Chapter 1

1.1. Introduction

Lymphatic filariasis (LF) is a chronic and debilitating disease that affects people in tropical and sub-tropical areas of Asia, Africa, Western Pacific and some areas of the United States of America. The disease is caused by the parasites *Wuchereria bancrofti* (*W. bancrofti*), *Brugia malayi* (*B. malayi*) and *Brugia timori* (*B. timori*), transmitted by *Culex*, *Anopheles* and other mosquitoes (Koroma *et al.*, 2012). Lymphatic filariasis (LF) is by far the most prevalent disease of the filarial infections (Chu *et al.*, 2010; Bockarie *et al.*, 2009; Melrose *et al.*, 2004; Molyneux *et al.*, 2001) commonly known as “elephantiasis,” affecting around 1.4 billion people globally with about 40 million disfigured and incapacitated by the disease in 73 countries. Current estimate shows that in India alone about 553 million people live in endemic areas with approximately 48 million having either circulating microfilariae (mf) or overt disease like hydrocele, lymphoedema and elephantiasis (WHO, March 2013).

Filariasis is one of the most common causes of permanent disability among tropical diseases worldwide creating the highest disease burden in terms of disability-adjusted life years (DALYs) (Lustigman *et al.*, 2012; Ottesen *et al.*, 2008). Those affected also suffer psycho social stigmatization and economic suffering as it can lead to job loss or “inability to work”. The disease is, therefore, a major cause of poverty as it causes economic burden for those affected, on their dependants, their communities and the country as a whole (Koroma *et al.*, 2012; Huppatz *et al.*, 2009; Ruberanziza *et al.*, 2009). World Health Assembly passed in 1997, a resolution for the elimination of LF as a
public health problem in the world by 2020 (Koroma et al., 2012). In 1998, World Health Organization (WHO) launched a global program for the “Elimination of LF” (Bockarie et al., 2009; Ottesen et al., 1997; Ruberanziza et al., 2009; Addiss et al., 2010).

The disease is transmitted by the circulating mf in the blood of patients. Therefore to reduce transmission, the communities were kept on annual mass drug administration (MDA) for 4-6 years to 80% of the entire at-risk population which can reduce to zero or close to zero (Gyapong et al., 2005). Before MDA implementation in a country, units were targeted through a rapid assessment study and also baseline data on LF level was obtained to monitor effectiveness of MDA. Few countries (Cape Verde, China, Costa Rica, Solomon Islands, South Korea, Suriname, and Trinidad and Tobago) with small population in density succeeded in eliminating the disease (Iboh et al., 2012) using a combination of strategies that included vector control and single annual doses of 2-drug treatments of albendazole along with ivermectin or diethylcarbamazine (Gyapong, et al., 2005). The currently preferred strategy for LF elimination recommended by WHO is the preventive chemotherapy using the available drugs (WHO 2006). The global LF elimination program has been strengthened by donation of albendazole by GlaxoSmithKline and continued donation of ivermectin by Merck & Co (Linehan et al., 2011). Still the upcoming resistance of ivermectin and poor drug compliance indicates towards the pressing need for a potential anti-filarial agent possessing both microfilaricidal and adulticidal activity (Mullner et al., 2011).

LF has a wide range of clinical manifestation from acute attacks of filarial fever, chronic conditions such as hydroceles, lymphoedema, elephantiasis of limbs, enlarged breasts and kidney damage, thus causing great morbidity and disability for those
affected (Iboh et al., 2012; Frimpong et al., 2012). The immunology of lymphatic filariasis is complex and is complicated by the anatomical compartmentalization of the immune response and the differing responses induced by the multiple stages of the parasite. The infection is initiated by mosquito-derived third-stage larvae (L₃) deposited in the skin, an immunologic organ, containing Langerhans’ cells (LC) and keratinocytes. The larvae enter through the skin into the body where, if successful, the parasite evades the primary line of defense. The adult female worm, once fertilized, develops into a fully formed, sheathed, first-stage larvae (L₁), a stage believed to mediate many of the systemic immunologic defects associated with chronic lymphatic filarial infection. As the initiation of infection occurs through the skin and the subsequent immune response, understanding the interaction between the filarial L₃ and the relevant antigen presenting cell (APC) in the skin is imperative. Between 9 and 14 days after infection, the parasites undergo a molt to the fourth larval stage (L₄) and then mature into lymphatic tissue dwelling adult male and female worms during the subsequent 3–12 months. Through this circuitous route from the skin to the lymphatics and lymph nodes (LNs) and ultimately to the systemic circulation, the various parasite stages interact with very different types of APCs that may induce quite distinct immune responses. Once the infection is fully established and microfilariae are produced. The nature of the APCs that come into contact with the parasites and the responses these APCs mediate are likely very different from what occurs initially.
Figure 1.1: Life cycle of *B. malayi* and its interaction with host antigen presenting cells (APCs).

The most widely used method for diagnosis of filarial infection is microscopic examination of blood for the presence of microfilaria (mf). This approach has limited sensitivity and is not suitable for large-scale mf screening of human populations in endemic areas because it is time-consuming and labor intensive. In epidemiological surveys, the alternate technique used to detect and characterize filarial parasites in vectors includes the collection, dissection and microscopy, is also not reliable because the mosquitoes may carry both animal and human filarial parasites, which cannot be identified biochemically or morphologically (Auty *et al.*, 2012; Sim *et al.*, 1986 a,b). In order to overcome some of these shortcomings, improved methods for diagnosis of filarial infections are needed to study the transmission, epidemiology and facilitate surveillance activities to monitor control efforts (Basáñez *et al.*, 2012; Chandrashekhar *et al.*, 1994). In addition, because the clinical manifestations of filariae vary greatly between individuals, an ideal diagnostic test which would not only reveal individuals that are
infected or have been exposed to infection, but would also differentiate between various clinical manifestations that the lymphatic-dwelling parasites, in particular, induce the infected population (Chandrashekar et al., 1997).

Recent advance in biotechnology have started revolutionizing the diagnosis of filarial; parasites not only in the host but also in their vector. The molecular biological techniques such as DNA probe development (Mohanka et al., 2009; Smith et al., 1999; Siridewa et al., 1994; Poole et al., 1990), and repeated DNA-polymerase chain reaction (PCR) method (Lizotte et al., 1994; McCarthy et al., 1996; Zhong et al., 1996) have been used in W. bancrofti or B. malayi detection and have provided the opportunity for improved diagnosis. However, these test encountered some problems as mosquitoes contains PCR inhibitor (Hunt 2011; Dissanayake et al., 1991) due to which DNA undergoes degradation during course of collection, transport and storage (Cooper, 1998).

Although, the PCR assay was found to be 100% specific but sensitivity was considerably low even in samples with low microfilarial counts (Utzinger et al., 2012; Ramzy et al., 2002; Lizotte et al., 1994; Zhong et al., 1996; Farid et al., 2001). Species-specific DNA probes have been developed for B. malayi, W. bancrofti, Onchocerca volvulus (O. volvulus), and Loa loa (L. loa). Furthermore, monoclonal antibodies have been developed that are specific for detection of circulating antigens in lymphatic filariasis. “For loiasis, immunodiagnostic methods have not been very successful but repetitive DNA sequences in the Loa genome have been found to be species specific. There have been no advances in the diagnosis of Mansonella perstans but both immunodiagnostic and PCR tests” (Walther et al., 2003) show promise in differentiating M. streptocerca, and the latter in differentiating M. ozzardi, from Onchocerca. Diagnostic antigens have been obtained by
cloning parasite DNA that codes for proteins recognized by infected individuals with only certain species of filariae. Recombinant antigens (rAgs) are available today which detect prepatent infections in onchocerciasis (Chandrashekar et al., 1997). Several laboratories developing new diagnostic tests for filariasis are currently evaluating these tests in the field with the collaboration of parasitologists, epidemiologists, and vector biologists. “Since the present global campaign to eliminate lymphatic filariasis, new diagnostic tools have emerged like PCR, antigen detection using finger-prick blood taken during the day and ultrasound to visualize adult worms. The last two can be applied in endemic countries” (Walther et al., 2003) with limited resources and enable the detection of early infections. As well as their worth in control schemes, the latter is mainly significant for the individual since recent research has shown that damage is usually caused long before symptoms appear” (Walther et al., 2003).

The gaps in the present tools suggested us to aim the present study towards the characterization of human and bovine filarial parasite by molecular, immunological and biochemical techniques to identify a promising diagnostic tool.
Objectives

• Collection and isolation of microfilaria (mf) from blood of human filariid infected experimental model and bovine parasite, isolation of filarial genomic DNA from mf & adult parasite and collection of serum samples from various categories of established filarial patients.

• Screening of DNA sequences from filarial DNA Genbank and EST’s for homologous sequences for primer designing and alignments of homologous sequences using CLUSTAL W & X multiple alignment program.

• Production of PCR product of homologous sequences using their primers.

• To characterize animal and human parasite by using immunological and biochemical techniques.