CHAPTER-1
INTRODUCTION

Leishmaniasis is a vector-borne infectious disease usually caused by a protozoan parasite belongs to genus *Leishmania*, of which nearly two dozen species are pathogenic to human beings (Murray *et al*., 2005). It is even today remains one of the major public health problem in tropical and subtropical countries of the world including India the effect of its morbidity on the social and economic development are staggering (Croft and Coombs, 2003). *Leishmania* parasite is transmitted through the bite of an insect vector *Phlebotomine* sand fly. It causes wide range of clinical manifestations including visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL) (Murray *et al*., 2005, Schwartz *et al*., 2006, Reithinger *et al*., 2007). The global incidence of leishmaniasis is approximately 12 million and is endemic in 88 countries (72 of which are developing countries), putting an estimated 350 million people at risk (Desjeux, 2004, Dujardin *et al*., 2008). Approximately 200 million peoples in 62 countries are on risk for VL with an estimated 0.5 million new cases along with 60,000 deaths each year (Desjeux, 2004). The risk of mortality by VL has recently been increased due to it’s association with HIV infection (Tiuman *et al*., 2011) and approximately 1 – 1.5 million cases of cutaneous leishmaniasis has been reported worldwide annually (Desjeux, 2004, Dujardin *et al*., 2008). The number of cases reported globally has increased over the past 10 years to an increase in anti-leishmanial drug resistance and a lack of adequate vector or reservoir control tools (Reithinger *et al*., 2007).

Visceral leishmanisis (VL) is mainly caused by the *L.L. donovani and L. infantum*. In India visceral leishmaniasis is a serious problem in Bihar, West Bangal and eastern part of Uttar Pradesh. Sporadic cases have been reported from Tamil Nadu, Pondicherry, Assam, Orissa and Gujarat (Pearson *et al*., 1995). *L. infantum* is a causative agent of visceral leishmanisis in the meditarian basin and more recently North America and Himachal Pradesh state of India (Robert *et al*., 2000). In man it causes infantile visceral leishmanisis, which is classically, restricted to the children, especially those below the age of 2 years. However, it may also cause infection to the adults; particularly those are infected with HIV (Alvar *et al*., 1997, Berman, 1997).

The recent emergence of VL in Rampur Bushahr of Himachal Pradesh appears to be due to construction activities for several hydroelectric power projects on River Sutlej, establishment of new residential colonies leading to destruction of forests and intrusion into
the sylvatic cycle of the vector and migration of the laborers from Bihar, Jharkhand, Uttar Pradesh and Nepal (WHO, 1990). It is still not clear whether the parasite has come from sylvatic cycle or introduced by migrant population. The cattle kept close to the houses by people in rural areas are also known to attract both anthropophilic, as well as zoophilic vectors (WHO, 1990).

In India localized cutaneous leishmaniasis (LCL) is mostly due to *L. tropica* and prevalent in the deserts of Rajasthan. Recently, Kinnaur district of Himachal Pradesh has been identified as a new endemic focus for the disease. It is rarely reported as a cause of LCL exists with sporadic VL. *Leishmania donovani* is the predominant pathogen along with *L. tropica* and the number of new cases of LCL has been increasing reaching almost epidemic proportions in Kinnaur district of Himachal Pradesh from last few years (Sharma *et al*., 2005).

At present, VL treatment relies on a number of drugs such as pentavalent antimonials, amphotericin-B and its formulations, paromomycin and the only orally administered drug miltefosine (Croft *et al*., 2006). However, none of these drugs are ideal for treatment due to their high toxicity, resistance issues, prohibitive prices, long treatment regimen and mode of administration (Alvar *et al*., 2006). Current control measures rely upon parental administration of sodium antimony stibogluconate, which has been the cornerstone of treatment for the last six decades with little evidence of resistance. (Sharma *et al*., 2000). The situation has been more complicated due to the spread of drug resistant strains of *Leishmania* resistant to Stibogluconate (V) (Thakur *et al*., 1998, Sundar, 2000, 2001). Stibogluconate (V) has been the most effective, safe and cost-effective drug for the treatment of leishmaniasis. Moreover, the control of leishmaniasis by insecticide sprays also has suffered a setback as sand fly vectors have been developed resistance to popular and cheap insecticide DDT (Sing *et al*., 2001). The newer insecticide like malathion and pyrethroids are several times more expensive. Thus the control of leishmaniasis has become a costly affair due to high cost of alternative antileishmanial and insecticides. The development of an effective vaccine against *Leishmania* will be a cost effective adjunct.

Over the past few decades significant improvements have been made in the number of treatments available for VL, with both new drugs and new formulations of old drugs that have been either recently approved or are in clinical trials are now available (Seifert, 2011). Recently, combination therapy using immunomodulators with antileishmanial compounds have become increasingly popular and several studies have reported benefits of co-administration of antileishmanial drugs with immunostimulants as they decrease the time of
course of treatment, and may prevent the emergence of resistance and increase the efficacy of current therapeutic regimen (Sundar et al., 2008, Musa et al., 2010, Sane et al., 2011, Shakya et al., 2012). Imiquimod, a novel immune response activating compound is currently being used as a combination therapy with paromomycin for the treatment of CL successfully (El-On et al., 2007). The other antileishmanial drugs like Quassin (Bhattacharjee et al., 2009), fucoidan (Kar et al., 2011), and curdlan (Ghosh et al., 2013) are well known immunomodulators that have recently been explored for their potential to kill the Leishmania parasites by boosting host immunity in experimental models of VL. The progression of VL infection is generally associated with down regulation of the host immune system (Olivier et al., 2005). Leishmania has developed several strategy to inactivate the functions of macrophage to survive inside the cells (Olivier et al., 2005). The outcome of the infection depends on the production and/or secretion of immunosuppressive molecules that includes, transforming growth factor (TGF)-β, interleukin (IL)-10 and prostaglandin E2 (PGE2) (Bogdan et al., 1996, Olivier et al., 2005). These molecules distort the normal immune response by suppressing host-protective immune response, including cytokines like interferon (IFN)-γ, IL-1, IL-12, and tumor necrosis factor-α (TNF-α), and reactive nitrogen and oxygen species (Olivier et al., 2005, Bogdan et al., 1996, Assreuy et al.,1994). Host immune response suggests that drugs that boost host cell activation by Th1 might be useful as potential therapeutic agents for treatment of experimental VL (El-On et al., 2007, Bhattacharjee et al., 2009, Kar et al., 2011, Ghosh et al., 2013).

The cytokines are able to activate macrophages, which is a major killing tool for Leishmania parasites(Reiner et al., 1995, Kemp et al., 1996). Patients with CL and ML have a strong type 1 immune response to Leishmania antigen, with high production of IFN-γ and TNF-α and decreased ability of IL-10 in down regulating IFN-γ production (Folladar et al., 2002). IFN-γ, secreted by Th1 cells, is the most potent macrophage-activating cytokine leading to host resistance to infection with Leishmania parasites (Scott, 1991, Fruth et al., 1995), whereas interleukin-4 (IL-4), secreted by Th2 cells, is associated with down-regulation of IFN-γ-mediated macrophage activation (Abbas et al., 1996, Himmelrich et al., 1998). IL-10 is an anti-inflammatory cytokine and is more potent than IL-4, it inhibiting the leishmanicidal activity of human macrophages. Inhibition of Leishmania killing by IL-4 and IL-10 correlated with decreased NO generation from macrophages. During infection it inhibits the activity of Th1 cells, NK cells, and macrophages, all of which are required for optimal pathogen clearance but also contribute to tissue damage. In consequence, IL-10 can both impede pathogen clearance and ameliorate immunopathology (Bamford et al., 1997).
We were interested in defining the role of humoral antibody response formed during *Leishmania* species infection and understanding the mechanisms leading to protection or pathology (i.e. IL-2, IL-4, IL-6, IL-10, IL-17A, TNF-α and IFN-γ). Such type of work was not previously studied from this region.

Keeping these points in view, the present study was planned to study the role of humoral antibody response formed during *Leishmania* species infection and understanding the mechanisms leading to protection or pathology (i.e. IL-2, IL-4, IL-6, IL-10, IL-17A, TNF-α and IFN-γ). The prevalence and understand the immune response in different age groups of individual as well as characterization of the strains prevalent in Sutlej valley of Himahcal Pradesh.
AIMS AND OBJECTIVES

The present study was aimed to study the prevalence and genotyping the strains prevalent in Sutlej river valley of Himachal Pradesh and to understand the role of immune responses in protection or pathogenicity of *Leishmania* infection. This aim was achieved by studying the following objectives:

**1.2 Objectives:**

1. The public health importance of this infection was quantified mainly by seroepidemiological study of *Leishmania* species

   a) The antibody response of IgG, and IgG subclass was assessed against *Leishmania* Species antigen in serum samples by using ELISA.

2. To evaluate the role of humoral antibody response formed during *Leishmania* species infection and understanding the mechanisms leading to protection or to pathology (Cytokines i.e. IL-2, IL-4, IL-6, IL-10, IL-17A, TNF-α and IFN-γ).

3. Characterization of the strains prevalent in Himachal Pradesh by using ITS-1 region segment as a marker

   (a) The ITS-1 region of *Leishmania parasite* was amplified by using PCR amplification method.

   (b) Selected Amplicones were sequenced to determine the species of *Leishmania* by using bioinformatics means.