LYMPHATIC FILARIASIS: MICROFILARIAE IDENTIFICATION AND EPIDEMIOLOGICAL SURVEY IN GANESHSTHAN VDC, NUWAKOT, NEPAL

A THESIS

SUBMITTED AS PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTER'S DEGREE IN ZOOLOGY WITH SPECIAL PAPER PARASITOLOGY

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OCTOBER, 2007

DECLARATION

I herby declare that the work presented in this thesis entitled "**Lymphatic Filariasis: Microfilariae Identification and Epidemiological Survey in Ganeshsthan VDC, Nuwakot, Nepal"** has been done by myself and has not been submitted else where for the award of any degree. All sources of information have been specifically acknowledged by reference to the authors or institution.

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RECOMMENDATION

This is to recommend that the thesis entitled **"Lymphatic Filariasis: Microfilariae Identification and Epidemiological Survey in Ganeshsthan VDC, Nuwakot, Nepal"** has been carried out by **Mr. Lil Bahadur Hamal** for the partial fulfillment of M.Sc. degree in **Zoology** with special paper **Parasitology**. This original work was conducted under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degrees.

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LETTER OF APPROVAL

On the recommendation of the supervisor, **Dr. Ranjana Gupta**, this thesis submitted by **Mr. Lil Bahadur Hamal** entitled **"Lymphatic Filariasis: Microfilariae Identification and Epidemiological Survey in Ganeshsthan VDC, Nuwakot, Nepal"** is approved for examination and submitted to the Tribhuvan University as partial fulfillment of the requirements for Master's Degree of Science in **Zoology** with special paper **Parasitology**.

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This thesis submitted by **Mr. Lil Bahadur Hamal** entitled **"Lymphatic Filariasis: Microfilariae Identification and Epidemiological Survey in Ganeshsthan VDC, Nuwakot, Nepal"** has been approved as a partial fulfillment of requirements for the master's Degree of Science in **Zoology** with special paper **Parasitology**.

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LIST OF ACRONYMS

AD	Anno Domini
ADL	Adeno-Lymphangitis
ALB	Albendazole
AM	Ante Meridiem
BS	Bikram Sambat
CBS	Central Bureau of Statistics
CDR	Crude Disease Rate
CDZ	Central Department of Zoology
DALYs	Disability Adjusted Life Years
DEC	Diethylcarbamazine
df	degree of freedom
DoHS	Department of Health Services
EDCD	Epidemiologial Disease Control Division
ER	Endemicity Rate
GIS	Geographical Information System
GPELF	Global Program to Eliminate Lymphatic Filariasis
HMIS	Health Management Information System
ICT	Immunochromatographic Card Test
IVT	Ivermectin
KAP	Knowledge, Attitude and Practice
LF	Lymphatic Filariasis
MDA	Mass Drug Administration
MF	Microfilariae
MoH	Ministry of Health
NG	Nepal Government
NTF-ELF	National Task Force for the Elimination of Lymphatic Filariasis
PCR	Polymerase Chain Reaction
PELF	Program to Eliminate Lymphatic Filariasis
PM	Post Meridiem
PARASED	Parasitological Research and Socio-Enviromental Development
SLC	School Leaving Certificate
STH	Soil Transmitted Helminthiasis
TB	Tuberculosis
TDR	Tropical Disease Research
TPE	Tropical Pulmonary Eosinophilia
TU	Tribhuvan University
VDC	Village Development Committee
WHO	World Health Organization

ABSTRACT

Lymphatic Filariasis (LF) is one of the world's disabling, disfiguring and most prevalent tropical diseases of human caused by the filarial nematode parasite of the family filaridae: *Wuchereria bancrofti, Brugial malayi* and *B. timori.* LF has been infected over 120 millions people in some 80 countries, leading more than 40 millions in disfigured state and over 2 billions at risk globally and is transmitted by blood sucking female mosquitoes. LF, in Nepal is one of the most neglected and hidden public health and socio-economic problem. The present study was proposed to determine the microfilarial species and the prevalence of the microfilarial infection in Ganeshsthan VDC, Nuwakot, Nepal. The study was carried out by the night blood collection, microscopical examination, questionnaire analysis, photomicrography and comparative morphological study of microfilariae (MF) taking a total duration of 7 months (April, 2006-October, 2006). All the MF observed were found to be *W. bancrofti.* Out of 502 total blood samples collected, 66 (13.14%) were found to be MF positive for LF and male (14.71%) were found to be more infected than female (11.39%) with the positivity ratio of 1.44:1. The highest prevalence of MF was found in the age group 61-70 years (21.05%) and least in the 2-10 years (4.16%).The highest percentage of knowledge on LF was found in the group of higher studies (81.48%) but the MF prevalence was most in the in the literate group (27.27%) and least in the primary group (7.78%). As per occupation teachers and labors were found to be most infected (28.57%). The people using no any precaution measures were found to be more infected than their counter parts of which people using mosquito mats were found least infected (5.55%). As per environmental status, the highest MF positive cases were recorded from the inhabitants around the stagnant water (30.0%). The average density of MF per smear (20µl) of blood was found to be 10.16. Hydrocele among the male (10.18%) and breast swelling among the female (8.43%) were the most prevalent clinical manifestations recorded. The total Crude Disease Rate (CDR) was recorded 23.10% and was found more in female (26.16%) than in male (20.37%). Similarly, total Endemicity Rate (ER) of LF was found 36.25% which was greater in female (37.55%) than in male (35.09%) and highest ER was recorded from the age group 61-70 years. The survey analysis showed that the LF is ignored to many people and need to be familiarized to improve health and hygiene. Prevention from mosquito bites; regular health checkup, treatment and control of vectors side by side throughout the elimination program were found to be indispensable.

I

INTRODUCTION

Lymphatic Filariasis (LF) is one of the world's major disabling, disfiguring and most prevalent disease of human caused by the filarial nematode parasite of the family filaridae; *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*. LF is the global problem and well established as a communicable endemic disease in tropics and sub-tropics. LF is transmitted from one person to another by the bite of female mosquitoes via the mode of inoculation. LF is a disease of poor environmental condition with low socio-economic status, low literacy rate and high percentage of illiterate health education status (WHO, 1997). High temperature and humidity as well as the stagnant water are the paradise condition of the vector multiplication and disease transmission. The rapid and unplanned growth of cities creates numerous breeding sites for mosquitoes, the vector. LF is a significant cause of acute and chronic illness in both sexes leading a disease burden in the poorer community of the society. LF prevents afflicted individuals from experiencing a normal working and social life, furthering the cycle of poverty (TDR, 2005). LF has been identified as the second leading cause of permanent and long term disability (WHO, 1995). The true amount of disability it caused is only the beginning to be quantified accurately (Ramu *et al.*, 1996). As LF is more prevalent in rural areas and slums of cities and as it affects predominantly the young and active working section of population resulting significant decreased productivity of the poorer sectors of the community-those who can least afford it.

In human filarial infection the adult worms are present in the lymphatic vessels, lymph nodes, connective tissues, and body cavities whereas the larval forms, the microfilariae (MF) are present in the peripheral blood and occasionally in chylous urine and hydrocele fluid. There are 9 species of filarial nematodes that commonly infect human beings and produce a pathological condition, the filariasis viz. *W. bancrofti*, *B. malayi*, *B. timori*, *B. pahangi*, *Onchocerca volvulus*, *Loa loa*, *Mansonella perstans*, *M. ozzardi* and *Dipetalonema perstans* (Smyth, 1996). Among them only the first 3 are responsible for the LF. *W. bancrofti*, the causative agent of Bancroft's filariasis is largely confined to the tropics and subtropics, occurs throughout a broad equatorial belt in the Nile Delta, Central Africa, Turkey, India, South-east Asia, the Philippines, many Pacific islands, Indonesia, Australia, the

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Caribbean islands and parts of South America. *B. malayi*, the causative agent of Malayan filariasis occurs extending from India to China, Japan, Formosa, Malaysia and Indonesia (Cheng, 2006). *B. timori* is chiefly reported from Indonesia.

The MF of *W. bancrofti* performs the nocturnal periodicity, since they are most plentiful in the peripheral circulation from 10:00 PM to 2:00 AM, whereas in the day time these are concentrated in the lungs (Smyth, 1994). Exceptionally in the Polynesian Islands and the Philippines there exists a variety, sometimes known as *W. bancrofti* Pacific, with a different periodicity pattern. The MF is ingested by a mosquito during a blood meal and the mosquito phase of the life cycle commences (Annex-IV). *W. bancrofti* can utilize *Culex spp., Aedes spp., Mansonia spp., Anopheles spp.*, and *Psorophora spp.* as vectors equally well. The 3rd stage filiform larvae (L_3) developed in the mosquito's gut is infective to human and entered into the human blood stream during the next blood meal of the mosquito vector via the punctured wound (Cheng, 2006).

The clinical features manifested by LF are not the initial effects but the result of long-standing infection. The chronic signs of LF do not usually develop before the age of 15 years. However, immigrants from areas where filariasis is not endemic tend to develop elephantiasis more often and much sooner than do the indigenous population of endemic areas. Pathogenesis in filariasis is heavily influenced by the immune responses to adult worms, the degree of inflammation and the stage in the course of illness. Infected patients are the usual reservoir. The incubation period varies from 5 to 10 months. The adult worms lodged in the lymphatic ducts, continuously increase in the number, coupled with the aggregation of connective tissue may result in complete blockage of the lymph flow; excess fluid behind the blockage then seeps through the walls of the lymph ducts into the surrounding tissues, causing edema. In time, the build up of scar tissue and fluid leads to enlargement of the limbs, breasts or scrotum, a condition known as 'elephantiasis' (Cheng, 2006). LF is characterized by massive swollen of limbs, thickened skin, enlargement of genitalia, grotesque enlargement of scrotum i.e. hydrocele (in male), swellen clitoris and breast (in the female), lymphodema of genitilia, Adeno-Lymphangitis (ADL), lymphadenopathy, chyluria, chylocele, lymphatic varix, chylothorax, chylous ascites and chylous diarrhoea. Acute infection causes fever, inflammation of the lymphatic system and

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bronchial asthmatic condition known as 'Tropical Pulmonary Eosinophlia' (WHO, 1995) which results in chronic pulmonary fibrosis. Tropical Pulmonary Eosinophilia (TPE) is likely to be due to MF trapped in the pulmonary capillaries and destroyed by allergic inflammation. Patients present with paroxymal cough, wheeze and fever (Boon *et al.*, 2006). The sequential stages occur in LF are: *Dilation of lymphatic vessels*, *Lymphangitis* and *Obstruction of the lymph nodes* (Parija, 1996).

The clinical phases of LF can be divided into *the incubation phase* (symptom less, but lymphatic inflammation accompanied by mild fever and malaise), *the acute phase* (intense lymphatic inflammation accompanied by chills, fever, and toxemia) and *the obstructive phase* (chyluria and elephantiasis) (Cheng, 2006). Four factors are central to the pathogenesis of LF: *the living adult worm* (cause lymphangiectasia, lymphatic dysfunction, lymphoedema and hydrocele), *the inflammatory response due to dead worms* (cause acute filarial lymphangitis), *the Microfilariae* (cause TPE) and secondary infections (cause tissue destruction) (Boon *et al.*, 2007). The pathogenic lesions in classical filariasis caused by *W. bancrofti* are: Inflammation, Dilation of lymphatics, Rupture of lymphangiovarix, Hyperplasia of skin and connective tissues and Secondary bacterial infection (Chatterjee, 2005). There is positive correlation between incidence of filarial disease and MF rate in a community but a negative correlation between elephantiasis and blood MF in an individual (Chandller and Read, 1961). In LF an attack of lymphadenitis is precipitated by hard physical works. Typically, each attack of fever and lymphadenitis lasts for several days and usually subsides spontaneously following best rest. Lymphoedema is quite often present in the legs (ankles, dorsum of the foot, calf and thigh) during the fulminate episodes. After 1 to 3 years, lymphoedema develops into 'elephantiasis' (Agrawal, *et. al.*, 2005).

Diethylcarbamazine (DEC) is being used from more than 40 years for the treatment of LF as a chemotherapic means. Repeated treatment with DEC results reduction in the incidence of acute and chronic attacks and the risk of developing chronic disease. Control with DEC involves either mass treatment of all the population of endemic community or selective treatments who are diagnosed as MF positive. Now-a-days treatment with single dose of DEC is of great practical interest. Excellent results have been achieved in some area of China with DEC medicated salt was used in the community during a period of six months or more.

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Global Situation of LF

LF is major global and socio-economical burden in tropics and sub-tropics and people living in those regions have long suffered under its yoke. Approximately one-third of people with this infection live in India, Africa and South-East Asian countries. LF is endemic in some 80 countries and more than 2 billions people are at risk globally (Erlanger *et al.*, 2005). It is found that 120 millions of people in the world are most infected by *W. bancrofti* (90.0%) followed by *B. malayi* (10.0%) and *B. timori* limited to Asia and some parts of Pacific (WHO, 1997). It is found that more than 15 millions World's people are suffered from elephantiasis/lymphoedema, 27 millions from hydrocele, 83 millions from lymphatic functional disability and 30 millions from the renal pathology (WHO, 1997). In South-East Asian region, about 600 millions people live in the endemic areas constituting 60.0% of the LF global burden where *Culex* is the key vector and Africa constituting 37.0% of the global burden where *Anopheles* is the key vector (Zagaria *et al.*, 2002). Approximately 45 millions of infected individuals live in the Indian Sub-continent. The global burden of LF is estimated at 5.78 millions Disability Adjusted Life Years (DALYs) lost annually (WHO, 2004). LF is ranked third among the TDR diseases in terms of DALYs after malaria and tuberculosis (TB). India and Africa together account for 85.0-90.0% of the estimated burden of diseases in terms of DALYs. LF is a major impediment to socio-economic development and is responsible for immense psychosocial suffering among the affected people (TDR, 2002). South-East Asia, Africa and South America are found to be the most endemic continents of LF. The problem of the LF is found to be graved in India, Indonesia and China; these 3 together account for more than two-third of the total infected population and India alone account for around 50.0% of the global cases. In India most of the cases are due to bancroftian filariasis (Ghai and Gupta, 1999).

LF is declared as one of the six eliminable infectious diseases (WHO, 1993). Global Program to Eliminate Lymphatic Filariasis (GPELF) was initiated by the year 1998 (Moleneux *et al.*, 2003). GPELF's goal is to eliminate LF as a public health problem by 2020. It mainly relies Mass Drug Administration (MDA) using Ivermectin (IVT)

or DEC+Albendazole (ALB) (Erlanger *et al.*, 2005). International programs were active in 38 of the 80 countries where LF is endemic reaching almost 90 millions people in the year 2002. The expansion required to meet the targets of reaching 350 millions people by 2005 and all 1.1 billions people at risk globally by the year 2020 is now the major challenge of what has become the global program to eliminate LF, coordinated by WHO and supported by a global alliance of partners (Ottesen, 2002). By the end of 2001, a total of 25,479,136 people around the world had received MDA in 22 countries participating in the Program to Eliminate Lymphatic Filariasis (PELF). This is marked increase compared to the year 2000 when only 20 countries participated and 3 millions people at risk were covered (WHO, 1999). Globally it is focused for the development and implementation of simple, cost effective and sustainable strategies for morbidity control (Ramachandran, 1993).

Current Situation of LF in South-East Asian Countries

South-East Asian Countries are said to be one of the most prevalent endemic areas of the LF, where 60.0% global burden is persisted. As per Annual Report of Department of Communicable Disease Control the current situation of LF in the South-East Asian Countries (last update 27, April, 2006) can be described as follows:

Bangladesh

W. bancrofti is the only filarial species prevalent in the country and about 70 millions of people are at risk of infection. In the country 32 out of 64 the districts have been found to be endemic of LF. –Bangladesh commented MDA by which 9 districts were covered with 18.6 millions people by annual DEC+ALB and now, the country is in the process of scaling up the program to cover the entire population. The MDA in 2005 was proposed to target 20.4 millions people. Community based LF disability alleviation services are expanded in all endemic districts.

India

Filariasis is a major public health problem next only to malaria and about 465 millions people are at risk. India contributes about 67.0% of endemic population and 81.0% of the disease burden. *W. bancrofti* is the most predominant infection comprises 99.4%.

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India has completed 87.8% of LF mapping. India commenced pilot testing of the two drugs strategy (DEC+ALB) in 11 districts in 2001 and has been expected to cover seven districts, targeting 21 millions people by 2005. Home-based morbidity management and Hydrocelectomies are performed in all the endemic districts.

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Indonesia

All the species viz. *W. bancrofti*, *B. malayi* and *B. timori* are prevalent in the country and 22 vectors have been incriminated. Twenty two millions people are estimated to be at the risk of LF. Eighty five out of 444 districts were mapped and all of them found to be endemic. MDA has been gradually scaled up in endemic district. Over 82.0% coverage was achieved by 2004. Community home-based disability alleviation in all endemic districts implementing MDA and Hydrocelectomies has been conducted in all the endemic districts.

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Maldives

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W. bancrofti infection has been prevalent for several decades in the country. In Maldives only one island, L. Fonadhoo found to endemic of LF. MDA was commenced in L. Fonadhoo in 2004 and 99.0% of the population was covered.

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Myanmar

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W. bancrofti is the only species prevalent in the country leading the 40 millions of people at risk. Out of 45 districts mapped so far, 39 were found to be endemic. MDA with DEC+ALB was commenced by 2004 and 17 millions people were covered under the annual MDA campaign. Hydrocele surgery and home based self help management of lymphoedema have been initiated.

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Sri Lanka

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W. bancrofti is the only LF infection being transmitted. The distribution of LF in the country and has been identified as endemic in 8 of the 25 districts with the risk of about 10 millions people. It commenced annual MDA using DEC+ALB by 2001 and the entire endemic population in the country has been targeted for MDA since 2002.

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Sri Lanka had also commenced a district-wide community home based prevention of disability and Hydrocelectomies.

Thailand

W. bancrofti and *B. malayi* are prevalent in the country borders and approximately 1,75,000 people have been found to be at risk. Thailand completed the country-mapping of LF. Thailand commenced MDA in 2003, with DEC+ALB, covering the entire endemic population. The elimination activities also included comprehensive coverage of prevention and alleviation of disability of lymphoedema cases.

Timor-Leste

Timor-Leste, the newest country is endemic with all the three species viz. *W. bancrofti*, *B. malayi* and *B. timori* of which *B. timori* is considered to be the most prevalent species accounting for about 95.0%. All districts have been categorized as endemic for LF. Timor-Leste commenced an integrated LF elimination, Soil-Transmitted Helminthiasis (STH) and yaws eradication program in 4 districts by 2005 and MDA with DEC+ALB was run to interrupt the transmission of LF.

Situation of LF in Nepal

LF has been known to be endemic in different areas of Nepal since a long as a major public health problem. In Nepal the distribution of LF is varied according topographic area ranging the altitude from 70m (in terai) to 1400m (in hills) and is more prevalent in the terai areas than in the hills. The distribution and magnitude of the disease is gradually increasing in rural as well (DoHS, MoH, NG, Nepal, 2004). As per recent mapping 60 out of the 75 districts of the country has been found to be endemic of LF and estimated to be the 24 millions of people at risk (DoHS, 2006). Nepal has a target to eliminate LF by the year 2018 AD. The policies to be carried out are interruption of transmission and morbidity management (DoHS, 2006).

MDA was carried out by co-administration of DEC+ALB commented in 2003 in 1 district (Parsa). This was scaled up in 2004 to cover 2 additional districts, covering a

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total of 1.5 millions people. Five more districts were proposed in 2005 covering a population of 3 millions. The achievement of MDA in the country by the year 2007 was found to be covered 11 millions of population (DoHS, 2006) and followings are the rounds of MDA conducted in the different districts of Nepal: 5th Round-1 district (Parsa), 3rd Round-4 districts (Makwanpur, Chitwan, Nawalparasi and Rupandehi) and 1st Round-15 districts (Nuwakot, Dhading, Gorkha, Tanahun, Shyangja, Palpa, Kapilbastu, Kavrepalanchock, Ramechhap, Sindhuli, Dhanusa, Mahottari, Sarlahi Rautahat and Bara). Home-based long term disability management has been initiated in Nepal. The disease is widespread throughout the country except mountainous and high hilly areas. Sherchand *et al.* (2003) first studied the mapping of LF in Nepal by Immunochromatographic Card Test (ICT) and Geographical Information System (GIS) methods who found that 33 districts (89.0%) were endemic out of 37 districts studied and average 13.0% population was found to be infected. LF impedes socio-economic development in many endemic areas of Nepal. It is a disease of poverty affecting the poorest of the poor. *W. bancrofti* is only the causative agent reported from Nepal (Thakur, 2000) and *C. quinquefasciatus* is only the mosquito vector. The situation analysis of national and regional cases of the LF can be presented as:

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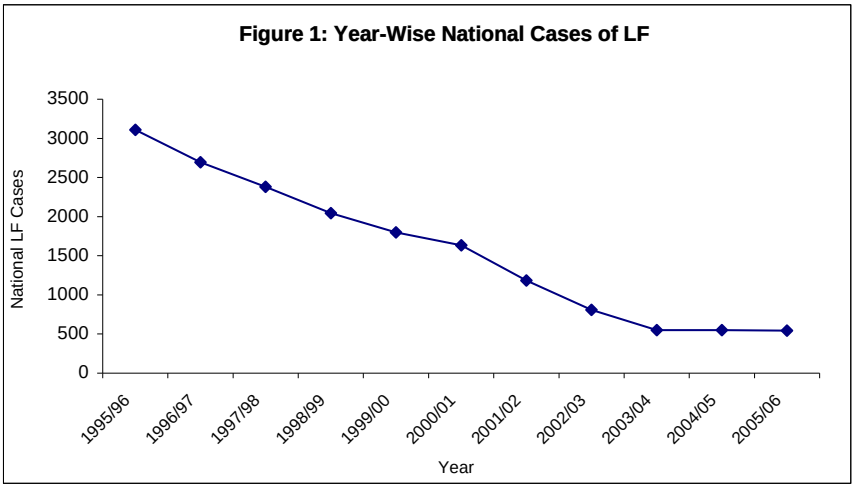
Table 1: National and Region-wise Filarial Cases in Nepal (1995/96-2005/06)

Year	National Cases	Eastern Region	Central Region	Western Region	Mid-Western Region	Far-Western Region
1995/96	3,110	493	849	789	662	317
1996/97	2,694	257	981	736	302	418
1997/98	2,381	328	605	976	317	155
1998/99	2,044	165	671	913	281	14
1999/00	1,797	209	718	632	195	43
2000/01	1,632	262	546	692	123	9
2001/02	1,183	142	173	733	79	56
2002/03	809	63	302	334	64	46
2003/04	550	47	246	221	20	16
2004/05	549	25	274	180	50	20
2005/06	542	82	193	175	89	3
Total	17,291	2,073	5,558	6,381	2,182	1,097

Source: DoHS (1995/96-2005/06), *Annual Report*. Ministry of Health and Population, Government of Nepal.



The national cases of LF from 1995/96 to 2005/06 are found to be decreased progressively in Nepal (Figure: 1).

With WHO's global strategy to eliminate lymphatic filariasis as a public health problem and the government's political commitment, the Epidemiology and Disease Control Division (EDCD), Department of Health Services (DoHS), Ministry of Health (MoH), has formulated a National Plan of Action (2003-2018 AD) for elimination of LF in Nepal. Government of Nepal, Ministry of Health and Population has also established a National task Force for the Elimination of Lymphatic Filariasis (NTF-ELF) headed by Directional General, DoH S. The present study has been done under the project work of Parasitological Research and Socio-Environmental Development (PARASED), Nepal a joint collaboration of WHO and EDCD and is said to be a part of LF elimination action project. The study site was selected as recommended by the district hospital, Trishuli, Nuwakot. Though, present study is like only a drop upon a large sea as compared to the national and global LF elimination program, but it is beneficial in mapping the LF prevalence so that MDA program can be implemented.

II

OBJECTIVES

General Objective

To determine the prevalence of microfilarial infection in Ganeshthan VDC, Nuwakot, Nepal by means of epidemiological survey and to provide the data essential to the planning, implementation and evaluation of services for the prevention, control and treatment of LF.

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Specific objectives

- To identify the microfilariae (MF) species.
- To determine the prevalence of microfilaria (MF) in relation to sex, age, educational status and occupation.
- To analyze the level of Knowledge, Attitude and Practice (KAP) of people about LF and prevalence of MF as per knowledge of LF and preventive measures.
- To calculate the prevalence of MF as per environmental status.
- To measure the density of MF per smear (20µl) of blood.
- To determine the sex-wise clinical manifestation and Crude Disease Rate (CDR).
- To determine the Endemicity Rate (ER) of LF in relation to sex and age.

III
LITERATURE REVIEW

Researches on LF in Global Context

The symptoms of bancroftian filariasis had been mentioned as 'elephantiasis arabicum' in the ancient Hindu literature (Susutra, 600BC)**.** Demarquay (1863) first demonstrated the MF in hydrocele fluid. Subsequently, Wucherer (1866) in Brazil described the MF in chylous urine. Lewis (1872) in India demonstrated the MF in the peripheral blood. Bancroft (1876-77) first demonstrated the adult female parasites and Sibthorpe (1888) first found adult males. Manson (1878) first demonstrated the *Culex* mosquitoes as the intermediate hosts and also described the nocturnal periodicity of MF in the peripheral blood (Parija, 1996). Cobbold (1877) named one of the MF that responsible for LF as *Filaria bancrofti* and later (1921) it was included in the genus *Wuchereria* (Arora and Arora, 2001).

Torres *et al.,* (2001) studied a microfilaremic situation in the province of small village Sorsogon, the Philippines by using Polymerase Chain Reaction (PCR) as the diagnostic tool. Out of 54 night blood samples 4(7.4%) were found to be microfilaremic as determined by combined direct blood film examination and membrane filtration followed by blood film examination. But use of the tool S_{sp}I PCR to detect *W. bancrofti* DNA sequences in human blood doubled the microfilaremic individuals to 8(14.81%).

Breg *et al.,* (2001) reported for the first time the confirmed cases of TPE in Pakistan in the indigenous patients as a result of infection with *W. bancrofti*. Following the clinical examinations total leukocytes and eosinophil counts were recorded. Parasitological examinations included blood for MF and stool and urine for eggs of intestinal parasites. Total immunoglobulin IgE and specific antifilarial IgG were measured. Suspected cases of TPE were treated with DEC, 6mg/kg for 4 weeks and were followed up to 2 and 4 weeks after treatment. 4 persons fulfilled the criteria for TPE and response to treatment was marked with clinical improvement, reduction in eosinophil count and reduced titers of specific antifilarial antibodies. 2 persons had *W. bancrofti* antigen in their sera confirmed by filariasis antigen detection test.

Esterve *et al.,* (2001) studied the impact of semi annual mass DEC chemotherapy combined with vector control program on the remote island of Maupit (French Polynesia) since 1955. The results of two surveys in 1985 and 1989 were reported to be 0.0% microfilaraemia. But with the combined parasitological criteria, immunological and molecular techniques and found only good control of the parasite. It was found that residual microfilaraemia in 0.4%, antigenaemia in 4.6% and specific IgG in 21.6% of the samples. In addition an infection rate of 1.4% was calculated in the *Aedes polynesiensis* vector population.

Babu *et al.,* (2001) determined the prevalence of LF by a cross section survey in Khurda district of Orissa, India. The total disease attributable to filariasis was significantly higher in males (14.79%) than females (10.04%). However 'elephantiasis' was more prevalent in females and 'adenolymphangitis' was more prevalent in males than their counterparts. The prevalence of various forms of disease were age dependent in both sexes and found that about one-seventh of men and women of higher age-groups suffered from chronic debilitation forms of the diseases.

Tayler *et al.,* (2001) reported that the lymphatic filarial nematodes were infected with the endosybiotic bacteria, *Wolbachia* and found that the lipopolysaccharide from these bacteria was the major activator of the innate inflammatory responses induced directly by the parasite. A mechanism was proposed by which *Wolbachia* initiated acute inflammatory responses associated with the death of the parasites, leading to acute filarial lymphangitis and adverse reactions to antifilarial chemotherapy.

Weerasooriya *et al.,* (2001) reported 4.1% of prevalence of micofilariaemia in 3 suburban areas of Matara, Sri Lanka. Prevalence was significantly lower in females than in males and in males aged <20 years than in older males. Overall, 9.5% of subjects had the clinical manifestations (6.4% had filarial fever, 3.0% had elephantiasis and 6.2% had hydrocele). The prevalence of elephantiasis was generally higher among females (4.2%) than among males (1.4%). This manifestation showed a linear increase in prevalence after the age of 40 years.

Witt *et al.,* (2001) reported that LF is acquired in childhood often with as many as one third of children were infected before 5 years of age. Initial damage to lymphatic system by the parasites generally remains sub clinical for many years or given risk

only to non-specific presentation of lymphodenopathy however, especially after puberty the characteristics clinical features of adult disease syndromes (lymphoedema, hydrocele) manifest themselves.

Chadee *et al.,* (2002) published a protocol for the collection of resting blood-engorged *C. quinquefasciatus* and their examination for MF, a way developed for detecting whether LF occurs in a particular locality. The protocol was first implemented in a pilot survey in Trinidad, West Indies.

Moleneux *et al.,* (2002) reviewed the Global Program to Eliminate Lymphatic Filariasis (GPELF) was an innovative, public-private partnership for health improvement that was initiated and progressed since 1998. The program was largely based on the regular mass administration of ALB with either IVT or DEC. The program has been expanded rapidly with the annual number of people treated rising from 2.9 millions (in 12 countries) in the year 2000 to 25.89 millions (in 22 countries) in 2001 and an estimated 80 millions (in 34 countries) in 2002.

Chandrasena *et al.,* (2002) estimated the sensitivity, specificity and cost effectiveness of an ICT card test for the diagnosis of LF against two standard parasitological techniques: Thick Blood Film (TBF) and Nucleopore Membrane Filtration (NMF). For which blood was collected from the individuals of endemic areas in the western part of Sri-Lanka, which shows the ICT to be more effective than TBF or NMF in diagnosing infection in LF.

Singh *et al.,* (2002) studied the filaria endemicity in Bagdora town, Darjeeling (West Bengal, India). Of 1,511 night blood smears examined, 35(2.31%) and were found to be positive for *W. bancrofti.* The MF rate of the males and females were 2.84% and 1.79% respectively. The age of these positive ranged from 5-45 years. The MF rate was highest (4.46%) in the age group of 22-29 years. *W. bancrofti* was only the infection encountered. Mean MF density was 7.71/20mm^3 of blood, whereas median MF density (MFD_{50}) was 21/mm^3 of blood. Disease and filarial endemicity rate were 0.33% and 2.65% respectively.

Ramzy *et al.,* (2002) evaluated the effect of a single dose of DEC on *W. bancrofti* infection in a low endemicity setting in Egypt where MF was 3.7 % and median MF was 34/ml. The subjects with MF or filarial antigenaemia were treated and restudied 1

year later .Treatment with DEC dramatically reduced blood MF counts with clearance in 69.0% of subjects. Mass treatment was administered in one village; 27 month later where MF prevalence had decreased 84.0% (4.9% to 0.8%).

Weerasooriya *et al.,* (2003) used ICT card test and ELISA to detect the Circulating Filarial Antigen (CFA) and filarial-specific urinary IgG4 respectively to screen 473 subjects from a community in Sri-Lanka where *W. bancrofti* is endemic. The ICT test was used as gold standard and ELISA was found to have a sensitivity of 91.2%. However, far more of the subjects were found ELISA-positive than ICT- positive (76.5% Vs 31.1%). The youngest children studied (aged 1-10 years) were similar to the adult subjects in terms of the prevalence of antigenaemia (33.8%) and the prevalence (72.1%) and concentration of filaria-specific IgG4 in their urine. The ELISA was found to be useful to screen the very young and school-age children taking the urine as sample instead of blood to estimate the current levels of transmission in a particular area.

Chai *et al.,* (2003) discovered the current situation of LF due to *B. malayi* in the southern island of Jeonaranum-do, including the Heugsan Island, S. Korea taking a small scale of survey. A total of 378 people, 151 male and 227 female living in 8 villages were subjected to a night blood survey for microfilaremia and physical examination for elephantiasis on the extremities. These were 6(1.6%) MF positive cases, all in females aged 57-72 years and from only two villages of Dacheugsan-do area. There were 4 patients reported with lower leg elephantiasis but they showed no microfilaraemia.

Engelbercht *et al.,* (2003) studied LF infection as part of a multi parasite survey in the Savanah of the Northern Nigeria. They analyzed serum samples from 341 individuals aged 5-70 years detecting CFA of *W. bancrofti* by the ICT card test. The prevalence of infections was found to be 10.0% and clearly age dependent, increasing from below 2.0% in children to over 20.0% in subjects older than 40 years. Measuring IgG4 antibodies against the recombinant *W. bancrofti* antigen SXP_1 showed that 36.0% of all tested individuals had been at least exposed to the parasite. Individuals with *O. volvulus* infections were found to be more often infected with *W. bancrofti* than expected statistically.

Gyapong *et al.,* (2003) investigated the geographical distribution of human infections with *W. bancrofti* in the 4 West African countries (Benin, Burkina Faso, Ghana and Togo) using ICT card test. 401 study communities were selected at random and the results subjected in Burkina Faso and Ghana revealed that prevalence in the adult population of the some communities exceeded 70.0% and that over a large areas of Burkina Faso community prevalence were between 30.0% and 50.0%. Most of Togo, Southern Benin and much of the Southern Ghana appeared completely free of the infection. Although, there were foci on the Ghanaian coast with prevalence of 10-30.0%, such high prevalence did not extend into the coastal Togo or coastal Benin.

Chadee *et al.,* (2003) studied on filariasis infections in Georgetown, South America. They conducted 1 year survey of febrile patients attending filariasis (night) clinic. Out of 769 thick blood smears collected of which 103(13.39%) were found to be positive for MF of *W. bancrofti*.

Kazura *et al.,* (2003) reported that the Bancroftian filariasis was the major public health problem in Papua, New Guinea where the level of transmission by the mosquito vector, human infection rate and clinical morbidity were found to be the highest in the world.

Tobian *et al.,* (2003) studied on risk factors for hydrocele as a consequence of *W. bancnfti* infection. 342 men more than 15 years of age in an endemic area in Papua New Guinea were evaluated. The observations suggested that filarial pathology of male genitalia was under reported when evaluated by physical examination alone and duration and intensity of infection were found to be the risk factors of hydrocele.

Yahathugoda *et al.,* (2003) conducted a study at a coastal community in Unawatuna (population sample 381) and an inland community in Baddegamma (population sample 236) in the Galle district (Sri Lanka) to know the level of knowledge on LF and response to mass treatment campaign. They were interviewed twice, 4 weeks before the MDA and 4 to 7 days after. The sample population of Unwatuna had a greater awareness of the clinical and parasitological features of the disease ($p=0.0003$) and drug treatment ($p=0.00380$) than that of Buddegamma. Only 5.5% of the combined sample attributed the cause of filariasis to a parasitic worm. However, over 70.0% of them knew that transmission was through the mosquito bites.

Anosike *et al.,* (2003) studied the infection rates, intensity and clinical manifestations of human filarial infection in Das area of Bauchi state, Nigeria. 1,628 persons were physically examined from a total population of 53,213. 215 (20.3%) of 1,059 males and 99(19.1%) of 569 females examined had filarial infections. Overall, 314(19.2%) persons were infected. MF of *O. volvulus, W. bancrofti, M. streptocerca, L. loa* and *M. perstans* were encountered. Altogether human filariasis varied significantly among communities, sex, ages and various occupational categories ($P<0.05$). While infection with *O. volvulus* increased with increasing host age. *L. loa* infections decreased with increase in the host age but there was no clear trend in age related prevalence of mansonelliasis due to *M. streptocerca*. Females of reproductive age had low *W. bancrofti* microfilaraemia.

Krishnamoorthy *et al.,* (2004) investigated a cohort of 2,187 laboratory reared *C. quinquefasciatus* fed on 69 human volunteers including 59 person with different levels of *W. bancrofti* MF and 10 without MF. Mosquito survival was analyzed in relation to the level of MF in the human and larval count in the dead mosquito vector mortality during the extrinsic incubation period (12 days post engorgement) was significantly higher in mosquitoes fed on microfilaraemic volunteers (50.0%) than in those fed on amicrofilaraemic (29.0%). Both the percentage infected and the geometric mean parasite density was significantly higher among mosquitoes which died before 13 days (45.0%) than these surviving beyond 13 days (39.0%). A large proportion of mosquitoes (62.0%) that died during the early phase of parasitic development were found to be 36.0% in low, 26.0% in medium and 90.0% in high human MF density.

Khan *et al.,* (2004) conducted a survey for LF among tea garden workers of Central Assam, India. Of the 656 night blood samples examined, 31(4.72%) were found to be positive for MF of W. *bancrotti.* Infection rate was higher in male (7.3%) than females (2.1%). *C. quinquefasciatus* was incriminated as vector mosquito.

Koyadun *et al.,* (2004) assessed a long term microfilaricidal effect of two year biannual mass treatment with a 300mg oral dose FILADEC tablet. The formulation of 6 mg/kg DEC, on clearance of the *W. bancrofti* adult worm CFA in Myanmar migrants at risk of emergence of imported bancroftian filariasis in South Thailand by using qualitative ICT and quantitative $Og4C_3$ ELISA. Out of 34 antigenemic

Myanmar index cases, 13 index cases were followed up, treated and monitored at DEC post treatment of 6, 12 and 18 months. At the 18 months post treatment residual antigenemias in 4 of 5 index cases (group I) with high antigen titres (99.7-181.6×10^3 AU/ml) were 54.44%, 33.58%, 27.43% and 9.97% significant decreases of the CFA level in only 3 out of 5 index cases were affected by the response to DEC treatments ($P< 0.007$). The treatments affects on clearance of the CFA in 8 index cases (group II) with low antigen titres (15.4-37.21×0^3 AU/ml) were shown for at least 6 months post DEC treatment and hence had 100.0% efficacy in the first 6 months of the first year of year round treatment.

Ramirez *et al.,* (2004) studied the complete census data collection in the rural villages of island Mindoro, Philippines for the mass treatment using DEC and ALB. A sample of individuals selected from each of two adjacent villages was examined for microfilaremia. MF were detected from thin smears in 34(12.5%) of 272 patients examined from the village of Bayanan and 10(3.4%) of 292 in the village of Mangangan ($P<0.01$). 33(97%) of 34 in Bayanan and 7(70%) of 10 in Mangangan. In children examined who were less than 10 years of age (n=165), girls were more commonly infected than the boys even though; the proportion of males in the general population was greater.

Boyd *et al.,* (2004) conducted a school-based assessment of the geographic distribution of *W bancrofti* infection in Leogane commune, Haiti using the ICT card test. In multivariate analyses performed using generalized linear mixed models, children attending schools in the foot hill and plains were 3.95 and 23.56 times as likely to be infected respectively as children attending mountain schools. Infection prevalence decreased with increasing altitude.

Murty *et al.,* (2004) carried out a night blood survey for parasitological evidence of Bancroftian filariasis in 45 rural areas belonging to National Filarial Control Program (NFCP) zones of East Godavari and West Godavari district of Andhra Pradesh, India during the period of 1998 to 2001. It was found that MF prevalence range was in between 2.9% - 10.2% and MF intensity in 20 mm^3 blood samples ranged from 1-281. They explained the trend of MF dynamics in the rural populations, where mass drug delivery has been implemented since 1997 and anti-larvicidal and adulticidal control measures have not been adopted.

Boggild *et al.,* (2004) presented 17 individuals with TPE during 1990-2003 at the Toronto General Hospital,USA. The study revealed that all cases of the TPE were of South Asian ancestry. All 17 received an incorrect diagnosis at presentation the most frequent of which was asthma (76.0%). Eosinophil count, serum immunoglobulin E levels, and anti-filarial antibody titers were elevated in all patients. 10 of 14 patients had an abnormal chest radiograph findings and 11 of 12 patients had abnormal results of pulmonary function test.

Mukoko *et al.,* (2004) investigated the transmission of *W. bancrofti* by taking the subjects from 12 villages in the Kwale district coastal province of Kenya, South of Mombasa. Night blood samples were 6,531 that taken from all the villagers aged ≥1 year and over all 16.0% of the villagers were found to be microfilaraemic, although the prevalence of MF in each village varied from 8.1%-27.4%. The geometric mean intensity of infection among the microfilaraemic was 322 MF/ml of blood. Clinical examination of 2,481 adults revealed that 2.9% had elephantiasis of the leg and that 19.9% of the adult men had hydrocele.

Leang *et al.,* (2004) assessed the filariasis disease burden in 4 North-Eastern provinces of Cambodia by using and validating a key-informant questionnaire. Validation surveys included clinical examination, a card test for *W. bancrofiti* and night blood finger prick examination of patients reported with clinical elephantiasis. A total of 220 patients were reported mostly from Stung treng (36.8%) and Rattanakiri provinces (35.0%). Key informants reported patients with LF with a sensitivity of 85.7% for leg and 97.0% for scrotum morbidity and with a specificity of 95.6%. However, substantial over reporting resulted in very low positive predictive values for elephantiasis of 19.4% for legs and of 23.7% for scrotum. As 97.4% of patients with clinical LF were older than 40 years. About 0.7% of 3,490 *W. bancrofti* card tests were positive, the prevalence was 1.94% in Rattnakiri, 0.38% in Stung treng and 0.22% in Preahvihear.

De Rochars *et al.,* (2004) reported the geographical distribution of LF in Haiti by collecting blood from the 50-250 schools children (6-11 years) in all 133 commences of country using an adaptation of lot quality assurance sampling method. Of 22,365 children tested, 901 (4.0%) were positive for CFA of *W. bancrofti* where as the overall national antigen prevalence in that age group was 7.3%. Infected children

were found in 147(87.9%) communes, the most heavily affected areas being concentrated in the northern part of the country. In only 16(12.1%) communes were all 250 children antigen negative. It was found that *W. bancrofti* infection in Haiti was much more widespread than previously realized and virtually the entire population of the country considered being at risk of infection.

Fox *et al.,* (2005) assessed the clinical findings associated with detection of adult *W. bancrofti* by ultrasound. 186 school children in a filariasis endemic area of Haiti under taken physical and ultrasonographic examinations. The Filarial Dance Sign (FDS) of adult *W. bancrofti* was detected in the inguinal and crural lymphatics of 28 (15.0%) children. FDS detection was more common in older children (P=0.003) and in those with a history of inguinal lymph node inflammation (P=0.002) or crural lymphodenopathy on physical exam (P=0.01). 25 FDS-positive children were examined after 3 annual cycles of mass treatment for LF. The total number of adult worm nests detected by ultrasound decreased from 29 to 4 (P≤0.0001). FDS and lymphangiectasia were detected in the intrascrotal (N=3) and inguinal (N=1) lymphatic vessels of 3 post pubescent boys.

Gbakima *et al.,* (2005) determined the potential of establishing urban transmission cycles of LF in Ghana's major cities. They clinically and immunologically assessed 625 individuals from the 3 major urban areas and found that the prevalence of infection with *W. bancrofti* ranged from 0-12.5%. The result of PCR based analysis of mosquitoes collected from those areas suggested that there was a low but detectable prevalence of mosquitoes infected with *W. bancrofti.*

Onapa *et al.,* (2005) assessed the geographical distribution of LF in Uganda by using a rapid ICT card test to check school aged children for *W. bancrofti*-Specific CFA. Overall 17,533 children from 76 sites were examined. CFA positive cases were detected at 31 of the sites with prevalence ranging from 0.4%- 30.7%. There appeared to be strikingly more LF in the North of the country than in the South. The main focus was North of the Victoria Nile where 27 (66.0%) of the 41 sites with high CFA-Positive cases were taken and 4 were along the western rift valley had relatively low CFA prevalence. Geostatistical interpolation was used to create a map showing the geographical distribution of CFA prevalence. It was found that approximately 8.7

millions people (35.3% of national population) lived in the areas where >1.0% of school aged children were CFA positive.

Ramaiah *et al.,* (2005) analyzed a situation of LF in a large urban area, Chennai, India so that to ensure the development of drug delivery strategies for high treatment coverage. The subjects interviewed came from households with high moderate, low or very low incomes. It was found that the cases of elephantiasis and hydrocele were detected in 2.0%-8.0% and 7.0%-20.0% respectively of the households investigated. Overall, 40.0% of the interviewees from very low income households and 78.0% of those from middle income households knew that elephantiasis was transmitted by mosquitoes. Only 4.0% of the subjects from high income areas and 1.0% of those from low income areas were aware that filarial infection was a major cause of hydrocele.

Yahathugoda *et al.,* (2005) studied the current situation of LF in 3 suburbs of Matara, SriLanka using in-depth interviews information on the current state of lymphoedema management. 101 cases of LF with lymphoedema were collected of which 32(31.6%) interviewees had severe lymphoedema and male had significantly more entry lesions than the female. 65.0% of the subjects paid no attention to limb care when bathing and 44.0% did not use foot wear. Over 80.0% made no effort to keep their afflicted limbs elevated and 95.0% did not exercise.

Das *et al.,* (2005) surveyed the bancroftian filarialsis for MF and CFA in two villages-Pista and Taroni, Madhyapradesh, India. The study was carried out by the clinical examinations, night blood smears collected for MF examination and estimation of CFA by O_g4C_3 ELISA, 38.0% serum of village Pista (n=332) and 47.7% from Taroni (n=88) were found positive for CFA. The overall disease rate was 57.9% by CFA while only 43.30% by night blood smear examination. A total of 14.5% individuals were found to be cryptic filarial infection detected by CFA.

Mathieu *et al.,* (2005) conducted an intense health-education campaign followed by an MDA with DEC and ALB in Leogane, Haiti. Questionnaire based interviews were used to explore the Knowledge, Attitude and Practice (KAP) of 304 subjects. The primary reasons given for failing to take the drugs were absenteeism during the distribution (17.0%), use of contraceptive drugs (12.0%) and pregnancy (11.0%). In a

multivariate analysis, being male, knowing that a mosquito transmits the disease, and having learned about the MDA through posters and banners were found to be positively associated with taking the drugs.

Singh *et al.,* (2005) studied the LF in Bilaspur district, Chhattisgarh. A total of 3426 night blood smears were collected from 24 randomly selected localities covering 25.0% known endemic areas and 75.0% reportedly non-endemic areas. 62 indigenous residents (1.8%) were found to be positive for *W. bancrofti.* Male (2.17%) were more affected than female (1.19%). The MF carriers were 2.05% in rural and 1.45% in urban communities. The mean density of MF was found to be 5.06.

Erlanger *et al.,* (2005) carried out a systematic literature review to establish global and regional estimates of population at risk of LF with particular consideration of water resource development project. They estimated that globally 2 billions of people are at risk of LF. Among them 394.5 millions were urban dwellers without access to improved sanitation and 213 millions rural dwellers living in close proximity to irrigation. The mosquito vectors density were found to be up to 25-folds higher in irrigated areas when compared to the irrigation free sites and infection prevalence. LF was found to be increased after the implementation of a water project.

McPherson *et al.,* (2006) studied the Interdigital Lesions (IDL) and frequency of Acute Dermatolymphangioadenitis (ADLA) lymphoedema in a filariasis endemic area. It was found that more than 50.0% of patients with lymphoedma had one or more IDL. The number of lesions was the strongest predictor of frequency of ADLA. Only 18.0% of the lesions had positive microscopy or culture for fungi.

Igbal *et al.,* (2006) determined the prevalence of LF among migrant workers in Kuwait by detecting CFA. A total of 1,050 migrant workers from filarial endemic countries and 260 individuals residing in Kuwait as controls were screened for filarial infection. All the specimens were tested for microfilaraemia by microscopy of Nucleophore Filtered Blood (NFB) and detection of CFA by ICT and Trop Bio-assay. The overall prevalence of filarial antigenemia was 18.3% using the ICT and 20.3% using the Trop Bio-assay 3.0% of *W. bancrofti* were detected by microscopy and the mean MF count in these cases was 816 MF/ml. CFA was detected only in 2 of the 260 control subjects.

Matheieu *et al.,* (2006) reported the result of participation in three consecutive MDA in Leogane, Haiti. During the third MDA, the overall surveyed coverage was 78.5%. A survey among adult population showed coverage estimates for persons >14 years old of 59.4%, 64.6% and 67.3% for the first, second and third MDA respectively. The coverage in rural areas was significantly higher than in urban areas.

Melrose *et al.,* (2006) mapped the distribution of LF in the democratic republic of Timor-Leste by the use of Brugia rapid dipstick and ICT test. 12 out of 13 districts were confirmed as being endemic Brugian filariasis predominates with an average prevalence of 11.6%. The average prevalence of Bancroftian filariasis was 1.1%. It was demonstrated that the Brugia rapid test can provide useful information about the distribution of Brugian filariasis in circumstances where it is difficult or impossible to obtain night blood samples for MF.

Kelly-Hope *et al.,* (2006) determined the relationship between human LF caused by *W. bancrofti* and falciparum malaria which are co-endemic through the West Africa. They used geographical information systems and spatial statistics to examine the prevalence of LF in relation to malaria prevalence, mosquito species distributions, vegetation and climate. A negative spatial association between *W. bancrofti* and falciparum malaria prevalence was existed.

Alison *et al.,* (2006) reported the results of two surveys of people's KAP regarding LF in Alor district, Eastern Indonesia. In the study area, the filarial parasites *B. timori* and *W. bancrofti* were highly endemic. The first KAP survey was conducted as a baseline pre-MDA with DEC and ALB and second as a post-intervention evaluation in order to obtain information on the impact of the communication campaign. Before the information campaign and the subsequent MDA, 54.0% of the study population had heard of one of the three main terms of LF, whereas after health education and MDA, 89.0% had heard of at least one of the three terms. Similarly, pre-MDA, 21.0% reported having had previously taken the treatment for filariasis, while post-MDA, 88.0% reported having taken the treatment during the pilot treatment period.

Researches on LF in Context of Nepal

Jung *et al.,* (1973) first reported LF from Nepal who studied all together 9 sites and showed that the 4.99%-6.15% infection of *W. bancrofti* in all age groups and both sexes in the urban population, 6.6%-10.3% in the semi urban population and 1.2%-17.8% in the rural population. Similarly, 7.1%-9.16% MF rate was found in the urban population, 10.03%-11.3% in the semi urban population and 0.8%-17.69% in the rural population.

Pradhan *et al.,* (1997) conducted an epidemiological study of LF in Gokarna VDC, Kathmandu. They reported 24.6% of endemicity rate (ER), 12.75% of MF infection and 11.95% of Crude Disease Rate (CDR) of *W. bancrofti.* The MF infection was found to be greater in male (15.09%) than that of female (8.9%) but CDR was found to be greater in female (16.59%) than that of male (8.49%). They identified 12species of mosquitoes from the study area of them *C. quinquifasciatus* was found to be more prominent.

Bista *et al.,* (2000) analyzed situation of LF and recorded the prevalence of LF of 13.2-23.4% in different regions of Nepal. The study was conducted in out patient clinics of different health institutions via the HMIS during the fiscal year 1995/96 to 1998/99.

Bhusal *et al.,* (2000) studied the prevalence of *W. bancrofti* infections in Tokha Chandeswori VDC of Katmandu in 1998. A survey of 978 nocturnal blood samples was collected. An overall prevalence of MF was found to be 5.8% and the CDR of *W. bancrofti* was recorded to be 13.0%. The highest MF infection rate was recorded as 11.8% in the group of 40-49 years and the highest CDR was recorded as 36.4% in the age group of 70 and above.

Manandhar (2001) conducted and epidemiological study of microfilariasis in three different geographical regions of Nepal. The study was conducted in Bhairahawa (Sipwa), Palpa (Dovan) and Bhaktapur (Golmadi, Byasi, Itachhen and Katunje). The study was carried out by the collection of night blood smears (thin, thick and buffy coat) and microscopic examination as well as the ICT card method. Out of total 512 samples taken the diseases prevalence was found to be 0.31%. The chronic clinical

manifestation in both male and female was found 21.67%. Elephantiasis was found to be more prevalent in female (59.45%) than in male (18.91%) and Hydrocele in male was found to be 21.62%.

Sherchand (2002) conducted an epidemiological survey to determine the prevalence of disease due to LF in Magargadi VDC, Bardia distrct of Nepal. The study population selected was above 15 years of age and the process used was ICT card test to screen the CFA. Out of 500 samples collected 141(28.2%) were infected with larvae of *W. bancrofti.* The vector for this parasite was found to be the *C. quinquefasciatus.* Out of 214 mosquitoes dissected 2 (0.93%) were found to infected with the larvae of *W. bancrofti.*

Sherchand *et al.,* (2003) studied the prevalence of infection by *W. bancrofti* in 37 districts of Nepal from July to December (2001). The study populations were selected above 15 years of age and the ICT-filariasis test was used to screen for CFA. The overall prevalence of LF was found to be 13.0% out of total sample of 4,488. Altogether 33 out of 37 districts were found to be endemic for LF. On the basis of geographical data, the highest numbers of cases were found at altitudes between 500-700m however, a substantial number of infected individuals were found in the highly populated Katmandu Valley at altitudes between 900-1500m. Prevalence rates above 20.0% were found in 11 districts (with the highest rate of 40.0%), 6.0-19.0% on 15 districts and 0.1-5.0% in 7 districts.

Ghimire and Thakur (2003) conducted the LF surveillance in Parsa district, Nepal. A total of 892 individuals were studied in two VDCs (Pokharia and Maniyari) by both night blood collection (thick and thin smears) and questionnaire survey, of them 13(1.45%) were found to be MF positive. MF positive from Pokharia was found to be 6 (1.30%) out of 460 and that of Maniyari was found to be 7 (1.62%) out of 432. Females were found to be more MF positive than that of males as it was found the MF positive of 1.97% and 0.59% in male and female respectively.

Agrawal *et al.,* (2004) conducted a study on the prevalence of LF in districts of eastern Nepal (Morang, Sunsari and Saptari); a population based cross-sectional household survey. A total of 10,000 samples were taken by the method of night blood collection and questionnaire interview. It was found that the total number of MF

positive cases were 10(0.1%). Of 3 districts Saptari was found to be more MF infection i.e. 8(0.2%) out of 4,000 samples taken than that of Morang i.e. 2(0.06%) out of 3,000 samples taken. But no MF positive cases were found in the Sunsari out of the 3,000 samples taken.

Jha *et al.,* (2006) conducted an epidemiological study for LF in 8 districts of Nepal representing 3 geographical regions i.e. terai, mid-terai and hilly region. Out of 958 samples collected 154(16.08%) were found to be positive for LF. The overall microfilariaemia, antigenaemia and LF rate was found to be 0.63%, 4.70% and 10.75% respectively. The highest CDR and ER were found in Bhaktapur district (23.88% and 28.36% respectively). Regarding region-wise prevalence it was found that the ER increased from terai to hill i.e., 12.54% in terai, 16.04% in mid terai and 24.05% in hilly region respectively.

Maharjan *et al.,* (2006) conducted an epidemiological surveillance of LF in Makwanpur, Chitwan, Rupandehi and Nawalparasi districts of Nepal. A total of 4,084 individuals were examined by night blood collection of them 103(2.58%) were found to be MF positive and overall CDR was found to be 67(1.68%). The overall ER of LF was reported to be 4.6%. The highest ER was reported from Rupandehi district (8.2%) and that of least from Chitwan district (0.79%).

Gupta *et al.,* (2006) conducted the baseline surveillance of LF in Nuwakot, Dhading, Kapilbastu, Bara and Rautahat districts. Out of 5,142 individuals studied both by night blood examination and questionnaire survey 331(6.44%) was found to be MF positive. Total CDR was reported to be 364 (7.08%) and overall ER was found to be 687 (13.36%). Among the 5 districts Dhading showed the highest MF prevalence (11.67%) followed by Nuwakot (10.89%), Kapilbastu (5.64%), Rautahat (3.23%) and Bara (0.6%). The ER was more in Nuwakot district (25.7%) than in Dhading district (23.14%).

IV
MATERIALS AND METHODS

Materials Required

Apparatus: Microscopical slides, Sterile lancets, Cotton, Gloves, Mask, Measuring cylinder, Dropper, Slide box, Compound binocular microscope, Micro photographic device, Tooth picks, etc.

Reagents: Methanol, Giemsa stain 5%, Distilled water, Oil immersion, etc.

A set of well-structured questionnaire.

Study Area

The study area is Ganeshthan VDC of the Nuwakot district. Nuwakot district, where the present study was carried, is a hilly district situated in Bagmati zone of the Central Development Region. Nuwakot district is surrounded by Rasuwa, Dhading, Kathmandu and Sindhupalchok districts in North, West, South and East directions respectively. The district consists of Bidur municipality and 61 VDCs. The total population of the district is 2, 88,478 (Census, 2058 BS). Nuwakot district is situated towards the northern side of Kathmandu valley and district head quarter, Bidur is only 70 km away from Kathmandu and Ganeshthan VDC is 75 km away from Kathmandu. The Nuwakot district is situated at 28^{o} 7' N from the equator and 85^{o} 28' E from the Greenwich meridian. The altitude ranges from 600m-1500m and climate is hot ranges from 10^{o}C-35^{o}C very similar to that of Terai region, even though it is the hilly region. The average annual rain fall in this district ranges from 100-200cm. The area concerned to present study, Ganeshsthan VDC (approximate area 9 km^2) is situated at the middle of the district and surrounded by Hallekalika, Khanigaun, Chaughada and Narjamandap VDCs at North, West, South and East respectively. The ethnic groups of Ganeshsthan VDC include Brahmin, Newar, Rai and others. The total households of the Ganeshsthan VDC are 718. The population of Ganeshsthan VDC is 3,898 among them 1,978 are males and 1,920 are females (Census, 2058 BS). As per Census 2058 BS in the Ganeshsthan VDC, the population growth rate is 1.33, sex ratio is 102.70, average family size is 5.4 and that of population density is 409.8/km^2. Among

the total population, 3,898 of the VDC 96.53% speak Nepali, 1.77% Tamang, 1.38% Newari and that of 0.30% speak some other language. As per religion 98.51% peoples are Hindu and 1.48% are Buddhists. The total literacy rate of the VDC is 51.8% and that of male is 65.3% but that of female is only 38.3%. According to the latest record the population of the VDC is recorded to be 4,109 (CBS, 2062). Most people in the Ganeshsthan VDC are farmers and the habitation is surrounded by poor sanitation.

Study Design

The design is the epidemiological descriptive study. An epidemiological survey method was applied which is the appropriate method to measure the prevalence of disease, chronic disease burden, sign and symptoms and asymptomatic MF carrier, KAP on the LF and the risk factors among community people.

Sampling Design and Sample Size

Basically the ward no. 4 and 2 were selected according to the presence of sign and symptoms of filariasis among the common people. Each and every individual (age >2 years) from each house hold was selected as the study unit in a continuous fashion to obtain the sample size of 502 both for the questionnaires and night blood.

Tools Used In the Study

i. Awareness or Mobilization Program

Community people were informed about the awareness program through health post staffs, health workers, community volunteers and leaders and requested to gather them at Health Post premises. The expert and team workers were introduced to them followed by the briefing of the program and its aim. Discussion on filariasis was also made and views were exchanged. And lastly they were requested to support, co-operate and help the research work by filling the questionnaires and permitting to collect night blood samples.

ii. Training to the Field Workers

One day training program was organized for all the field workers at the Health Post. All the field members were made well skilled with the methods and technique for the administration of the questionnaires in the field and collection of the blood samples from the ear lobes with the help of theoretical and practical approach.

ii. Administration of Questionnaires

A set of well structured questionnaire was developed in order to determine the potential risk factors for filarial transmission. The survey team visited in each of the household and administered structured questionnaire from the household head and others during the day time.

iv. Night Blood Sample Collection

Since, W. *bancrofti* is nocturnally periodic in nature, the night blood sample is the only reliable method available for the diagnosis of filarial infection. For this, the survey team visited the households in between 10:00PM-2:00AM for the blood sample collection. The ear lobe of each household members were pricked by a sterilized blood lancet and extracted total of 6 drops (60 µl) of blood to prepare 3 thick blood smears on a glass slide each containing 2 drops of blood (20 µl). The blood smears were made by spreading the blood drops with the help of toothpicks. The blood smears were air dried, labeled according to questionnaire with the code number and slide symbol number and then stored safely in the slide box.

v. Laboratory Diagnosis

- **Dehaemoglobnization of thick blood smears**

The thick blood smears were dehaemoglobinized by putting the distilled water on the blood smears for about 30 minutes. It was waited until the time that the thick smear seems to be spongy in appearance. The slides were washed properly by the drop wise distilled water upon the inclined slide. The washed slides were dried on the room temperature.

- **Fixing and staining of slides**

The blood smears were fixed by dipping the slide in methanol for a few seconds. Then the dehaemoglobinized and fixed slides were stained with 1/10 Giemsa stain for 25-30 minutes. The stain was washed with distilled water, dried and safely stored in the slide box to be examined under microscope.

- **Microscopic examination of slides**

The slides were examined by using binocular compound microscope with 10X, 40X and 100X objectives. Each and every field of the smear were thoroughly examined first in low power for screening and then in high power for the confirmation. The slides were rechecked by the supervisor and final conclusion for MF positivity and density were drawn.

vi. Photomicrography and Comparative Morphological Study

The MF positive slides were marked as +ve and each positive slide was fitted on special microscope upon of which a camera was fitted on the eye piece. Each parasite from each smear was observed and clear parasites were subjected to take the photographs. The photomicrography was taken under the power of 10X, 40X and 100X of the same parasites. The photomicrography under the power of 100X was taken only after applying the oil immersion on the slide for the clarity of external and internal structures of the parasite. The film was washed in the digital color lab to develop the negatives and then positives. A number of photomicrographs were taken and morphological comparative study was carried out. The comparative morphological study was carried out among the human infecting microfilariae (*W. bancrofti, B. malayi, B. timori, L. loa, M. ozzard,. M. perstans, M. streptocerca* and *O. volvulus*) by comparing the photographs with the authorized published photographs. The taxonomic keys of the *Monitoring and Epidemiological Assessment of the Program to Eliminate LF, WHO (2002)* was used for the identification of MF (Annex: I).

vii. Data Processing, Analysis and Interpretation

Data were edited as soon as possible to detect errors and to make sure that the data were accurate, uniform and well arranged. Informations were coded so that they were easily classified and tabulated. All the data were classified according to the need of

the objectives and tabulated for summarizing the data and displaying them statistically. The interpretation of data was made with the statistical tools.

viii. Validity and Reliability of the Study

Quality control on specimen collection, processing and conformation of the species was maintained out of the test. All reagents, equipments and laboratory methods were standardized. The study was properly instructed and guided by the well-trained and long experienced supervisor. Questionnaires were filled by the respondents in the presence of well-trained research team member or the investigator. The microfilarial species identification was carried out by the comparative morphological studies of the MF in the presence of long experienced taxonomist.

V
RESULTS

The research work was completed within 7 months (April, 2006-October, 2006). Different results obtained from the questionnaire analysis, microscopic examination of night blood smears and morphological comparative study can be presented as follows:

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- **Microfilarial Species Identification**

The MF studied in all the slides were found to be *W. bancrofti.* It is supported and proved by the following morphological structure of the MF, as seen in the stained specimen (Annexes-I, II and III) (Plates: IX, X, XI, XII, XIII, XIV, XV, XVI, XVII and XVIII):

i) The body of the MF was provided by the hyaline sheath. The structure less sac like sheath was much longer than the larval body.

ii) Cuticle was lined by subcuticular cells.

iii) Nuclei were appeared as granules but which were not extended up to the tail tip. The tail was uniform some 5.0% of the tail tip was devoid of the nuclei but was provided with the hyaline sheath.

iv) The anterior end of the MF was provided by a space, the cephalic space. The cephalic space was as broad as it was long.

- **General Prevalence of Microfilariae (MF)**

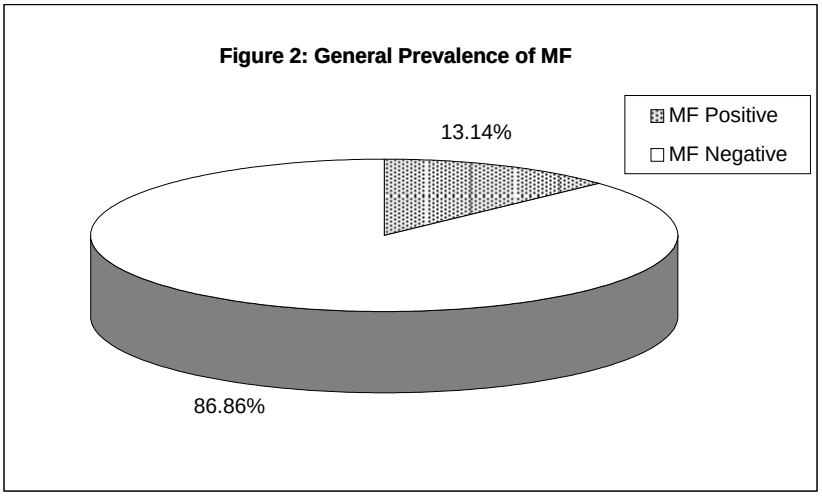
Out of 502 human blood samples examined for identification of microfilariae (MF) infection, 66 were found to be MF positive Hence; general prevalence of MF was found to be 13.14% (Table: 2).

Table 2: General Prevalence of Microfilariae (MF)

Sample size	MF positive cases	Percentage
502	66	13.14%

Source: *Field survey, 2006*

Figure 2: General Prevalence of MF



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- **Sex-wise Prevalence of MF**

As depicted in the table no. 3, out of total of 502 samples taken, 265 (52.78%) were male and 237(47.22%) were female. Male and female MF positive case were found to be 39(14.71%) and 27(11.39%) respectively with the male and female positivity ratio 1.44:1. This explains the longer outdoor exposure of male than the female. However, the prevalence of MF in male was found to be slightly greater than in female, statistically it was significantly indifferent (χ^2=1.39, df =1, P<0.05) (Table: 3).

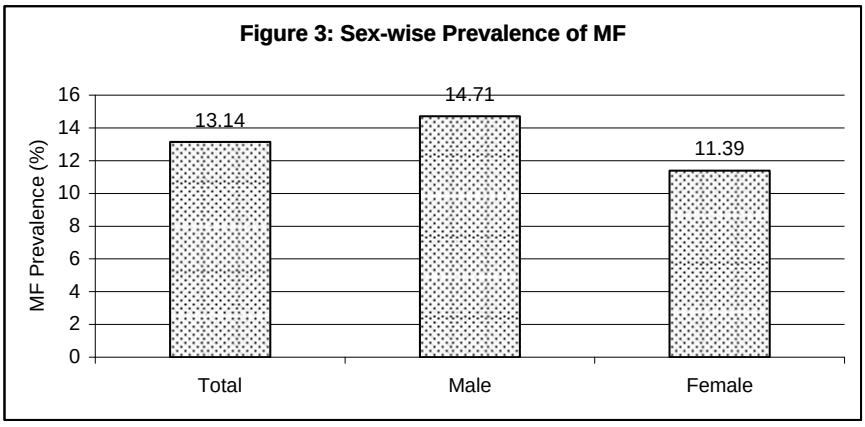
Table 3: Sex – wise Prevalence of MF

Sex	Sample Size	Positive Cases	Percentage
Male	265(52.78)	39	14.71%
Female	237(47.22)	27	11.39%
Total	**502**	**66**	**13.14%**

Source: *Field Survey, 2006.*

(The figures in the parenthesis indicate the percentage)

Figure 3: Sex-wise Prevalence of MF



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• Age and sex-wise Prevalence of MF

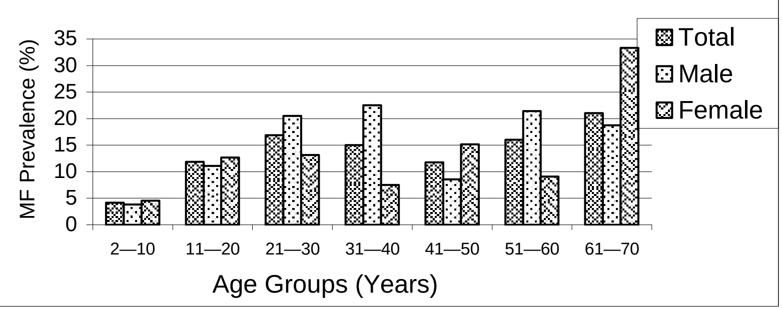
Among, total of 502, blood samples collected, majority was from the age group of 11-20 years (31.87%) and the least was from the age group of 61-70 years (3.78%). The highest percentage (21.05%) of the MF positive cases was recorded from the age group of 61-70 years and the least (4.16%) from the age group of 2-10 years. The prevalence of MF was found to be increased with the increase of age in both the sexes. In case of the male, the highest prevalence of MF (22.50%) was found to be in the age group of 31-40 years and the least (3.84%) was in the age group of 2-10 years. Similarly, in case of the female, the highest prevalence of MF (33.33%) was found in the age group of 61-70 years and the least (4.54%) was found to be in the age group of 2-10 years. Statistically, the MF prevalence in different age group was found to be significantly different ($\chi2$=21.17, df =6, p>0.05) (Table: 4).

Table 4: Age and Sex-wise Prevalence of MF

Age group (years)	Sample size			MF Positive Cases		
	Total	Male	Female	Total	Male	Female
2-10	48(9.56)	26	22	2(4.16)	1(3.84)	1(4.54)
11-20	160(31.87)	81	79	19(11.87)	9(11.11)	10(12.65)
21-30	77(15.33)	39	38	13(16.88)	8(20.51)	5(13.15)
31-40	80(15.93)	40	40	12(15.00)	9(22.50)	3(7.50)
41-50	68(13.54)	35	33	8(11.76)	3(8.57)	5(15.15)
51-60	50(9.96)	28	22	8(16.00)	6(21.42)	2(9.09)
61-70	19(3.78)	16	3	4(21.05)	3(18.75)	1(33.33)
Total	**502(100.00)**	**265**	**237**	**66(13.14)**	**39(14.71)**	**27(11.39)**

Source: *Field survey, 2006.*
(The figures in the parenthesis indicate the percentage)

Figure 4: Age and Sex- wise Prevalence of MF



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- **Educational Status-wise Prevalence of MF**

Out of 502 samples taken, the most (36.85%) of the respondents were found to be illiterate and the least (4.38%) were literate (persons with informal education). The maximum MF positive cases were found in the literate group (27.27%) and the least in the primary group (7.78%). The MF positive cases were found to be increased with the increase in educational status. Among the male, the highest MF positive cases were found in the literate group (46.15%) and the lowest cases were found in the primary group (9.63%). Similarly, among the female, the highest MF positive cases were found in the secondary level (25.0%) and none of the cases were found in the literate and higher studies level. Statistically, the prevalence of MF in the different educational status was found to be significantly different (χ^2=32.53, df=5, P>0.05) (Table: 5).

Table 5: Educational Status-wise Prevalence of MF

Educational status	Sample size			MF Positive cases		
	Total	Male	Female	Total	Male	Female
Illiterate	185(36.85)	84	101	27(14.59)	10(11.90)	17(16.83)
Literate	22(4.38)	13	9	6(27.27)	6(46.15)	0(0.00)
Primary	167(33.26)	83	84	13(7.78)	8(9.63)	5(5.95)
L. Secondary	53(10.55)	28	25	5(9.43)	3(10.71)	2(8.00)
Secondary	48(9.56)	36	12	10(20.83)	7(19.44)	3(25.00)
Higher studies	27(5.37)	21	6	5(18.51)	5(23.80)	0(0.00)
Total	**502(100.00)**	**265**	**237**	**66(13.14)**	**39**	**27(11.39)**

Source: *Field survey, 2006.*
(The figures in the parenthesis indicate the percentage)

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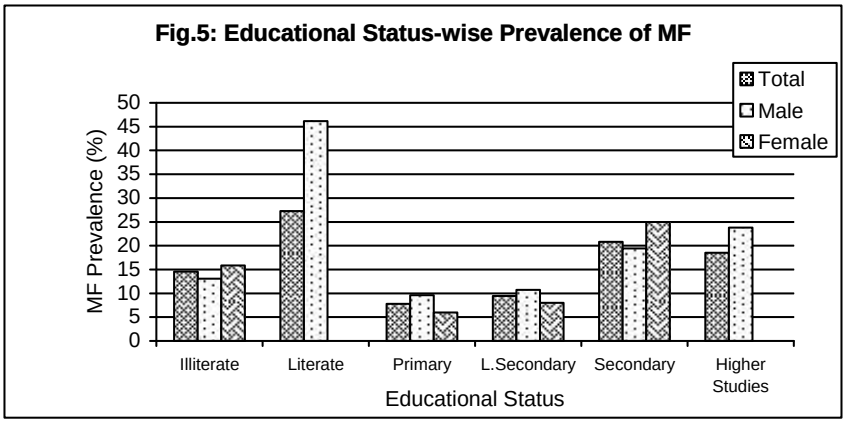


Fig.5: Educational Status-wise Prevalence of MF
Total
Male
Female
MF Prevalence (%)
50
45
40
35
30
25
20
15
10
5
0
Illiterate
Literate
Primary
L.Secondary
Secondary
Higher Studies
Educational Status

- **Occupation-wise Prevalence of MF**

Out of the 502 samples taken, the most (48.0%) of the people were found to be farmers and then the students (33.46%). The least were found as the Job holders (working for monthly salary) (0.79%). The highest prevalence of MF was found in the labors and teachers each constituting 28.57% and no case was found in the child (pre-school age i.e. 2-4 years). Among the male, the highest MF prevalence was found to be in the teachers (30.76%). Similarly, among female, the highest MF prevalence was found in the businessmen (25.0%). Statistically, the prevalence of MF in different occupational group was found to be significantly different (χ^2=50.86, df=7, P>0.05).

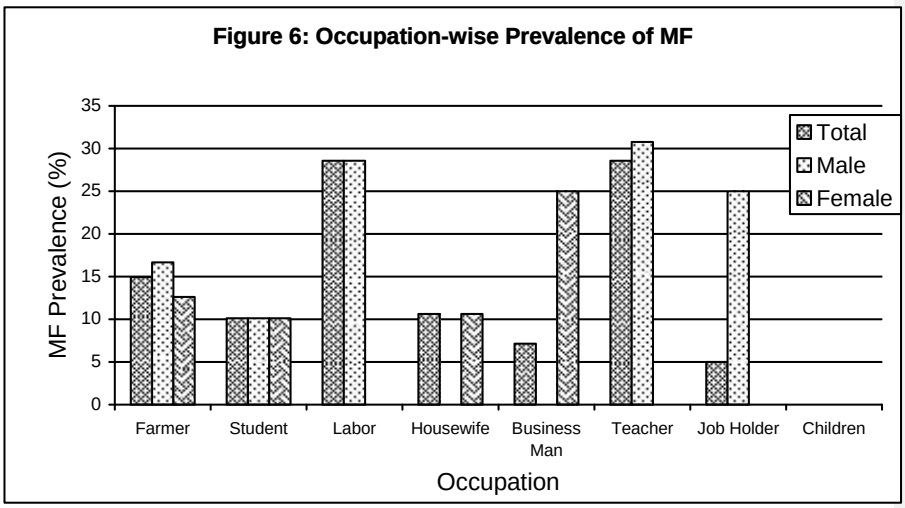
Table 6:Occupation-wise Prevalence of MF

Occupations	Sample size			MF Positive cases		
	Total	Male	Female	Total	Male	Female
Farmers	241(48.00)	138	103	36(14.93)	23(16.66)	13(12.62)
Students	168(33.46)	89	79	17(10.11)	9(10.11)	8(10.12)
Labors	7(1.39)	7	0	2(28.57)	2(28.57)	0(0.00)
Housewives	47(9.36)	0	47	5(10.63)	0(0.00)	5(10.63)
Businessmen	14(2.78)	10	4	1(7.14)	0(0.00)	1(25.00)
Teachers	14(2.78)	13	1	4(28.57)	4(30.76)	0(0.00)
Job holders	4(0.79)	4	0	1(25.00)	1(25.00)	0(0.00)
Child	7(1.39)	4	3	0(0.00)	0(0.00)	0(0.00)
Total	**502(100.00)**	**265**	**237**	**66**	**39(14.71)**	**27(11.39)**

***Source**: Field survey, 2006.*

(The figures in the parenthesis indicate the percentage)

Figure 6: Occupation-wise Prevalence of MF



- **Prevalence of MF as per Educational Status and Knowledge on LF**

The highest percentage of knowledge was found in the group of higher studies (81.48%) and least in the group of illiterate respondents (24.32%). But for MF positive cases, the highest percentage was found in the literate group (27.27%) and the least in the group of primary level (7.78%). Increase of MF positive cases was found in the more educated group. Statistically, the knowledge of LF at different educational status was found to be significantly different (χ^2= 60.28, df =5, P>0.05)

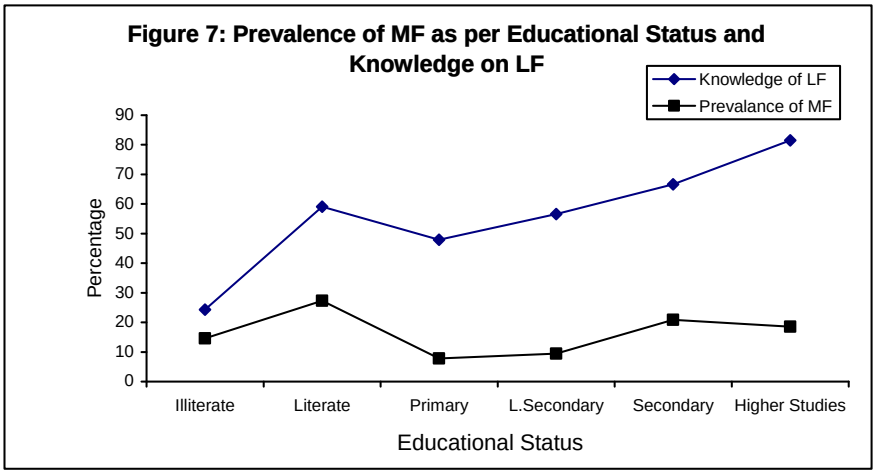
Table 7: Prevalence of MF as per Educational Status and Knowledge on LF

Educational Status	Sample Size	Knowledge of LF		MF Positive Cases.
		Yes	No	
Illiterate	185(36.85)	45(24.32)	140(75.67)	27(14.59)
Literate	22(4.38)	13(59.09)	9(40.90)	6(27.27)
Primary	167(33.26)	80(47.90)	87(52.09)	13(7.78)
L. Secondary	53(10.55)	30(56.60)	23(43.39)	5(9.43)
Secondary	48(9.56)	32(66.66)	16(33.33)	10(20.83)
Higher Studies	27(5.37)	22(81.48)	5(18.51)	5(18.51)
Total	**502(100.00)**	**222(44.22)**	**280(55.77)**	**66(13.14)**

Source: *Fields survey, 2006.*

(The figures in the parenthesis indicate the percentage)

Figure 7: Prevalence of MF as per Educational Status and Knowledge on LF



- **Prevalence of MF in Relation to Precaution Measures**

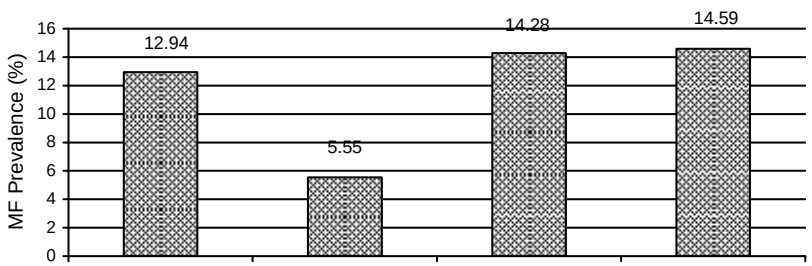
The highest number of the study population was found to have used the mosquito net (67.72%) and the least was found to have used the smoke (1.39%) to be protected from the mosquito bite. The prevalence of MF was found highest in the group who were not using any precaution measures (14.59%) and the least in the group who were using the mosquito mats during night sleeping (5.55%). This indicates that the mosquito mat is far better than the mosquito nets and smoke. Statistically, the usage of various precaution measures was found to be significantly different (χ^2=113.83, df=3, P>0.05) (Table: 8).

Table 8: Prevalence of MF in Relation to Precaution Measures

Precaution Measures	No. of respondents	Percentage (%)	MF Positive cases	Percentage (%)
Mosquito Nets	340	67.72	44	12.94
Mosquito Mats	18	3.58	1	5.55
Smoke	7	1.39	1	14.28
None	137	27.29	20	14.59
Total	**502**	**100.00**	**66**	**13.14**

***Source**: Field survey, 2006.*

Figure 8: Prevalence of MF in Relation to Precaution Measures



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- **Prevalence of MF as per Environmental Status**

Out of 502, the highest sample (43.82%) was collected from the inhabitants of the dirty environment and the lowest (0.99%) from the inhabitants of the environment of open ground night soil disposal. The highest MF positive cases were recorded from the inhabitants around the environment of the stagnant water (30.0%) since that provides the suitable breeding sites of the mosquito vectors and no case was recorded from the inhabitants of environment of open ground night soil disposal. Statistically, the MF positive cases as per different environmental status were found to be significantly different (χ^2=71.98, df=5, p>0.05) (Table: 9).

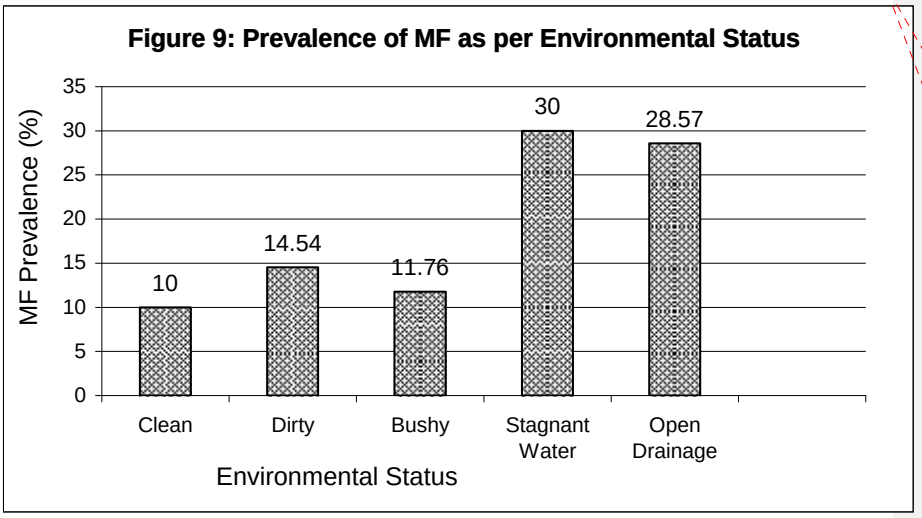
Table 9: Prevalence of MF as per Environmental Status

Environmental status	Sample size	MF positive cases
Clean	90(17.92)	9(10.00)
Dirty	220(43.82)	32(14.54)
Bushy	170(33.86)	20(11.76)
Stagnant Water	10(1.99)	3(30.00)
Open drainage	7(1.39)	2(28.57)
Open ground night soil disposal	5(0.99)	0(0.00)
Total	**502(100.00)**	**66(13.14)**

***Source**: Field survey, 2006.*

(The figures in the parenthesis indicate the percentage)

Figure 9: Prevalence of MF as per Environmental Status



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- **Density of MF per Smear (20 µl) of Blood**

The density of MF can be expressed as the number per smear (20 µl) of the blood. Out of the 66 positive slides (3960 µl of blood), total MF count was 2012 and the average density of the MF was found to be 10.16 MF per smear (20 µl) of blood (Table: 10). Out of the 66 positive cases, 3(4.54%) were found to be of the highest density (containing >30MF/smear) and the least density (containing 1-5 MF /smear) were found in 29 (43.93%). In the male, highest density of MF was found in 66.66% cases and least density of MF was found in 55.17% cases. Similarly, in the female the highest density of MF was found in 33.33% case and least density of MF was found 44.82% cases. Thus, density of MF was found to be greater in male than in the female (Table: 11).

Table 10: Average Density of MF per Smear (20µl) of Blood

Total No. of MF	Volume of Blood	Density(No./ 20 µl)
2012	3960 µl	10.16

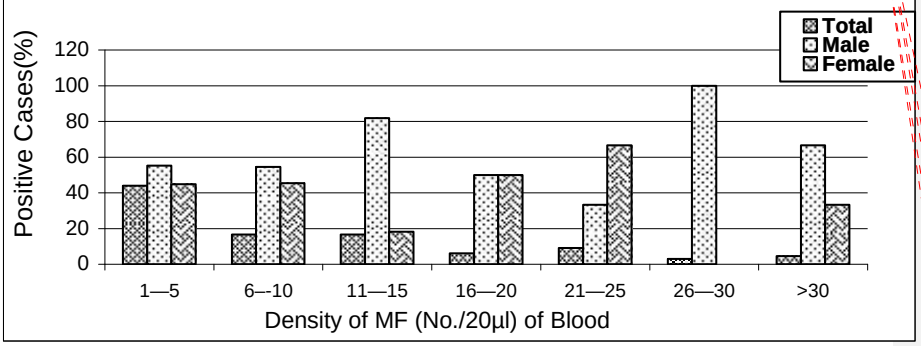
Source: *Field survey, 2006.*

Table 11: Density of MF per Smear (20µl) of Blood

Density of MF (No./20 µl)	Total No. of Positive Cases	Male Positive cases	Female Positive cases
1-5	29(43.93)	16(55.17)	13(44.82)
6-10	11(16.66)	6(54.54)	5(45.45)
11-15	11(16.66)	9(81.81)	2(18.18)
16-20	4(6.06)	2(50.00)	2(50.00)
21-25	6(9.09)	2(33.33)	4(66.66)
26-30	2(3.03)	2(100.00)	0(0.00)
>30	3(4.54)	2(66.66)	1(33.33)
Total	**66(100.00)**	**39(59.09)**	**27(40.90)**

Source: *Field survey, 2006.*
(The figure in the parenthesis indicates the percentage)

Figure 10: Density of MF per Smear (20µl) of Blood



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- **Sex-wise Clinical Manifestations and Crude Disease Rate (CDR)**

The chronic sign and symptoms of the LF describes about the Crude Disease Rate (CDR). Total Elephantiasis was found 7.96% and was less in male (7.92%) than in female (8.01%). Hydrocele, the most prevalent symptom among the male was found 10.18% and breast swelling in female was found 8.43%. The CDR in male was found to be 20.37% and that of female 26.16%. The greater CDR in female explains about the more prevalence of chronic sign and symptoms in female than that of male. Statistically, the CDR in both sexes was found to be significantly indifferent (χ^2=0.54, df=1, p< 0.05) (Table: 12).

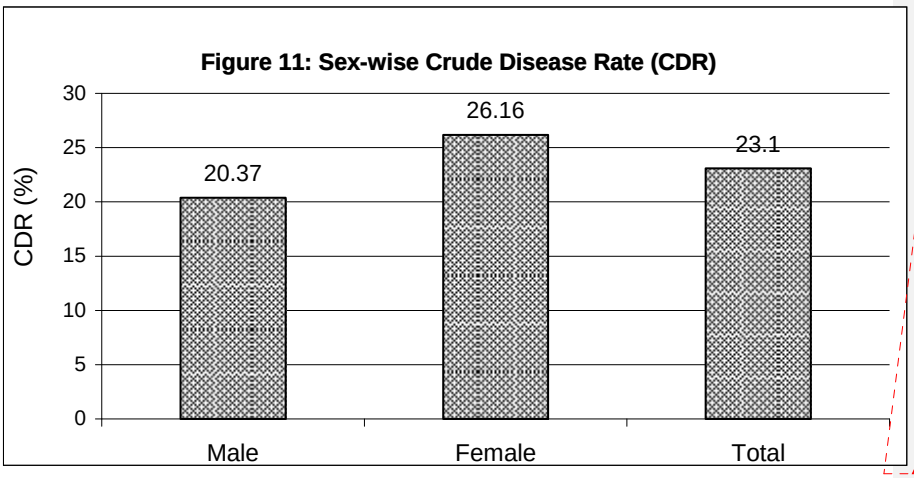
Table 12: Sex-wise Clinical Manifestations and Crude Disease Rate (CDR)

Sign and Symptoms	Male			Female			Total		
	Cases	Sample Size	CDR	Cases	Sample Size	CDR	Cases	Sample Size	CDR
Elephantiasis	21(7.92)	265	54(20.37)	19(8.01)	237	62(26.16)	40(7.96)	502	116(23.10)
Hydrocele	27(10.18)			_			27(5.37)		
Breast Swelling	_			20(8.43)			20(3.98)		
Chyluria	_			7(2.95)			7(1.39)		
Limb Swelling	3(1.13)			11(4.64)			14(2.78)		
Thickening of Skin	3(1.13)			5(2.10)			8(1.59)		
Total	**54(20.37)**	**265**	**54(20.37)**	**62(26.16)**	**237**	**62(26.16)**	**116(23.10)**	**502**	**116(23.10)**

Source: *Field Survey, 2006.*

(The figures in parenthesis indicate the percentage)

Figure 11: Sex-wise Crude Disease Rate (CDR)



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- **Total Endemicity Rate (ER) of LF**

Out of 502 samples of human population studied by both microscopic night blood smear examination and questionnaire survey method, 10.15% was found to be pure MF positive , 23.10 % pure CDR , 2.98% was combined of MF and CDR and 36.25% was found to be total Endemicity Rate (ER) of LF. (ER=A+B+C, ER%= $\frac{A+B+C}{X}$ ×100%) (Table: 13).

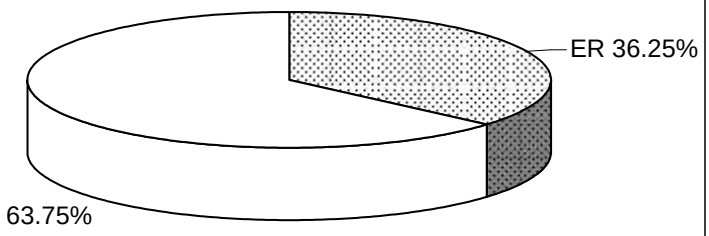
Table13: Total Endemicity Rate(ER) of LF

Total sample (X)	MF (A)	CDR (B)	MF+CDR (C)	ER
502	51(10.15)	116(23.10)	15(2.98)	182(36.25)

Source: *Field survey, 2006.*

(The figure in the parenthesis indicates the percentage)

Figure 12: Total Endemicity Rate (ER) of LF



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- **Sex-wise ER of LF**

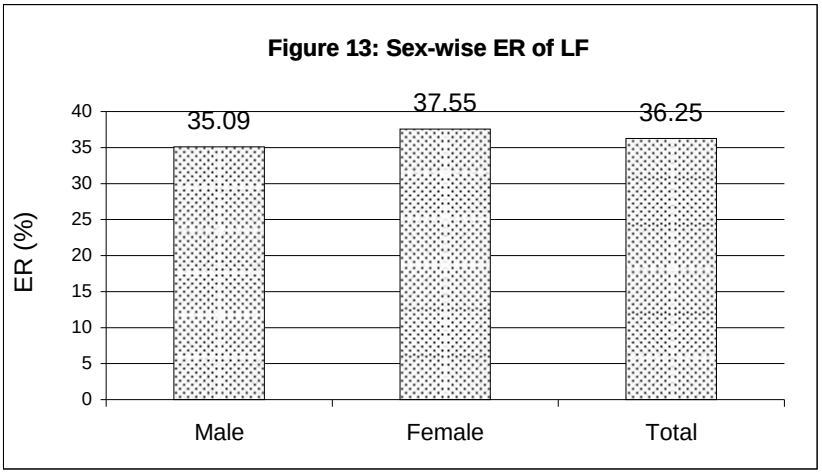
Out of 265 male samples taken, ER was found to be 35.09%, and similarly, out of 237 female samples taken, the ER was found to be 37.55%. Thus, it is obvious that the ER in male is less than in female. It is due to the fact that females are more susceptible with low resistance to the parasites. Statistically, the ER in both sexes found to be significantly indifferent ($\chi 2$ =0.08, df=1, p<0.05) (ER%= $\frac{A+B+C}{X}$ ×100%) (Table: 14).

Table14: Sex-wise ER of LF

Sex	Sample Size(X)	MF(A)	CDR(B)	MF+CDR(C)	ER (%)
Male	265	30(11.32)	54(20.37)	9(3.39)	93(35.09)
Female	237	21(8.86)	62(26.16)	6(2.53)	89(37.55)
Total	**502**	**51(10.15)**	**116(23.10)**	**15(2.98)**	**182(36.25)**

***Source**: Field survey, 2006.*

(The figure in the parenthesis indicates the percentage)



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- **Age-wise ER of LF**

As shown in the table: 14, the highest ER (57.89%) was found in the group of 61-70 years and the least (8.33%) in the age group of 2-10 years. Statistically, the ER at different age group was found to be significantly different (χ^2 =51.09, df=6, p>0.005)

(ER%= $\frac{A+B+C}{X}$ ×100%) (Table: 15).

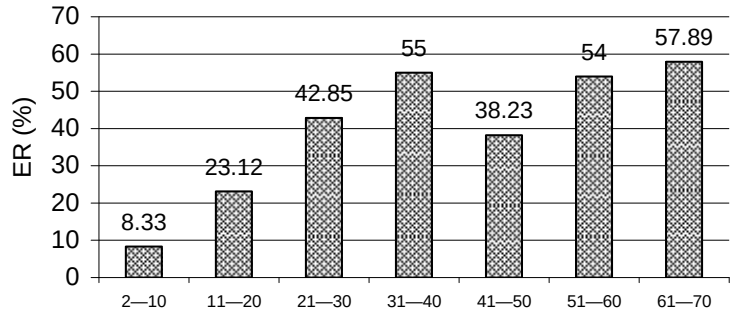
Table 15: Age–wise ER of LF

Age group(years)	Sample size (X)	MF(A)	CDR(B)	MF+CDR(C)	ER (%)
2-10	48	2(4.16)	2(4.16)	0(0.00)	4(8.33)
11-20	160	18(11.25)	18(11.25)	1(0.62)	37(23.12)
21-30	77	9(11.68)	20(25.97)	4(5.19)	33(42.85)
31-40	80	9(11.25)	32(40.00)	3(3.75)	44(55.00)
41-50	68	6(8.82)	18(26.47)	2(2.94)	26(38.23)
51-60	50	5(10.00)	19(38.00)	3(6.00)	27(54.00)
61-70	19	2(10.52)	7(36.84)	2(10.52)	11(57.89)
Total	**502**	**51(10.15)**	**116(23.10)**	**15(2.98)**	**182(36.25)**

***Source**: Field survey, 2006.*

(The figure in the parenthesis indicates the percentage)

Figure 14: Age-wise ER of LF



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VI
DISCUSSION AND CONCLUSION

Lymphatic Filariasis (LF) is one of the most important public health and socio-economic problem prevalent in many tropical and sub tropical countries in the developing World and is considered as an indicator of poverty (Durrheim *et al.*, 2004). LF is also said to be the disease of a poor environmental condition with low socio-economic status, low literacy rate and high percentage of illiterate situation (WHO, 1997). It is world wide in distribution as an endemic disease of some 80 tropical and sub tropical countries (South east Asia, Africa and South America) leading the infection of 120 millions and risk of 2 billions of people (Erlanger *et al.*, 2005).

The microfilariae (MF) were found to be only the *W. bancrofti.* In the present study the comparative morphological study of the MF was carried out. It is supported by Thakur (2000) who found only the *W. bancrofti* as the causative agent of LF in Nepal, Torres *et al.,* (2001) who reported the *W. Bancrofti* from the province of small village Sorsogon, Philippines by using the PCR as diagnostic tool and Singh *et al.,* (2002) who reported only the MF of *W. bancrofti* from the Bagdora town of Darjeeling, West Bengal, India. Similarly, Gyapong *et al.,* (2003) reported all the MF encountered as *W. bancrofti* from the 4 West African countries, Tobian *et al.,*(2003) found only *W. bancrofti* as the risk factor of hydrocele in Papua, New guinea, Boyd *et al.,* (2004) reported only the geographic distribution of *W. bancrofti* by using ICT in Leogane commune, Haiti, Mukoko *et al.,*(2004) determined only the transmission of *W. bancrofti* from the Kwale district, Kenya and Singh *et al.,*(2005) found all the positive cases of *W. bancrofti* in Bilaspur, India.

LF has been known to be endemic in Nepal since a long time. The first report of LF in Nepal was given by Jung (1973) from the central Nepal. The present study was conducted in the semi-urban population of hilly area at Ganeshsthan VDC, Nuwakot, Nepal. The general prevalence of MF in present study was found to be 13.14% which is comparable to the result of Jung (1973) who found 10.03%-11.3% of MF in semi-urban population of Central Nepal, Pradhan *et al.,* (1997) who reported 12.75% of MF from Gokarna, Kathmandu, Nepal, Gyapong *et al.,* (2003) who found 13.39% of MF in the 4 West African countries, Sherchand *et al.,* (2003) who reported 13.0% of MF

from Nepal. The prevalence of MF in present study was found to be obviously greater than as reported by Lamichhane (2006), Bhat Chhetri (2006), Maharjan *et al.*,(2006), Agrawal *et al.,* (2004), Khan *et al.,* (2004) Ghimire *et al.,* (2003) Chai *et al.,* (2003), Singh *et al.,* (2002) and Bhusal *et al.,* (2002) who reported the prevalence of MF as 9.90% ,7.54% , 2.58% , 0.1% , 1.45%, 4.72%, 1.6%, 2.31% and 5.8% respectively. On the other hand, the present result of MF prevalence was found less than as reported by Byanju (2006) who reported 22.67% of MF from Salyantar, Dhading, Nepal, Sherchand (2002) who reported 28.2% of MF and Anosike *et al.,* (2003) who reported 19.2% of MF from Bauchi, Nigeria. The MF prevalence of present study was found to be greater than that of prevalence in terai regions. It is due to the reason of similarity in climatic condition to that of terai and being the unawared and unreported virgin area of study, where no any control and treatment campaigns were administered before the present study.

Both the sexes, male and female are equally susceptible to the infection of LF. As per sex-wise prevalence of MF, present study revealed that male (14.71%) were more infected than the female (11.39%) and found the male and female MF infection ratio being 1.44:1. This result is supported by Byanju (2006), Lamichhane (2006), Singh *et al.,* (2005), Khan *et al.,* (2004), Ramirez *et al.,* (2004), Singh *et al.,* (2002), Weerasooriya *et al.,* (2001), Babu *et al.,* (2001) and Pradhan *et al.,* (1997) who reported greater prevalence of MF in male than in female. The reasons of greater male infection are: long outdoor exposure, outdoor working during the peak biting hours of mosquitoes, outdoor sleeping habit without bed nets and clothing of just a half pant and a vest. On the other hand females involve mostly in the indoor household work, indoor sleeping using the bed nets along with their children and use of more protective clothing thus remaining lower chances for transmission of MF via mosquito bites while comparing to males. However, this result is contradicted with the result of Ghimire *et al.,* (2003) and Bhat Chhetri (2006) who found that the females were more infected than the males.

All the age-groups are equally susceptible to the infection of LF. Present study revealed that the rate of prevalence of MF and clinical features have increased with the increase in age. Age is equivalent to the length of the exposure (WHO, 2001).

Although, LF is first acquired in childhood but clinical features occur after the puberty and hence increase with the age (Witt and Ottesen, 2001). In the present study highest prevalence of MF was found to be in the age group 61-70 years (21.05%) and the least in the age group 2-10 years (4.16%), that indicates an increase of MF prevalence with increase of age. It is supported by the result of Lamichhane (2006) Byanju (2006), Bhat Chhetri (2006) and Bhusal *et al.*, (2000) who found the highest MF positive cases in the age group of 61-70 years (20.0%) and >70 years (36.36%), 41-50 years (20.68) and 40-49 years (11.8%) respectively.

The study revealed that there was a rise in MF positive cases with an increase of educational status as highest MF cases were found in the literate group (27.27%) and lowest in the group of primary level (7.78%). Even though, more educated people are more aware of the disease, in the endemic area the infection takes place in the childhood and advanced as the age and educational level increased without being any control measures and treatment. Similar results were reported by Lamichhane (2006), Byanju (2006), and Bhat Chhetri (2006) who found the highest prevalence of MF in the group of literate (14.74%), Lower secondary (31.25%) and lower secondary (11.76%) respectively.

Occupation which is responsible to expose the individual outdoor is the most important risk factor for the distribution of MF. Present study revealed that the Labors (28.57%) and Teachers (28.57%) were most infected with the MF but child were found to be non-infected at all. It is due to the reason that the labors and teachers by their occupation are most outdoor exposed to mosquito bite but child are prevented from the biting of mosquitoes since they are kept under the care of mother or seniors, well-clothed and sleep under the bed nets. This result is supported by the study of Bhat Chhetri (2006), Lamichhane (2006) and Byanju (2006) where Labors and Businessmen were found the most infected.

It is the universally accepted fact that the increase of educational status definitely leads to the increase of the knowledge regarding the disease. The analysis of Knowledge, Attitudes and Practice (KAP) provides the clear picture of the risk factors of infection and facilitate for the improvement of positive attitudes to participate in the controlling and eliminating programs of LF. More people knew about the main

symptoms of LF such as lymphoedema, hydocele than about the disease name filariasis (Eberhard *et al.,* 1996). Male individuals appear to be more aware of the symptoms, especially hydrocele than females (Babu, *et al.,* 2004). In most communities it is not widely known that mosquitoes transmit the disease agent and very fewer individuals know that worms in the blood cause the disease (Eberhard *et al.,* 1996, Babu *et al.,* 2004, Das *et al.,* 2005). The cause of LF due to parasitic worm was known to only a few (5.5%) than transmission due to mosquito bites (70.0%) (Yahathugoda *et al.,* 2003). In the present study the highest percentage of knowledge of LF was found to be in the group of higher studies (81.48%) but highest percentage of MF was found in the group of literate (27.27%) and least in the group of primary level (7.78%) not in the group of higher studies (18.51%). In general the level of knowledge and MF cases were found to be in the relation of inversely proportional. It is not necessary to decrease the MF positive cases when the knowledge about LF increased; since the peoples are living in the endemic area get continually exposed even form the childhood but no treatment at all. Knowledge may initiate the strategy for treatment, control and elimination but it is not the means of so! This result is supported by Byanju (2006) and Lamichhane (2006) who reported more prevalence of MF in the knowledgeable people than their counterparts.

Environmental condition plays an important role to assure the endemicity of the disease (LF). Environment such as dirty, bushy, stagnant water, etc. are responsible for the flourishment of the mosquito vectors and definitely lead to more prevalence of MF. Present study revealed that the stagnant water was the most pronounced risk factor to distribute the MF positive cases and highest MF cases (30.0%) were reported from the inhabitants around the stagnant water. Similar result was also provided by Bhat Chhetri (2006) and Jha *et al.,* (2006) who reported the highest cases of MF from the inhabitants surrounding of stagnant water.

The MF count per smear (20µl) of blood determines the density. The average density of MF was found to be 10.16/20µl of blood. Similar results were reported by Singh *et al.,* (2002), Ramzy *et al.,* (2002), Mukoko *et al.,* (2004) Singh *et al.,* (2005) who found the density MF 7.71/20mm^3, 34/ml 322/ml and 5.05/µl respectively. The result associated with density analysis revealed that males were with greater load of

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parasites than females. Not only the higher prevalence but also the higher density of MF in male revealed that the males were more exposed to the biting of mosquito vectors than the female as determined by their occupation clothing and sleeping habits.

Functional impairment is one of the most pronounced aspects of disability in LF and the genitalia are considered as the most vulnerable organs in the infection of LF. About 66.0% of people are said to be hampered in their occupational activities by the disease (Ramaiah, *et al.,* 1997). People infected from LF become disabled, disfigured also consist of the sexual problem and totally devoid of physical as well as sexual thought (WHO, 1997). The clinical morbidity from the Papua, New Guinea is found the highest in the world (Kazura *et al.,* 2003). The chronic sign and symptoms of LF eg. Elephantiasis, Hydrocele, Breast swelling, Chyluria, etc. are the result of long lasting period of LF and describe the CDR. Present study revealed that the total elephantiasis was 7.96% with slightly more prevalent in female (8.01%) than in male (7.92%) and is supported by Babu *et al.,* (2001) and Weerasooriya *et al.,* (2001) who found that the Elephantiasis was more prevalent in female than their counterparts. Hydrocele among the male was found 10.18% which is obviously higher than the result of Lamichhane (2006) but less than the result of Byanju (2006) and Mukoko *et al.,* (2004) who found total hydrocele cases of 11.5% and 19.9% respectively. Breast swelling among the female was found to be 8.43% which is similar to the result of Byanju (2006) and Lamichhane (2006). Similarly, Chyluria among the female was found 2.95% and was obviously greater than the result of Byanju (2006) and Lamichhane (2006) who reported 1.01% and 0.42% of chyluria respectively. The total CDR was found to be 23.1% which is comparable to the study of the Jha *et al.,* (2006), Byanju (2006) and Manandhar *et al.,* (2001) who found the CDR as 23.88%, 22.09% and 21.62% respectively. The CDR was found to be increased with the increase of age, since the advancement of disease and manifestations of sign and symptoms is directly proportional to the time period. CDR in female (26.16%) was found to be greater than in male (20.37%). This is supported by the result of Lamichhane (2006), Byanju (2006), Manandhar *et al.,* (2001), Weerasooriya *et al.,* (2004) and Pradhan *et al.,* (1997) due to the reason of that the females are with the low resistivity of the disease.

The total sum of MF, CDR and MF + CDR cases describe the Endemicity Rate (ER) of the disease in the specified population. Greater the ER reveals the greater burden of disease. Present study revealed the total ER of 36.25%. It is obviously greater than the result of Jha *et al., (*2006), Lamichhane (2006) and Pradhan *et al.,* (1997), who reported total ER of 16.08%, 11.68% and 24.60% respectively but is less than the result of Byanju (2006) who reported 44.76% of total ER at Salyanter VDC of Dhading, Nepal. As Jha *et al.,* (2006) reported that the ER in hilly region was greater than that of terai region (12.54% in terai, 16.04% in mid terai and 24.05% in hilly region) the present study conducted in the hilly region found the ER to be greater than in terai. It is also strongly supported by the result of Gupta *et al.,* (2006) who found total ER in hilly region was far greater than that of terai regions. Higher prevalence of LF in hilly region than the terai region is due to the fact that being the historical problem of mosquito borne diseases in terai, people adopt the vector controlling methods as well as chemotherapy but not that of people at the hilly regions. Similarly, present study revealed that ER in female (37.55%) is slightly greater than that of male (35.09%). This is supported by the result of Jha *et al.,* (2006) but contradicted to the result of Byanju (2006), Lamichhane (2006) and Maharjan *et al.,* (2006) who found the ER in male is greater than in female. Even though, sex-wise ER was found to be more or less equal but slightly greater in females describes the low development of resistivity against the disease and manifestation of high percentage of sign and symptoms. On the other hand both the CDR and ER are age dependent (WHO 200, Witt and Ottesen, 2001). The result of present study revealed that the highest CDR (40.0%) and ER (57.89%) were found in the age group of 31-40 years and 61-70 years respectively. It is comparable to the result of Lamichhane (2006) who found the highest CDR in the age group of 41-50 years and highest ER in 61-70 years and Byajnu (2006) who found highest CDR in the age group of 41-50 years and the ER in >70 years of age group. Similarly, Bhusal *et al.,* (2000) found that the highest CDR in the age group of >70 years. As revealed by the present study the highest CDR in the middle age (31-40 years) is due to the manifestation of clinical features after the puberty of the childhood infection (Witt and Ottesen, 2001). This is also supported by Massaga *et al.,* (2000) who reported that the prevalence of clinical manifestations and MF increases with age.

By the Epidemiological survey of LF at Ganeshsthan VDC, Nuwakot, Nepal, it can be concluded that humid climate with high temperature, red soil having high water holding capacity, bushy and dirty surroundings with the water filled pits were the characteristics of the study area that were considered as the paradise of mosquito breeding and found as the major risk factors of LF. Similarly, Illiteracy, lack of awareness of disease, vectors and impact of LF were also found to be non neglected risk factors. Common habitat of cattle and human was also found to be next risk factor that was responsible for the direct contact of human and mosquito vectors. Dung pits and irrigation channels were regarded as the chief sites of vector breeding. Poor socio-economic status was found to be the number one risk factor of contributing the epidemicity of LF. It has been found that the community awareness and mobilization play the key role in the success of community based health program and realized that the high scale extensive study should be undertaken to formulate the epidemiological and aetiological factors of LF. MDA and Vector Control Campaign are found to be the instant requirement of the study area to solve the number one health problem.

VII

RECOMMENDATIONS

With the present study the followings are the important recommendations:

- The lymphatic filariasis is not much known to many people. Thus, people need to be familiar with this disease. For this awareness programs through mass media, radio and television must be expanded for protecting vector borne LF and to improve health and hygiene.
- The cause of the disease, early symptoms, detection and preventive measures and control of disease should be made familiar to the people.
- People should be made conscious to use mosquito net, mosquito coil, mosquito mats, bagon spray and ointment for protection from mosquito bite.
- Regular active surveillance should be carried out to know the prevalence of LF.
- Health education should be emphasized to improve the health status of the community. Public health education should necessarily be included from the primary level of the school and both the teachers and students should be well known about the LF as they can aware the society.
- Regular health check-up and treatment of filarial patient should also be brought about. For this specific filariasis clinic should be run in the endemic areas for the proper diagnosis of the cases. The MDA and the control program must be regularized along with the monitoring of the same study population.
- The specific taxonomic research should be carried out at the molecular level.

REFERENCES

Agrawal, C.S.; Jha, N. and Agrawal, S. (2004). Prevalence of lymphatic filariasis in districts of eastern Nepal: A population based cross-sectional household survey. *A Report Submitted to Nepal Health Researc Council,* Nepal.

Anosike, J.C.; Onwuliri, C.O.E. and Onwuliri, V.A. (2003). Human filariasis in Das local government area of Bauchi state, Nigeria. *Tropical Ecology,* Nigeria, **44(2):** 215-225.

Arora,D.R. and Arora, B. (2001). *Wuchereria bancrofti. Medical Parasitology,* 1st Ed. CBS publishers and Distributors, New Delhi. ISBN: **81-239-0729-X:** 173-179.

Babu, B.V.; Hazra, R.K.; Chhotray, G.P. and Satyanarayana, K. (2004). Knowledge and beliefs about elephantiasis and hydrocele of lymphatic filariasis and some socio-demographic determinants in an endemic community of Eastern India. *Public Health,*India, **118:** 121-127.

Bhat Chhetri, B.B. (2006). An epidemiological sentinel survey of filariasis in Vishnura VDC, Rupandehi district, Nepal. *A Dissertation Submitted to Central Department of Zoology*, T.U., Kathmandu, Nepal.

Babu, B.V.; Acharya, A.S.; Mallick, G.; Jangid, P.K.; Nayak, A.N. and Satyanarayan, K. (2001). Lymphatic filariasis in Khurda district of Orissa, India: An epidemiological study. *South East-Asian Journal of Tropical Medicine and Public Health,* India, **32(2):** 240-243.

Bhusal, K.P.; Joshi, A.B.; Mishra P.N. and Bhusal, K.(2000). Prevalence of *Wuchereria bancrofti* infections in Tokha-Chandeshwori VDC, Kathmandu. *Journal of the Institute of Medicine,* Nepal, **22**: 204-211.

Bista, M.B.; Banerjee, M.K.; Thakur, G.D. and Shrestha, S.B. (2000).Lymphatic filariasis: Review of literature and epidemiological analysis of the situation in Nepal. *An Annual Report, Epidemiology and Disease Control Division, Department of Health Services, Ministry of Nepal.*

Boggild, A.K.; Keystone, J.S. and Kain, K.C. (2004). Tropical pulmonary eosinophilia: A case series in a setting of non- endemicity. *Clinical Infectious Disease*, Toronto, USA, **39(8):** 1123-1128.

Boon, N.A.; Colledge, N.R.; Walker, B.R. and Hunter, J.A.A. (2006). Lymphatic filariasis. *Davidson's Principles and Practice of Medicine*, 20th Ed. Churchill Livingstone Press, London, UK. ISBN: **13978-0-443-10133-5:** 363-364. Chatterjee, K.D. (2005). Superfamily Filarioidea. *Parasitology*, Twelfth Ed., Chatterjee Medical Publisher, Calcutta, India: 187-202.

Boyd, H.A.; Walker, L.A.; Flanders, W.D.; Beach, M.J.; Sivilus, J.S.; Lovince, R.; Lammie, P.J. and Addis D.G. (2004). Community and individual level determinants of *W. bancrofti* in Leogane commune, Haiti. *American Journal of Tropical Medicine and Hygiene*, Haiti, **70(3):** 266-272.

Breg, M.A.; Naqvi, A.; Zaman, V. and Hussain, R. (2001). Tropical pulmonary eosinophilia and Filariasis in Pakistan. *South-east Asian Journal of Tropical Medicines and Public Health*, Pakistan, **32(1):** 73-75.

Byanju, R. (2006). Lymphatic Filariasis: Epidemiological analysis of the situation in Salyantar VDC of Dhading, Nepal. *A Dissertation submitted to Central Department of Zoology*, T.U., Kathmandu, Nepal.

Chadee, D.; Samuel, C.; Rawlins, B. and Tiwari, T.S. (2003). Concomitant malaria and Filarial infections in George town, Guyana, S. America. *Tropical Medicine and International Health*, South America, **8(2):** 125-130.

Chadee, D.D.; Williams, S.A. and Ottesen, E.A. (2002)**.** Xenomonitoring of *Culex quinquefasciatus* mosquitoes as a guide for detecting the presence or absence of lymphatic filariasis: A preliminary protocol for mosquito sampling. *Annals of Tropical Medicine and Parasitology*, Trinidad, West Indies, **96(2):** 47-53.

Chai, J.Y.; Lee, S.H.; Choi, S.Y.; Lee, J.S.; Young T.S.; Park, K.J.; Yang, K.A.; Lee, K.H.; Park, M.J.; Park, H.R.; Kim, M.J. and Rim, H.J. (2003). A survey of *Brugia Malayi* infection on the Heugsan Island, Korea. *Korean Journal of Parasitology*, Korea, **41(1):** 69-73.

Chandler, A.C. and Read C.P. (1961). Filariae, Spiruroids and Guinea worm. *Introdution to Parasitology,* 10 th Ed. Toppan Printing Company, Tokyo, Japan: 473-513.

Chandrasena, T.G.; Premaratna, R.; Abeyewickrema, W. and Desilva, N.R. (2002). Evaluation of ICT whole–blood antigen card test to detect infection due to *W. bancrofti* in Sri-Lanka. *Transaction of the Royal Society of Tropical Medicine and Hygiene*, Srilanka, **96(1):** 60-63.

Cheng, T.C. (2006). Supertamicy filariodea. *General Parasitilogy* , 2nd Ed. Academic press , California, USA. ISBN: **81-312-0163-5:** 536-544.

Das, D.; Kumar, S.; Dash, A.P. and Babu, B.V. (2005). Knowledge of lymphatic filariasis among the population of an endemic area in rural Madhya Pradesh, India. *Annals of Tropical Medicine and International Health,* India, **9:** 843-845.

Das, D.; Kumar, S.; Sahoo, P.K. and Dash, A.P. (2005). A Survey of bancroftian filariasis for microfilariae and circulating antigenaemia in two villages of Madhya Pradesh. *Indian Journal of Medical Research*, India, **121(6):** 771-775.

DoHS, Ministry of Health and Population, (2005/06)**.** Lymphatic Filariasis. *An Annual Report*, Nepal, 139-141.

Durrheim, D.N.; Wynd, S.; Liese, B.; and Gyapong, J.O. (2004). Editorial: Lymphatic filariasis endemicity-an indicator of poverty. *Tropical Medicine and International Health*, **9:** 843-845.

Eberhard, M.L.; Walker, E.M.; Addiss, D.G. and Lammie, P.J. (1996). A survey of knowledge, attitudes and perceptions (KAPs) of lymphatic filariasis, elephantiasis, and hydrocele among residents in an endemic area in Haiti. *American Journal of Tropical Medicine and Hygine,* USA, **54:** 299-303.

Engelbercht, F.; Oettl, T.; Herter, U.; Link, C.; Philipp, P.; Edeghere, H.; Kaliraj, P. and Enwezor, F. (2003). Analysis of *W. bancrofti* infections in a village community in Northern Nigeria: Increased prevalence in individuals infected with *Onchocerca volvulus*. *Parasitology Interational,* Nigeria, **52(1):** 13-20.

Erlanger, T.E.; Keiser, J.; Castro, M.C.; Bos, R.; Singer, B.H.; Tanner, M.; and Utzinger, J. (2005). Effect of water resource development and management on Lymphatic Filariasis and estimates of populations at risk .*American Journal of Tropical Medicine and Hygiene*, USA, 73(3): 523-533.

Esterve, P.; Catherine, P.; Yves, S. and Ngoc, L.N. (2001). The impact of 34 years of massive DEC chemotherapy on *W. bancrofti* infection and transmission. The Maupit cohort. *Tropical Medicine and International Health*,Polynesia, **6(3):** 190-195.

Fox, L.M.; Furness, B.W.; Haser, J.K.; Brissau, J.M.; Charles, J.L.; Wilson, S.F.; Addiss, D.G.; Lammie P.J. and Beach, M.J. (2005). Ultrasonographic examination of Haitian children with lymphatic filariasis: A longitudinal assessment in the context of antifilarial drug treatment. *American Journal of Tropical Medicine and Hygiene*, USA, **72(5):** 642-648.

Gbakima,A. A.; Appawu, M.A.; Dadzie, S.; Karikari, C.; Sackey, S.O.; Wilmot, A..B.; Gyapong, J. and Scott, A.L. (2005). Lymphatic filariasis in Ghana: establishing the potential for an urban cycle of transmission. *Tropical Medicine and International*,Ghana, **10(4):** 387-392.

Ghai, O. P. and Gupta, P. (1999). Filariasis. *Essential Preventive Medicine*, 18th Ed., Vikas Publishing House,New delhi, India. ISBN: **81-259-0633-9:** 293-332.

Ghimire, P. and Thakur, G.D. (2003). Lymphatic filariasis surveillance in Parsa District, Nepal. *A Report Submitted to Central Department of Microbiology*., Nepal*:* 7-9.

Gupta, R.; Jha, S. C.; Bam, A.B.; Subedi, J.R.; Bhusal, K.P.; Hamal, L.B. and Byanju, R.; Byanju, J. (2006). Baseline surveillance of lymphatic filariasis in Nuwakot, Dhading,Kapilbastu, Bara and Rautahat districts of Nepal. *A Final Report submitted to Filariasis elimination program, EDCD, Teku, Kathmandu, Nepal.*

Gyapong, J.O.; Kyelem, D.; Kleinschmidt, I.; Agbo, K.; Ahouandogho, F.; Gaba, J.; Owusu-Banahene, G.; Sanou, S.; Sodahlon, Y.K.; Biswas, G.; Kale, O.O.;

Molyneux, D.H.; Roungou, J.B.; Thomson, M.C.; Rem,me, J. (2002). The use of spatial analysis in mapping the distribution of bancroftian filariasis in four West African countries. *Annals of Tropical Medicine and Parasitology*, West Africa, **96(7):** 695-705.

Igbal, Jumshaid, and Alisher (2006). Determiation of lymphatic filariasis among migrant workers in Kuwait by detecting circulating filarial antigen. *Journal of Medical Microbiology*, Kuwait, **55(4):** 401-405.

Jha, S.C.; Gupta, R.; Sherchand, J. B. and Jha, R.G. (2006). Microfilarial infection in eight districts of Nepal. *Nepalese Journal of Zoology*, Nepal, **1(1):** 26-32.

Jung, R.K. (1973). A brief study on the epidemiology of filariasis in Nepal. *Journal of Nepal Medical Association*, Nepal, **11**: 155-168.

Kazura, J. and Bockarie M.J. (2003). Lymphatic filariasis in Papua, New Guinea: Interdisciplinary research on a national health problem. *Trends in Parasitology*, New guinea, **19(6):** 260-263.

Kelly- Hope, L.A.; Diggle, P.J.; Rowlingson, B.S.; Gyapong, J.O.; Kyelem, D.; Coleman, M.; Thomson, M.C.; Obsomer, V.; Lindsay, S.W.; Hemingway, J. and Moleneux, D.H. (2006). Short communication: Negative spatial association between lymphatic filariasis and malaria in West Africa. *Tropical Medicine and International Health*, West Africa, **11(2):** 129-135.

Khan, A.M.; Dutta, P.; Khan S.A. and Mahanta, J. (2004). A focus of lymphatic filariasis in a tea garden worker community of Central Assam, India. *Jojurnal of Environmental Biology*,India, **25(4):** 437-440.

Koyadun, S.; Bhumiratana, A. and Prikchu, P. (2003). *Wuchereria bancrofti* antigenemia clearance among Myanmar migrants after biannual mass treatment with diethylcarbamazine, 300mg oral- dose FILADEC tablet, in Southern Thailand. *South- East Asian Journal of Tropical Medicine and Public Health*, Thailand, **34(4):** 758-767.

Krentel, A.; Fisher, P.; Supali, T.; Servais, G. and Ruckret, P. (2006). Using knowledge, attitudes and practice (KAP) surveys on lymphatic filariasis to

prepare a health promotion campaign for mass drug administration in Alor District, Indonesia. *Journal of Tropical Medicine and International Health,* Indonesia, **11(11):** 1731-1740.

Krishnamoorthy, K.; Subramanian, S.; Van, G.J.; Oortmarssen, J.D.; Habbema, F. and Das, P.K. (2004). Vector survival and parasite infection: The effect of *Wuchereria bancrofti* on its vector *Culex quinquefasciatus. Parasitology*, India, **129(Part I):** 43-50.

Lamichhane, J. (2006). A prospective study of lymphatic filariasis in an endemic village of Kapilbastu district, Nepal. *A dissertation submitted to Central Department of Zoology*, T.U., Kathmandu, Nepal.

Leang, R.; Socheat, D.; Bin, B.; Bunkea, T. and Odermatt, P. (2004). Assessment of disease and infection of lymphatic filariasis in North-eastern Cambodia. *Tropical Medicene and International Health,* Cambodia, **9(10):** 1115-1120.

Maharjan, M.; Jha, S.C.; Khanal, M.; Bhat Chhetri, B.B.; Bam, A.B.; Dhakal, D.N.; Chaudhary, B.; Pokhrel, Y.B. and Jha, R.G. (2006). Epidemiological surveillance of lymphatic filariasis in Makwanpur, Chitwan, Rupandehi and Nawalparasi districts of Nepal. *A Final Report,Submitted to Filariasis Elimination Program, Epidemiology and Disease Control Division (EDCD),Teku, Kathmandu, Nepal.*

Manandhar, R.; Tuladhar, N.R. and Sherchand, J.B. (2001). Epidemiological study of Microfilariasis in three different geographical regions of Nepal. *Journal of Department of Microbiology, Institute of Medicine,* Nepal, **112:** 1-10.

Massaga, J.J.; Salum, F.M. and Sareal, Z.X. (2000). Clinical and parasitological aspects of bancroftian filariasis in Hale, Northern Tanzania. *Central Africa Journal of Medicine,* Tazania, **46:** 236-241.

Mathieu, E.; Direny, A.N.; Rochars, M.B.; Streit, T.G.; Addiss, D.G.; and Lammie, P.J. (2006). Participation in three consecutive mass drug administrations in Leogane, Haiti. *Tropical Medicine and International Health,*Haiti, **11(6):** 862-868.

Mathieu, E.; Lammie, P.J.; Radday, J.; Beach, M.J.; Streit, T.; Wendtnd, J. and Addiss, D.G. (2004). *Annals of Tropical Medicine and Parasitology*, Haiti, **98(7):** 703-714.

Mcpherson, T.; Singh, S.; Fay, M.P.; Addiss, D.; Nutman.T.B. and Hay, R. (2006). Interdigital lesions and frequency of acute dermato-lymphangioadenitis in lymphoedema in a filariasis endemic area. *British Journal of Dermatology*, UK, **154(5):** 933-941.

Melrose, W. and Rahmah, N. (2006). Use of Brugia rapid dipstick and ICT test to map distribution of lymphatic filariasis in democratic republic of Timor-Leste. *South-East Asian Journal of tropical Medicine and Public Health*, Timor-Leste, **37(1):** 22-25.

Moleneux, D.H. and Zagaria, N. (2002). Lymphatic filariasis elimination: progress in global program development. *Tropical Medicine and Parasitology*, **96(2):** 15-40.

Mukoko, D.A.N.; Pedersen, E.M.; Masese, N.N. and Estambale, B.B.A. (2004). Bancroftian filariasis in 12 villages in Kwale district, coast province, Kenya: Variantion in clinical and parasitological patterns. *Annals of Tropical Medicine and Parasitology*, Kenya, **98(8):** 801- 815.

Murty, U.S.; Praveen, B.; Satyakumar, D.V.R.; Sriram, K.; Rao, K.M. and Sai, K.S.K. (2004). A baseline study of rural Bancroftian filariasis in Southern India. *South- East Asian Journal of Tropical Medicine and Public Health,*India, **35(3):** 583-586.

Onapa, A.W.; Simonsen, P.E.; Baehr, I. and Pedersen, E. M. (2005). Rapid assessment of geographical distribution of lymphatic filariasis in Uganda by screening of school children for circulating filarial antigens. *Annals of Tropical Medicine and Parasitology*, Uganda, 99 **(2):** 141-153.

Parija, S.C. (1996). Filarial Nematod Parija, S.C. (1996). Filarial Nematodes. *A text book of medical parasitology*, 1st Ed. All India publisher and distributors, Chennai, India. ISBN: **81-8004-006-2:** 314-330.

Pradhan, S.P.; Shrestha, I.; Palikhey, N. and Uprety, R.P. (1997). Epidemiological study of lymphatic filariasis in Gokarna VDC of Kathmandu valley. *Journal of Nepal Health Research Council,* Nepal, **2**: 13.

Ramachandran, C.P. (1993). Lymaphatic filariasis and Onchocerciasis. *TDR Eleventh Program Report*, **9593:** 37-46.

Ramaiah, K.D. Vijaya Kumar, K.N., Ravi, R. and Das, P.K. (2005). Situation analysis in a large urban area of India, Prior to branching a programme of mass drug administration to eliminate lymphatic filariasis. *Annals of Tropical Medicine and Parasitology,*India, **99(3):** 243-252.

Ramaiah, K.D.; Vijay Kumar, K.N.; Ramu, K.; Pani, S.P.; and Das, P.K. (1997). Functional impairment caused by lymphatic filariasis in rural areas of South India. *Tropical Medicine and International Health,* India, **9(2):** 832-838.

Ramirez, B.L.;Hernander, L.; Alberto, F.F.; Collins, M.; Nfonsam, V.; Punsalan, T. and Kron, M.A. (2004). Contrasting *Wuchereria bancrofti* microfilaria rates in two Mangyan Populated Philippine villages. *American Journal of Tropical Medicine and Hygiene,* Philippines, **71(1):** 17-23.

Ramu *et al.* (1996). Impact of lyamphatic filariasis on the productivity of male weavers in a south Indian Village. *Transition of the Royal Sociaty of Tropical Medicine and Hygiene,* India, **90:** 669-670.

Ramzy, M.R.; Setouhy, M.E.; Helmy, H.; Kandil, A.M.; Ahmed E.S.; Farid, H.A.; Faris, R. and Weil, H.J. (2002). The impact of single dose diethyl carbamazine treatment of bancroftian filariasis in a low-endemicity setting in Egypt. *American Journal of Tropical Medicine and Hygiene,* USA, **67(2):** 196-200.

Rochars, D.; Beau, M.V.E.; Mollord, M.D.; Jean Y.ST.; Desormeaux, A.M.; Dorvil, J.J.; Lafontant, J.G.; Addiss, D.G. and Streit, T.G. (2004). Geographic distribution of lymphatic filariasis in Haiti. *American Journal of Tropical Medicine and Hygiene,* USA, **71(5):** 598-601.

Sherchand, J.B.; Obsomer, V.; Thakur, G.D. and Hommel, M. (2003). Mapping of lymphatic filariasis in Nepal. *Electronic Journal, Filaria Journal of WHO,* Nepal, **2(7):** 16-26.

Sherchand, S. (2002). Demonstration of strategy for elimination of lymphatic filariasis (*W. bancrofti*) in Nepal. *Journal of Nepal Health Research Council,* Nepal, **1(2):** 25-29.

Singh, S.; Raina, V.K.; Bora, D.; Dariwal, A.C. and Lal, S. (2005). Lymphatic fialariasis in Bilaspur district, Chattisgarh. *Journal of Communicable Disease*, India, **37(2):** 125-130.

Singh, S.V.; Bora, D.; Sharma, R.C. and Datta K.K. (2002). Bancroftian filariasis in Bagdora town, Darjeeling (West Bengal, India). *Journal of Communicable Disease,* India, **34(2):** 110-117.

Smyth, J.D. (1994). Human filariasis. *Animal Parasitology*, 3rd Ed. Cambridge Unuversity Press, UK. ISBN: **0-521-56696-7:** 625-628.

Taylor, M.J.; Helen, F.C.; Louise, F.; Williams, H.M.; Prasad, K. S. and Bilo, K. (2002). *Wolbachia* bacteria in filarial immunity and disease. *Parasite Immunology (Oxford),* UK, **23(7):** 401-409.

TDR (2002). Research on Tropical disease. Strategic emphasis for LF research in TDR. *Diseases Burden and Epidemiological Trend.*

TDR News (2005). **06:** 19-28.

Tobian, A.R.; Nandao, T.; Moses, B.; James, W.K. and Christopher, L.K. (2003). *American Journal of Tropical Medicine and Hygiene,* USA, **68(6):** 638-642.

Torres, E.P.; Bernadette L.R.; Ferdinad S.; Macelio, J.P.; Judith, G.A.; Mario L.S. and Julius Clemence, R.H. (2001). Detection of bancroftian filariasis in human blood samples from Sorsogon province, the Philippines by polymerase chain reaction. *Parasitilogy Research*, Philippines, *87***(8):** 677-679.

Weerasooriya, M.V.; Itoh, M.; Mudalige, M.P.S.; Qiu, X.G.; Kimura, E.; Gunawardena, N.K. and Yfujimaki, Y. (2003). Human infection with *W. bancrofti* in Matara, Sri-Lanka: The use in parallel of an ELISA to detect filarial- specific IgG_4 in urine and of ICT card test to detect filarial antigen in whole blood. *Annals of Tropical Medicine and Parasitology,* Sri Lanka, **97(2):** 179-185.

Weerasooriya, M.V.; Weerasooriya, T.R.; Gunawardeha, N.K.; Samarawickrema, W.A. and Kimura, E. (2001). Epidemiology of bancroftian filariasis in three sub-urban areas of Matara, Sri-Lanka. *Annals of Tropical Medicine and Parasitology*, Sri Lanka, **95:** 263-273.

WHO (1993). International Task Force for Disease Eradication. Recommendations of the International Task Force for Disease Eradication. *MMWR Recommendation Report*, 42: 1-38.

WHO (1995). Lymphatic filariasis and Onchocerciasis. *TDR Twelfth Program Report*, **10532:** 87-100.

WHO (1997). Lymphatic filariasis Research on Hope. *CTD/FIL*, **4:**1-20.

WHO (1999). Initial Assessment , Monitoring And Certification. *A Report of WHO Informal Consultation on Epidiomiological Approaches to Elimination of Lymphatic filariasis.*

WHO (2000). Eliminate filariasis attack poverty. *CDS/SPE*, **5:**1-35.

WHO (2001). Regional strategic plan and for elimination of lymphatic filariasis. *SEA/FIL*, **28:** 1-6.

WHO (2004). *The World Health Report 2004-Changing History.* Geneva: World Health Organization.

Witt, C. and Ottesen, E.A. (2001). Lymphatic filariasis: An infection and childhood. *Tropical Medicine and International Health*, **6:**582-606.

Witt, C. and Ottesen, E.A. (2001). Lymphatic filariasis: An infection in childhood. *Tropical Medicine and International Health*, **6:** 582-606.

Yahathugoda, T.C.; Wickaramasinghe, D.; Liyanage, T.S.; Weerasooriya, M.V.; Mudhalige, P.S.; Waidyaratna, I. and Samarawickrema, W.A. (2003). Knowledge on lymphatic filariasis and response to July 2002 mass treatment in two communities in the Galle district, Sri Lanka. *Ceylon Medical Journal*, Sri Lanka, **48(3):** 74-77.

Yahathugoda, T.C.; Wickramasinghe, D.; Weerasooriya, M.V. and Samarawickrema, W.A. (2005). Lymphoedema and its manangement in cases of lymphatic

filariasis is the current situation in three suburbs of Matara, Sri Lanka, before the introduction of a morbidity-control program. *Annals of Tropical Medicine and Parasitology,* Sri Lanka, **99(5):** 501-510.

Zagaria, N. and Savioli, L. (2002).Elimination of Lymphatic Filariasis: A Public Health Challenge. *Annal of Tropical Medicine and Parasitology,* 96(2):3-13.

ANNEX-I

Taxonomic Keys of Microfilariae

ANNEX-II

Structure of Microfilariae

ANNEX-III

Possible Causes of Misidentification of Microfilariae (MF)

a. Broken or Folded Tail

If the tail of *W. bancrofti* is broken or folded over it appears to have nuclei extending to the tip like *L. loa*.

b. Torn or Colorless Sheath

Sometimes the sheath is torn or almost colorless. In *L. loa* for example, the sheath appears as a colorless space in between the tail and blood cells.

c. Usually Large or Small Microfilariae

Some small microfilariae e.g. *M. perstans* are very long (200µm) and small some large microfilariae e.g. *W. bancrofti* and *L. loa* are very small (250µm) appearing almost the same.

d. Badly Made Smears (Films)

If it is damaged when the smear (film) is being made, *W. bancrofti* may appear twisted and *L. loa* may show a few curves.s

ANNEX-IV

Questionnaires for Lymphatic Filariasis: Microfilariae Identification and Epidemiological Survey in Ganeshsthan VDC, Nuwakot, Nepal

Date: ----------

House No.: ----------

S.No.: ----------

1. **Name of respondent:** ----------
2. **Address:**

   District: ----------

   VDC/Municipality: ----------

   Ward No.: ---------- Tole: ---------- Block No.:----------
3. **Age:** ---------Years.
4. **Sex:** I) Male ( ) II) Female ( )
5. **Education:**

   I) Illiterate ( ) II) Literate ( ) III) Educated ( )

   If educated:

   a) Primary ( ) b) Lower secondary ( )

   c) Secondary ( ) d) SLC ( )

   e) Intermediate ( ) f) Bachelor ( )

   g) Master ( ) h) Others ( )
6. **Occupation:**

   I) Farmer ( ) II) Businessman ( )

   III) Teacher ( ) IV) Labor ( )

   V) Housewife ( ) VI) Student ( )

   VII) Unemployed ( ) VIII) Others ( )

   IX) Child ( )
7. **Marital Status:** I) Married ( ) II) Unmarried ( )
8. **Relationship with the head of household:** ----------
9. **Respondent's current residence status:**

   I) Birthplace ( ) II) Migration ( )

   III) Temporary ( ) IV) Others ( )

10. Surrounding environmental status:

I) clean ( ) II) Dirty ( )

III) Bushy ( ) IV) Open drainage ( )

V) Stagnant water ( ) VI) Open ground night soil disposal ( )

11. Do you use any means to protect mosquito biting?

I) Yes ( ) II) No ( )

If yes, which one:

a) Use of anti mosquito cream ( ) b) Use of mosquito coil ( )

c) Use of smoke ( ) d) Use of mosquito net ( )

e) Use of domestic herbs ( ) f) Others ( )

12. Do you know about the disease Lymphatic filariasis?

I) Yes ( ) II) No ( )

If yes, how does this disease transmit?

a) By mosquito biting ( ) b) By contact of the diseased patient ( )

c) From mother to fetus ( ) d) Others ( )

13. How can we prevent this disease? May you give any suggestion?

I) ----------

II) ----------

14. What you think is the disease lymphatic filariasis more in parent's time or now?

I) Parent's time ( ) II) Now ( ) III) Don't know ( )

15. Respondent's current heath status:

I) Healthy ( ) II) Unhealthy ( )

If unhealthy, since when? ----------years.

Do you have any symptoms listed as follows?

a) Fever ( ) b) Headache ( )

c) Swollen of genital organ ( ) d) Swollen breast ( )

e) Swollen lymph node ( ) f) Hydrocele ( )

g) Swollen limbs ( ) h) Thick skin ( )

i) Chyluria ( ) j) Swollen nerves ( )

k) Weakness ( ) l) Epigastric pain ( )

m) Nausea ( ) n) Abscess formation ( )

If yes, have you used any medicine? i) Yes ( ) ii) No ( )

If yes, which medicine? ----------

16. Have you seen the person suffering from the disease lymphatic filariasis?

I) Yes ( ) II) No ( )

If yes, a) Where? ---------- b) When? ----------

c) How many? ----------

17. Is there any person suffering from the disease lymphatic filariasis in your family or in relatives?

I) Yes ( ) II) No ( )

If yes, who is he/she? ---------- (Your relation).

Signature of consent participation.

(Signature of the respondent)

Thank you, very much for providing your valuable time.

Result of blood examination: Positive ( ) Negative ( )

If Positive, Number of micrifilariae per 20µl of blood----------.

Signature of Investigator

ANNEX-V

Life cycle of *Wuchereria bancrofti*

Adult worms are found in the lymph glands and associated ducts of humans. Gravid female give birth to sheathed microfilariae (MF) that are 127-320 µm long. Nocturnal periodicity i.e., occurrence of microfilariae in the peripheral circulation at night has been reported in many instances. However, certain strains do not demonstrate this phenomenon .For example, in the pacific strain of *W. bancrofti*, found on various Polynesian islands in the south Pasific, the microfilariae show only slight periodicity, and it is diurnal i.e., they appear in the greater numbers in the peripheral blood during the day. The South-Pacific strain is said to be sub periodic.

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In strains that demonstrate nocturnal periodicity microfilriae are most plentiful in the peripheral circulation between 10:00 PM and 2:00 AM If these microfilariae are ingested by a mosquito during a blood meal, the mosquito phase of life cycle commences. Unlike the malaria organisms, filarial worms show little specificity in regard to mosquito hosts. *W. bancrofti* can utilize *Culex spp., Aedes spp., Mansonia spp., Anopheles spp.,* and *Psorophora spp.* as vectors equally well.

On reaching the mid gut of the mosquito, the microfilariae lose their sheaths within 2 to 6 hours, penetrate the gut wall, and migrate to the thoracic muscles. At this site, the organism becomes shortened into a

Short, sausage-shaped body measuring 124-250 µm. At this stage of development, the first true molt occurs, after which the tail portion atrophies and the intestinal tract becomes well defined. A second molt follows, and the resulting filiform L_3, measuring 1.4-2 mm in length, migrates anteriorly into the proboscis sheath of the mosquito's mouth parts.

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When an infected mosquito feeds again, the larvae can enter the human host through the puncture wound, i.e., they are not inoculated as the malarial parasites. The last two molts occur within man. It has been determined that the infection of mosquitoes can only occur if 15 or more microfilariae are present in every 20 mm^3 of blood. If there are 100 or more microfilariae in every 20 mm^3 of blood, the mosquito is commonly killed. Under experimental conditions, development in the mosquito takes 2 weeks at 27^0C and 90% humidity.

ANNEX-VI

Method of Preparing the Dilute Giemsa Solution

Giemsa stain:

Giemsa stock stain is an alcohol based Romanowsky stain. It is highly inflammable with flash point 12^0C, which requires dilution in buffered water (P^H 7.1-7.2) before its use. It gives best staining of the microfilarial parasites in both the thick and thin films of blood. The concentration of the stain is low and the staining time is long. The care must be taken to prevent the entering of water into the stock stain.

Preparation of 10% working Giemsa solution

1. Take a measuring cylinder of the capacity of 100ml.
2. 90 ml of distilled water and 10 ml of Giemsa stock solution is poured in the same cylinder and mixed thoroughly.
3. Thus the prepared solution is now ready to be used.

Preparation of reagents for Giemsa stain (stock solution)

To make the 500 ml of the Giemsa stain the following are the chemicals required and the procedures of the preparation:

Chemicals required:

Giemsa……3.8 grams.
Glycerol……...250ml.
Methanol……..250ml.

Procedures:

1. Take the 3.8 gm. of Giemsa and put it into the clean and dry beaker of the capacity of 500 ml. that contains some glass beads.
2. Take a measuring cylinder and measure 250 ml of Glycerol and pour it into the beaker along with the Giemsa.
3. Similarly measure 250ml of Methanol and pour it in the same beaker together with the Giemsa and Glycerol.
4. Shake well to mix all the contents thoroughly.
5. Place the resultant mixture in bottle and place it into the water bath at the temperature 50-60^0C for 5-10 minutes or up to 2 hours at the temperature of 37^0C to dissolve the stain thoroughly.
6. Pour the prepared stain in a clean bottle and label it as an inflammable and toxic.
7. Store the preparation in the room temperature in the dark and dry condition for the long term storage.
8. For use filter a small amount of stain into a dry-dispensing container.

Caution:

1. Giemsa stain will be spoilt if water enters into the stock solution during its preparation or storage.
2. Methanol is toxic and highly inflammable. Therefore, it should be handled with the great care and should be kept away from the open fire.

Controlling of stains, reagents and equipments:

Giemsa stain is used mainly for staining microfilaria, malarial parasites, trypanosomes and leishmanial parasites. The stains, reagents and equipments are controlled as follows:

1. Only reliable and if possible ready made and standardized stain should be used.
2. The stock stain should be stored in a dark bottle and precautions should be taken to avoid moisture entering.
3. For routine use, a small amount of the stock stain should be transferred to a dry dispensing bottle (that can be closed tightly after use).
4. The quality of all new batches of Giemsa should be checked by using it to stain microfilarial parasite for the control purpose.
5. Thick and thin blood film should be prepared from the fresh blood. The dried films are folded individually in the paper, sealed in a plastic bag and stored in a freezer at 20^0C.

Characteristics of Giemsa stain:

Resulting color of different organelles of the parasite after staining with Giemsa stain are as follows:

1. Chromatin of parasite……Dark red.
2. Cytoplasm of the parasite …….Blue.
3. Schuffner's dots…….Red.
4. Maurer's dots (Clefts)……Red mauve.
5. Red cells……Grey to pale mauve.
6. Reticulocytes…….Grey blue.
7. Nuclei of neutrophils……..Mauve purple.
8. Granules of eosinophils……..Red.
9. Cytoplasm of mononuclear cells……Blue green.

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