shown that kinetoplastids are probably related to the euglenids (Dooijes et al., 2000). Both belong to the eukaryote supergroup Excavata, whose fossils suggest their appearance during the Ordovician period (Roger & Hug, 2006). In the Old World, Indian physicians applied the Sanskrit term *kala-azar* (meaning ‘black fever’) to an ancient disease later defined as VL. It was first noticed in Jessore in India in 1824, when patients suffering from fevers that were thought to be due to malaria failed to respond to quinine. In 1901, Sir William Boog Leishman (1865–1926) identified certain organisms in smears taken from the spleen of a patient who had died from "dum-dum fever". Initially, these organisms were considered to be trypanosomes, but in 1903 Charles Donovan (1863–1951) described them as being new. The link between these organisms and kala azar was eventually discovered by Ronald Ross, who named them *Leishmania donovani* in memory of these two researchers.
In 1921, brothers Edouard and Etienne Sergent succeeded in proving that the vector for transmission of *Leishmania* parasites to mammals is the sand fly of the genus *Phlebotomus*. Swaminath et al (Swaminath et al., 2006) proved using human volunteers that the Leishmania parasite could be transmitted by *Phlebotomus* sandflies in the Old World. In 1922, it was found that *Lutzomyia* is the vector of New World Leishmaniasis by Ralph Lainson and his colleagues (Cox, 2002).

### 1.2 Types of Leishmaniasis

Leishmaniasis is divided into three general clinical patterns according to the form of the disease.

#### 1.2.1 Cutaneous Leishmaniasis (CL)

This is the most common form of Leishmaniasis, also known as ‘Oriental sore’ which first appears as a persistent insect bite. Simple skin lesions occur on the site of sandfly bite which self-heal within few months but leaves scars. The incubation period can last from few days to months. Gradually, the lesion enlarges, remaining red, but without noticeable heat or pain. Resolution of the lesion involves immigration of leucocytes, which isolate the infected area leading to necrosis of the infected tissues, and formation of a healing granuloma in the floor of the lesion.

The disease is mostly prevalent in Mediterranean region, Central Asia and many places of Central Africa (Chatterjee & Ghosh, 1957). Man is the definitive host whereas gerbils, cats, dogs, and rodent act as the natural reservoir of CL. Sandflies of genus *Phlebotomus* serve as transmitter for this disease. CL is usually caused by *L. major*, *L. tropica*, *L. aethiopica*, in old world and by *L. mexicana*, *L. venezuelensis*, *L. amazonensis*, *L. braziliensis*, *L. panamensis*, *L. guyanensis* and *L. peruviana* in new world.

*Figure 1.1:* Typical skin lesions due to localized CL..
1.2.1.1 Variation of CL:

1.2.1.1.1 Diffuse cutaneous leishmaniasis (DCL):
DCL is a rare form of the disease caused by *L. aethiopica* in the old world and *L. mexicana* complex specifically *L. amazonensis* in the new world (Mehlhom, 2004; Silveira et al., 2005). It is a chronic, progressive, polyparasitic variant that develops in context of leishmanial-specific anergy and is manifested by disseminated non-ulcerative skin lesions, plates or lumps, on the face, arms and legs which can resemble lesions of lepromatous leprosy (Fig. 1.2). The disease does not heal spontaneously and tends to recur (WHO, 1990b).

![Figure 1.2: Symptoms of disfiguring DCL.](image)

1.2.1.1.2 Mucocutaneous Leishmaniasis (MCL)
This form of disease, also known as “espundia”, causes extensive destruction of oral-nasal and pharyngeal cavities with hideous disfiguring lesions, mutilation of the face and great suffering for life. MCL is occasionally reported from Sudan and other Old World foci. Classical MCL is, however, restricted to *L. braziliensis* infections in which, following the apparently complete resolution of the initial oriental sore, sometimes many years later, metastatic lesions appear on the buccal or nasal mucosa. MCL usually exists as an azoonotic infection in which lifecycle is being transmitted from rodent to rodent and mammal by the forest sandfly *Lutzomyia* spp. The reservoir hosts include rodents, opossums, anteaters, sloths and dogs etc. Human infection occurs when human invade the forest habitats.

The causative agents of MCL in old world are *L. aethiopica* (rare), and in new world are *L. braziliensis, L. guyanensis, L. mexicana, L. amazonensis*, and *L. panamensis*. 
1.2.2 Visceral Leishmaniasis (VL) or Kala-azar:

VL also known as kala-azar, black sickness, black fever, Burdwan fever, Dumdum fever or Sarkari Bimari etc. is characterized by prolonged fever, splenomegaly, hepatomegaly, substantial weight loss, progressive anemia, pancytopenia, and hypergammaglobulinemia and is complicated by secondary opportunistic infections (Herwaldt 1999; Guerin et al. 2002). It is the most severe form of the disease and is usually 100% fatal if left untreated. The parasite invades and multiplies within macrophages (free mononuclear phagocytic cells) throughout and affects the reticuloendothelial system including spleen, liver, bone marrow, and lymphoid tissue. *L. donovani* is the causative organism for VL in the Indian subcontinent and East Africa. *L. infantum* causes VL in the Mediterranean basin and *L. chagasi* is responsible for the disease in Central and South America (Desjeux, 2004).

The Post kala-azar Dermal Leishmaniasis (PKDL) is a type of non ulcerative cutaneous lesion (Fig. 1.5) which is developed in about 10% of kala-azar patients (Rees et al., 1984; Salotra & Singh, 2006). It is characterized by a macular, maculo-papular or nodular rash and is a complication of VL (Zijlstra et al., 2003). It can also occur in immunosuppressed individuals in *L. infantum*-endemic areas. PKDL lesions develop 1-13 months post antimony treatment in Sudan, and 1-3 years post antimony treatment in India. It may also develop on individuals who do not have previous history of VL or its treatment. PKDL is common in India, Bangladesh and China, less frequent in Africa (regularly in Sudan and Kenya) and rare in the new world.
1.3 Geographical distribution of Leishmaniasis

Leishmaniasis occurs in tropical and temperate regions and it has been estimated that 90% of CL cases occur in 7 countries: Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia and Syria whereas MCL is endemic in Mexico and Central and South America (Fig. 1.6). Human Leishmaniasis is a worldwide infectious disease, it occurs mainly in the tropics and subtropics (Croft & Coombs, 2003). The annual estimate for the incidence and prevalence of kala-azar cases worldwide is 0.5 million and 2.5 million, respectively (Croft et al., 2006; Desjeux, 2004) and of these, 90% cases occur in India, Nepal, Bangladesh and Sudan. PKDL is prevalent in India, Sudan and Kenya. A summary of clinical manifestations and geographic distribution of the *Leishmania* species is summarised in Table 1.1.

Table 1.1: Summary of clinical manifestations and geographical distribution of *Leishmania* species

<table>
<thead>
<tr>
<th>Species</th>
<th>Clinical manifestation</th>
<th>Geographical Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. donovani</em></td>
<td>Visceral</td>
<td>Old World: China, India, Bangladesh.</td>
</tr>
<tr>
<td><em>L. infantum</em></td>
<td>Visceral</td>
<td>Old World: North Central Asia, Northwest China, Uzbekistan, Middle East.</td>
</tr>
<tr>
<td><em>L. chagasi</em></td>
<td>Visceral</td>
<td>New World: South and Central America.</td>
</tr>
<tr>
<td><em>L. major</em></td>
<td>Cutaneous</td>
<td>Old World: Africa, Middle East, Northern Asia</td>
</tr>
<tr>
<td><em>L. mexicana</em></td>
<td>Cutaneous, Diffuse cutaneous, Mucocutaneous</td>
<td>New World: Southern Mexico, Belize, Northern Guatemala, Southern Texas.</td>
</tr>
<tr>
<td><em>L. amazonensis</em></td>
<td>Cutaneous, Mucocutaneous</td>
<td>New World: South and Central America.</td>
</tr>
<tr>
<td><em>L. braziliensis</em></td>
<td>Mucocutaneous, Cutaneous</td>
<td>New World: Throughout South America.</td>
</tr>
</tbody>
</table>

### 1.3.1 Global status of visceral leishmaniasis

Leishmaniasis is a public health problem in 98 countries or territories, with more than 350 million (82% of which are developing countries) people at risk (Fig. 1.5). India, Bangladesh and Nepal together account for an estimated 250 000 to 300 000 cases per year, comprising 60% of the global burden of VL. The disease burden associated with VL, measured in disability-adjusted life years (DALYs) was estimated to be 1,980,000 (1,067,000 for male and 744,000 for female (Guerin et al., 2002) in year 2000. However, it is felt by many authorities that the number of sufferers may be a few times. About 90% of all VL cases occur in Bangladesh, Brazil, India, Nepal and Sudan. A major decade-long epidemic of VL occurred in Sudan from 1984 to 1994 and as this was the first epidemic in the area, populations were highly susceptible.

The number of cases is increasing, mostly because of gradually more transmission in cities, displacement of populations, exposure of people who are not immune, deterioration of social and economic conditions in outlying urban areas, malnutrition (with consequent weakening of the immune system), and coinfection with HIV. Currently, Leishmania-HIV co-infections have been reported from at least 35 of the 88 countries in which the disease is endemic (WHO, 1990b).
1.3.2 National status of VL

In India, VL is caused by *L. donovani*. Indian VL is anthropootic and is transmitted chiefly through the bites of the female sandfly, *P. argentipes*. The Indian subcontinent (India, Nepal & Bangladesh) is one of the main areas affected by VL worldwide. It accounts for about 67% of total cases reported with almost 200 million people at risk of contracting the disease. Most of the affected population are very poor and illiterate (Mondal et al., 2009). Epidemics of VL occurred in Bengal in the years 1832, 1857, 1871, 1877, and 1899. The reported number of cases is around 20,000 and number of deaths about 200 per year. Estimated number of cases is much higher. Bihar state is the worst affected with 90% of India’s disease burden and an estimated incidence of 22 per 10,000 of population (Mondal et al., 2009) (Fig. 1.6). It is also found in the neighboring states of West Bengal with ten districts affected, Jharkhand with five districts endemic and Uttar Pradesh with four.

The governments of India, Bangladesh and Nepal have launched a joint programme to eliminate VL by 1 per 10,000 by the year 2015 (Mondal et al., 2009). The Indian National Vector Bourne Disease Control Program has decided to launch a pilot project for Kala Azar with support from WHO in 2007. This will cover six endemic districts in Bihar, two in Jharkhand and two in West Bengal. Since, VL is more relevant in Indian context; so the research work embodied in this thesis was carried out on this form of the disease.

![Figure 1.5: World map showing distribution of Leishmaniasis](https://www.izb.unibe.ch/res/roditi/Lehr/2007/07_Leish.pdf)
1.4 Disease risk assessment

Until recently, the public health impact of leishmaniasis was grossly underestimated. An estimated 2 million new cases (1.5 million cases of CL and 500,000 of VL) occur annually, with about 12 million people currently infected (Desjeux, 2004), a death toll that is surpassed among the parasitic diseases only by malaria (The World Health Report, <http://www.who.int/whr/2002/en/whr02_en.pdf). Both figures are approximations as VL is
frequently not recognized or not reported (Singh et al., 2006). Migration, lack of control measures and HIV–VL co-infection are the three main factors driving the increased incidence of VL (Boelaert et al., 2000; Desjeux et al., 2001). The disease is mostly endemic in countries that are among the least developed in the world (such as Nepal) or in the poorest regions of so-called ‘middle income’ countries (such as Bihar State in India where people have a daily income of less than 2 USD and poor economic level (Murray, 2005). India, Nepal and Bangladesh, harbour an estimated 67% of the global VL disease burden (Hotez, ; Hotez, 2009) and patients and families affected by VL become poorer because of the high direct costs (for example, the costs of VL diagnosis and treatment) and indirect costs (for example, loss of household income) of the disease (Ahluwalia et al., 2003; Alvar et al., 2006; Rijal et al., 2003).

1.5 Leishmania/HIV co-infections

Beyond the damage VL causes on its own, it can also accelerate the onset of disease progression in HIV-positive people by suppressing the immune system and allowing viral replication to occur. Conversely, HIV infection substantially increases the risk of acquiring VL in endemic areas. Epidemiology of VL is further changing due to widespread migration of population and emerging HIV/VL co-infection which is emerging as an extremely serious problem. Recently, it has been reported that the risk of VL among AIDS patient’s increases by 320 times (Alvar et al., 2008). To date, it has been reported from 31 countries, with most of the cases from Southern Europe, where 25–70% of adult patients with VL are co-infected with HIV (Desjeux, 1995). The first case of VL/HIV co-infection in India was identified from the State of Bihar in the year 2000 (Sinha et al., 2003). Since, AIDS epidemic is looming large on the horizon of new millennium in India (Sinha et al., 2006), the State of Bihar needs to be looked seriously for VL/HIV coinfections. The clinical outcome of *Leishmania* infection depends on the balance between Th1 and Th2 cytokines (Bacellar et al., 2000; Bourreau et al., 2003; Wolday et al., 2000) and HIV infection alters the Th1/Th2 balance in VL patients, resulting in increased Th2 cytokine responses and decreased levels of interleukin 12 (IL-12) and IL-18 (Wolday et al., 2000). Leishmaniasis with HIV co-infection is an emerging condition that demands urgent attention. Even when coinfected
patients receive proper treatment, they relapse repeatedly and the outcome frequently is fatal.

1.6 Morphology and digenetic Life cycle of *Leishmania donovani*

*Leishmania* exists in two forms (i) promastigotes: these are extracellular, elongated, flagellated, motile and ranges in size from 2 mm × 2-20 μm (Fig. 1.7). It shuttles between sandfly vector (subfamily *Phlebotominae*) where it multiplies as free promastigotes in the gut lumen and mammalian host where it propagates as intracellular amastigotes in the mononuclear phagocytes (Kedzierski et al., 2006). Following the sandfly bite, some of the flagellates entering the circulation are destroyed while others enter the cells of the reticuloendothelial system. Here they undergo change into amastigote form which multiplies by binary fission, with the multiplication continuing until the host cell is packed with the parasites and ruptures, liberating the amastigotes into circulation. This form resides and multiplies within the phagolysosomes of macrophages of reticuloendothelial system of the vertebrate host (Handman, 1999). The released amastigotes are taken up by additional macrophages and so the cycle continues (Fig. 1.8). Ultimately all the organs containing macrophages and phagocytes are infected, especially the spleen, liver and bone marrow.

**Figure 1.7:** Two stages of *Leishmania* parasite (A) Intracellular, non-motile stage called amastigotes (small dots) observed in Giemsa stained dab smear from the spleen of *L. donovani* infected hamster. (B) Extracellular and motile form called promastigotes.
1.7 Vectors and transmission of the disease

VL is transmitted by the *Phlebotomus* spp. in the old world and *Lutzomyia* spp. in the new world. Only 60 of about 600 sand fly species are vectors for *Leishmania* (Mehlhorn, 2004). *P. argentipes* is the proved vector of VL in India (Kishore et al., 2006; Swaminath et al., 2006). Sandflies are very small in size (< 3.5 mm) (Fig. 1.9) and may be hard to see. They are usually most active in the twilight, evening and night hours (from dusk to dawn) and less active during the hottest time of the day. Only the female sandfly transmits the parasites and need blood for their eggs to develop, and become infected with the *Leishmania* parasites when they suck blood from an infected person or animal. Female sandfly lays its eggs in the burrows of certain rodents, in the bark of old trees, in ruined buildings, in cracks in house walls, in animal shelters and in household rubbish (http://www.who.int/en). High incidence of VL is reported during pre-monsoon season that coincides with vector abundance and
increased man-vector contact due to sleeping habits of children in open space (Kishore et al., 2006; Singh et al., 2006). The main reservoir hosts for *Leishmania* are domestic animals (e.g. dogs, cats and horses), peridomestic animals (e.g. mice and rats) and wild animals (e.g. rodents, hyraxes, sloths, bats, oppossums, kangaroos, wolves and foxes) (WHO, 1990a).

![Picture of a sand fly biting a human arm, source: CDC/Frank Collins](image)

**Figure 1.9:** Picture of a sand fly biting a human arm, source: CDC/Frank Collins

### 1.8 Clinical symptoms of Visceral Leishmaniasis

VL patients are heavily infected throughout the mononuclear phagocyte system and develop life-threatening disease after an incubation period of weeks to months. Symptoms include fever, severe cachexia, hepatosplenomegaly ([Fig. 1.10](image)), pancytopenia, anaemia, thrombocytopenia, leucopenia with neutropenia, marked eosinopenia, a relative lymphocytosis and monocytosis and hypergammaglobulinaemia (mainly IgG from polyclonal B-cell activation) with hypoalbuminaemia (Herwaldt, 1999). VL encompasses a broad range of manifestations of infection which remains asymptomatic or subclinical in many cases, or can follow an acute, subacute, or chronic course. Active VL may also represent relapse (recurrence 6-12 months after successful treatment) or late reactivation of subclinical or previously treated infection.
1.9 Diagnosis of Visceral Leishmaniasis

Laboratory diagnosis of leishmaniasis can be made by light microscopic examination of the stained specimen, by *in vitro* culture, by animal inoculation, detection of parasite DNA in tissue samples, detection of parasite antigen or specific antileishmanial antibodies (Sundar & Rai, 2002a). Prevalence and incidence data for assessing the full impact of leishmaniasis are unreliable.

Direct visualization of amastigotes in clinical specimens is the diagnostic gold standard in regions where tissue aspiration is feasible and microscopy and technical skill are available. Diagnostic sensitivity for splenic, bone marrow, and lymph node aspirate smears is >95%, 55.97%, and 60%, respectively (Sundar, 2003). Elsewhere, serum antileishmanial immunoglobulin G in high titre is the diagnostic standard, primarily with direct agglutination tests or other laboratory-based serological assays (Abdallah *et al.*, 2004; Desjeux, 2004; Herwaldt, 1999). Freeze-dried antigen and rapid detection of anti-K39 antibody with fingerstick blood in an immunochromatographic strip test have advanced field
serodiagnosis. In symptomatic patients, anti-K39 strip-test sensitivity is 90-100\% (Boelaert et al., 1999; Boelaert et al., 2004; Sundar & Rai, 2002b; Sundar et al., 1998; Veeken et al., 2003). This test can safely substitute for invasive diagnostic procedures in VL and is also useful for PKDL. Testing urine for leishmanial antigen or antibody is a new approach (Islam et al., 2004; Sundar et al., 2005). Various immunodiagnostic tests include antibody detection, complement fixation test, immunodiffusion test, countercurrent immunoelectrophoresis (CCIEP), indirect hemagglutination, indirect fluorescent antibody (IFA) test, DAT, ELISA with crude soluble antigen (CSA), ELISA with fucose-mannose ligand, ELISA with rK39 antigen and rapid strip test with rK39. In addition, antigen detection test called KATEX is 68-100\% sensitive. DNA detection by PCR with LDI primer using blood, bone marrow and skin are currently under use (Salotra et al., 2001). Different DNA sequences in the genome of *Leishmania* have been documented in diagnosis and prognosis of VL (Schallig et al., 2002; Sundar et al., 2006). Leishmanin skin test, a form of DTH, is also widely used as a diagnostic tool for leishmaniasis (Handman, 2001; Khalil et al., 2000; Khamesipour et al., 2006; Reed, 1996).

1.10 Control strategies of the disease

Efficient case management based on early diagnosis and treatment is the key to limit morbidity and prevent mortality. In rural areas where dwellings are more dispersed and surrounded by large, untargeted "reservoir" populations of sandflies, residual spraying of houses may be both impractical for logistic reasons and ineffective. Impregnated bednets may offer the best solution in rural areas where transmission is largely intradomiciliary. This measure has the advantage that it can be employed at the individual household level and affords collateral benefits such as privacy and control of other biting insects such as mosquitoes, fleas and bedbugs. In foci where sandflies bite at night, impregnated bed-nets have decreased the incidence of leishmaniasis. Effective treatment of patients is also a measure to control reservoir and transmission in anthroponotic foci, particularly for cases of PKDL, which are thought to act as a long term reservoir of the disease. In addition, vector control should be implemented wherever feasible. Spraying of houses with residual insecticides has been an important measure in the past in India but is not much used now. Therefore, protection from sandfly bite can be achieved by avoiding outdoor activities, using...
protective clothing and insecticide treated nets and insect repellents (Davies et al., 2003). Insecticides used in malaria control programmes are also effective on sandfly. Indian government started Leishmaniasis Elimination Programme in 2001 with the targets of prevention of death by 2004, zero level incidence by 2007, zero level prevalence by 2010 and elimination by 2012 (Das et al., 2005).

1.11 Drugs against VL

VL, though localized in certain areas only, has gained immense importance because of high mortality rate, mainly in children. The drugs currently recommended for the treatment of VL include the pentavalent antimonials viz. sodium stibogluconate (SSG) and meglumine antimoniate, amphotericin B and its lipid formulations (AmBisome®, Abelcet, Amphocil), pentamidine, miltefosine and paromomycin. Pentavalent antimonials were introduced 60 years ago as first-line drug and remain the mainstay of treatment in most parts of the World except some parts of India and Bangladesh where the clinical value of SSG is being seriously eroded by the emergence of widespread resistance (Sundar et al., 2001). In Bihar, about 37-70% of cases are currently reported to be non responsive to SSG (Croft & Engel, 2006; Croft et al., 2006; Sundar et al., 2000). Second line drugs, such as Pentamidine is more effective than antimonials but also induces serious disorder in glucose metabolism and nephrotoxicity (Jha, 2006; Jha et al., 1998). Another drug, Amphotericin B and its derivatives are used successfully, though its applicability in the field is limited, due to toxicity, long-term therapy and complicated mode of administration. Liposomal formulation of Amphotericin B is expensive and toxic too. Miltefosine, a new low cost oral drug with tolerable toxicity too has teratogenicity and is contradicted in pregnancy. It was registered in India in 2002 for clinical use. The other new drugs-Aminosidine and Parmomycin are undergoing clinical trials for Indian VL. The risk of development of resistance in the parasite is theoretically feasible against all drugs (Yardley et al., 2005). Thus, mere chemotherapy is not sufficient to combat the disease. Hence, in addition to the therapeutic measures, vaccination is the point of serious consideration (Table 1.2).
### Table 1.2: Dose, duration, cost and drawback of drugs currently in use for VL

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose</th>
<th>Duration</th>
<th>Cost ($)</th>
<th>Side effects</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentavalent antimonials</td>
<td>20 mg/kg</td>
<td>For 30-40 days</td>
<td>634.4</td>
<td>Arthralgia, myalgia, nausea, vomiting, abdominal pain, headache, rash, leucopenia, thrombocytopenia, reversible renal insufficiency and cardiotoxicity.</td>
<td>Drug resistance in Bihar, India.</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.75-1.0 mg/kg</td>
<td>15 to 20 infusions daily/ alternate</td>
<td>439</td>
<td>Fever, nausea, vomiting, malaise, anemia, phlebitis, hypokalemia and nephrotoxicity.</td>
<td>Costly</td>
</tr>
<tr>
<td>ABCL</td>
<td>2mg/kg/day</td>
<td>5 days</td>
<td>792.9</td>
<td>Infusion-related reactions</td>
<td>High cost</td>
</tr>
<tr>
<td>Liposomal Ampho B</td>
<td>5 mg/kg</td>
<td>1 day</td>
<td>667.9</td>
<td>mild but reversible rise in serum creatinine</td>
<td>High cost</td>
</tr>
<tr>
<td>Pentamidine</td>
<td>4 mg/kg</td>
<td>i.m/ i.v once alternately for upto 15 doses</td>
<td></td>
<td>Hypoglycemia followed by diabetes mellitus, hypotension, nausea, vomiting, abdominal pain and headache.</td>
<td>Low frequency, long duration</td>
</tr>
<tr>
<td>Miltefosine</td>
<td>1.5-2.5 mg/kg</td>
<td>28 days orally</td>
<td>246.9</td>
<td>Vomiting, nausea and diarrhea.</td>
<td>Costly</td>
</tr>
<tr>
<td>Paramomycin</td>
<td>15mg/kg/day</td>
<td>21 days</td>
<td>116</td>
<td>nephrotoxicity and ototoxicity.</td>
<td>-</td>
</tr>
</tbody>
</table>

#### 1.12 Vaccines against Leishmaniasis

The development of a vaccine against leishmaniasis is a long term goal in both human and veterinary medicine. In the past decade, various subunit and DNA antigens have been identified as potential vaccine candidates in experimental animals but none have so far been approved for human use. To date there is no vaccine against VL in routine use anywhere in the world though several vaccine preparations are in more or less advanced stages of testing. However, there is consensus that in the long term, vaccines ought to become a major tool in the control of this group of diseases. Unfortunately, the development of vaccines has been hampered by significant antigenic diversity and the fact that the parasites have a digenetic life cycle in at least two hosts.
Although a great number of antigens have been tested for protection against the cutaneous
disease with *in vitro* cell or mouse models, no effective vaccine against human kala-azar is
yet available. Though, the solid immunity observed following cure of kala-azar has suggested
that the vaccination to prevent leishmaniasis is within the reach of conventional
immunization methods, only few reports in literature deal with vaccines viz., FML, FML-
QuilA Saponin etc., against canine VL (Da Silva et al. 2000; Santos et al. 2002; Borja-
Cabrera et al. 2004). Immune mechanisms involved in VL and various vaccines candidates
evaluated in experimental models for VL has been extensively reviewed by Modabber
(Brodskyn *et al.*, 2003), (Scott, 2003), (Ravindran & Ali, 2004), (Goto & Lindoso, 2004),
(Nyame *et al.*, 2004), (Kaye *et al.*, 2004), (Awasthi *et al.*, 2004), (Wilson *et al.*, 2005), Garg