

**AN EPIDEMIOLOGICAL ANALYSIS OF LYMPHATIC
FILARIASIS IN KABAHIGOTH VDC, KABAHI, BARA
DISTRICT OF NEPAL**



A THESIS

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

BY

MR. TRAILOKYA NATH JOSHI



TO

**CENTRAL DEPARTMENT OF ZOOLOGY
INSTITUTE OF SCIENCE AND TECHNOLOGY
TRIBHUVAN UNIVERSITY, KIRTIPUR,
KATHMANDU, NEPAL**

2008

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RECOMMENDATION

This is to certify that **Mr. Trailokya Nath Joshi** has completed his dissertation work entitled “**AN EPIDEMIOLOGICAL ANALYSIS OF LYMPHATIC FILARIASIS IN KABAHIGOTH VDC, KABAHI, BARA DISTRICT OF NEPAL**” as a partial fulfillment of the Master’s Degree of Science in Zoology with special paper Parasitology under our supervision. His work is an original research study deserves to recommendation and has not been submitted for any other degree.

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APPROVAL LETTER

On the recommendation of supervisor **Dr. Ranjana Gupta**, Associate Professor Central Department of Zoology T.U., Kirtipur, this thesis of **Mr. Trailokya Nath Joshi** is approved for examination and is submitted to the Tribhuvan University in partial fulfillment of the requirements for Master's Degree of Science in Zoology with Parasitology as a special paper.

Prof. Dr. Vasant Kumar Thapa
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Date:

CERTIFICATE OF APPROVAL

We, the members of expert committee, evaluated the dissertation work entitled “**An Epidemiological Analysis of Lymphatic Filariasis in Kabahigoth VDC, Kabahi, Bara District of Nepal**” presented by **Mr. Trailokya Nath Joshi** and found it to be satisfactory in scope and quality as a dissertation for the partial fulfillment of Master’s Degree of Science in Zoology with Parasitology as a special paper.

Expert Committee

Prof. Dr. Vasant Kumar Thapa

Head of Department
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ACKNOWLEDGEMENTS

I am deeply indebted to my honorable supervisor **Dr. Ranjana Gupta**, Associate Professor of Central Department of Zoology, T.U., for her warm support, encouragement and guidance during the whole dissertation work.

I would like to express my gratitude to the Head, Central Department of Zoology **Prof. Dr. Vasant Kumar Thapa** and all the teachers of Zoology Department for their time to time valuable suggestions.

I would like to express my sincere gratitude to respected teachers **Mr. Janak Raj Subedi** and **Mr. Satish Chandra Jha** for lab work proceedings and management. I would like to give hearty thanks to **Mr. Pitambar Dhakal** and **Mr. Bhoj Bahadur Bhat Chhetri** for useful suggestions. No word is enough to express my gratitude to **Mr. Ashok Bahadur Bam** who guided and inspected the whole work in a systematic way. I feel glad to express deep thanks to all the staffs of Zoology who punctually helped to manage the equipment on time without hesitation and delay. It is a pleasure to respect all of my friends who gave encouragement to complete this work, especially **Mr. Rupak Shah** to manage Camera, **Mr. Bhim Dahal** for photographing, and **Mr. Lil Bahadur Hamal** for managing valuable records. Thanks to **Mr. Binod Adhikari**, **Mr. Hom Kumar Dawadi**, **Mr. Rawet Ranjan Thakur** and other friends for their supportive helping hand and encouragement until the completion of this dissertation work. I am thankful to “PARASED, NEPAL” for training and management. I am thankful to all the staffs of Primary Health Care Centre of Kabahigoth of Bara District for good cooperation and Nepal Government for making peaceful environment.

Last but not the least, I would like to extend my deepest gratitude to my respectable parents and other family members, without their support, I would not have been able to complete this work and achieve the present academic position.

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ABSTRACT

Lymphatic filariasis is one of the most prevalent tropical and subtropical diseases that lead to permanent and long term disability. It is a infectious disorder caused by threadlike nematode parasite, *Wuchereria bancrofti*. The present study was conducted from 15 December 2006 in Kabahigoth VDC of Bara district. A total of 506 night blood samples were collected from the ear lobes of the respondents. The respondents were informed about the study through mass orientation program. The questionnaires were filled up during day time in order to take information about the participating respondents before night blood sample collection. Microscopical examination showed 1/506 (0.19%) microfilaria +ve cases. The only microfilarial +ve case was found in a male/female of 41-50 years as group. Crude disease rate was reported to be 31(6.13%) and the total endemicity rate was 32(3.32%). The main factors affecting the prevalence of the disease were lack of awareness about the disease and its transmission carelessness of using mosquito nets and carelessness about their health. Most of people were not familiar to LF. Thus people should be made familiar to LF by organizing awareness programme through mass media such as pamphlets, radio, television etc. Control of vector mosquito is also necessary.

LIST OF ACRONYMS

ADL	-	Adenolymphagitic
CBOS	-	Central Bureau of Statistics
CDR	-	Crude Disease Rate
CFA	-	Circulating Filarial Antigen
DEC	-	Diethyl Carbamazine
DOHS	-	Department of Health Service
EDCD	-	Epidemiological Disease Control Division
ELISA	-	Enzyme Linked Immunosorbent Assay
ER	-	Endemicity Rate
FILDAC	-	Filarial Diethyl Carbamazine
NG	-	Nepal Government
ICT	-	Immunochromatographic test
LF	-	Lymphatic filariasis
MDA	-	Mass Drug Administration
MF	-	Microfilaria
MOH	-	Ministry of Health
PARASED	-	Parasitological Research and Socio-Environmental Development Nepal
PMDH	-	Per man hour density
VDC	-	Village Development Committee
WHO	-	World Health Organization

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I

INTRODUCTION

The nematodes of the super family “Filarioidea” are generally referred to as the filarial worms or simply filariae. An infection caused by any of the parasitic filariae is referred to as “filariasis.” Eight filariae, viz., *Wuchereria bancrofti*, *Brugia malayi*, *Brugia timori*, *Onchocerca volvulus*, *Loa loa*, *Mansonella perstans*, *Mansonella streptocerca* and *Mansonella ozzardi* are known to infect humans. Dipteran vectors transmit all these (Cheng, 1986). Among the eight human parasitic filariae, *W. bancrofti*, *B. malayi* and *B. timori* are known to cause lymphatic filariasis (LF). *W. bancrofti* is found exclusively in man while other species infect animals too. (Rozendaal, 1999).

WHO has identified LF as the second leading cause of permanent and long term causing disability in the world after leprosy. WHO has named filariasis as one of the six “potentially eradicable” infectious diseases and initiated a program in 1997 to eliminate LF globally as a public health program.

The symptoms of bancroftian filariasis has been mentioned as “elephantiasis arabicum” in the ancient literature “Sushruta Sangita” (Sushruta, 6000 B.C.). The term “Malabar leg” was applied to the condition by Clark in Cochin in 1709 A.D. Microfilariae (1st stage larva) were first demonstrated by Demarquay in 1863 in the hydrocoele fluid of a patient from Cuba. In 1968 Otto Wucherer in Brazil found Microfilariae in the urine of the patient with haematochyluria, Lewies (1872) in India demonstrated the same in the peripheral blood. Adult worms were found by Bancroft in

Brisbane in 1876 and named *Filaria Bancrofti* by Cobbold in 1877. In 1921 this species was included in the genus *Wuchereria* (Arora and Arora, 2001).

Sir Patrick Manson, working in China in 1878, observed the development of *Wuchereria bancrofti* in the mosquito *Culex quinquefasciatus* and established the essential role of the vector. A year earlier he confirmed the hypothesis that this nematode was the cause of elephantiasis (Cheng, 1986). In 1881 he described the nocturnal periodicity of *W. bancrofti*, the microfilariae present in greatest numbers in the peripheral blood during night hours (Cook, 1996).

Recently, lymphatic filariasis is endemic in 80 countries and more than 2 billions people are at risk globally (Erlanger *et al.*, 2005).

But in South East Asia, 60% global burden is seen. Annual report of Department of Communicable Disease Control showed the current lymphatic filariasis in South East Asian countries is increasing so 60% global burden is seen (27, April 2006).

About 1.1 billion people (20% of the world's population) in some 80 endemic countries located in tropical areas of the world are at risk of infection by *Wuchereria bancrofti* and *Brugia malayi* (Michael *et al.*, 1996).

More than 15 millions people suffered from elephantiasis Lymphoedema/filariasis, 27 million from hydrocoele, 83 million people from lymphatic functional disability and 30 millions from renal pathology (WHO, 1997). Approximately one third of people with infection live in India, Africa and South East Asian countries where 90% infection is due to *W. bancrofti* and 10% by *Brugia malayi* is seen (WHO, 1997). In South East Asian region, about 600 million people live in the endemic areas constituting about 60% of the global burden. Nocturnal periodic *W. bancrofti* is endemic in tropical Americas, Caribbean, Tropical Africa, Egypt, Middle East, India,

South East Asia, Southern and Eastern China the far East and New Guinea. The diurnal sub periodic variant of *W. bancrofti* is found mainly in the eastern pacific (Polynesia). The nocturnal subperiodic variant is found especially in Thailand and Vietnam (Cheesbrough, 1998).

The problem of lymphatic filariasis is found to be grave in India, Indonesia and China. These three countries together account for more than two third of the total infected population. India alone accounts for about 50% of the total cases occurring worldwide. In India most of the cases are due to bancroftian filariasis (Ghai and Gupta, 1999). Filariasis represent a significant impediment to economic development, as a result of lost working hours, and the cost of treating the sick and controlling the vectors of disease (Rozendaal, 1999).

The infection is seen hidden largely due to several years of disease proliferation (Dreyer, 1997). So, its magnitude and public health impact are often not recognized by government.

It is estimated that the LF is endemic in 80 countries including 120 million people (WHO, Geneva, 2000). It is due to socio-economical burden in the tropics and subtropics.

Nepal is bordered along its eastern, southern and western sides by the Indian states of Sikkim, west Bengal, Uttar Pradesh (UP) and Uttaranchal. Bihar and UP are among the states of India most affected by Lymphatic filariasis (Park, 2000). Since the border of Nepal and India is open, the movement of people from both the sides across the boarder is very common and climatic condition is also similar so transfer of disease and vector is made possible.

In Nepal out of 75 districts, 60 districts are seen infected with lymphatic filariasis (DOHS, MOH, NG, Nepal, 2004). Report showed 24 million peoples are at risk (DOHS, 2006).

Out of total population of world 23.2 million approx 60%, 13.9 million are estimated at risk of filariasis. Out of 3 species (*W. bancrofti*, *B. malayi* and *B. timori*) of lymphatic filarial parasites only one species i.e. *W. bancrofti* has been reported in Nepal (Thakur, 2000).

The previous work on filariasis were done by Jung (1973), Pradhan *et al.*, (1997), Bhusal *et al.*, (2000), Bista *et al.*, (2000), Manandhar (2001), etc. More detail work recently done by Sherchand *et al.*, (2002) studied mapping of lymphatic filariasis in Nepal on the basis of 37 districts of Nepal. The average of 13% people were infected. The study showed that 33 districts were endemic for lymphatic filariasis. Out of the 33 districts, 11 districts were having above 20% prevalence rate, 15 districts between 6-19% and 7 district between 1-5%.

Significance of the Study

In 1993 when the “International Task force for Disease Eradication” identified lymphatic filariasis as one of only six diseases meeting the criteria for being eradicable or potentially eradicable. Since that time, efforts towards that goal have moved ahead rapidly. Recognizing that this disease of disability was a major health drain on the economics, wellbeing and development of the 80 mostly poor nations where lymphatic filariasis remained endemic and that tools were available that could eliminate it, the World Health Assembly passed a resolution in 1997 proposing as a public health goal for the global elimination of lymphatic filariasis.

In 2002, national programs were active in 38 of the 80 countries where LF is endemic, reaching almost 90 million people. The expansion

required to meet the targets of reaching 350 million people by 2005 and all 1.1 billion at risk globally by 2020 is now the major challenge of what has become the global program to eliminate lymphatic filariasis, coordinated by the WHO and supported by a global alliance of the partners (Ottesen, 2002).

For any country to be successful in eliminating lymphatic filariasis from its territory, the first epidemiologic approach to be adopted is to assess the current magnitude and geographic distribution of the disease. Prevalence of filariasis is seen at different places of Nepal including Terai and Hilly areas. It may be due to climatic and geographic variations. A study conducted by Sherchand *et al.*, 2001/2002 in 37 districts of Nepal had shown that about 13% of the Nepalese population were infected with lymphatic filariasis that indicated filariasis as a public health problem of Nepal.

With WHO's global strategy to eliminate lymphatic filariasis as a public health problem and government's political commitment, EDCD, Department of Health services, MOH has formulated a National plan for action (2003-2015 A.D.) for elimination of lymphatic filariasis in Nepal. On this context, the study on lymphatic filariasis in Kabahigoth VDC of Bara district has been undertaken with the project work held by "PARASED", Nepal having joint venture with the government of Nepal and WHO to find the prevalence of infection on the basis of age, sex, occupation, education, attitude of people towards the disease which contributes to obtain baseline situation of lymphatic filariasis in the districts. This will help in the formulation of strategies and its implementation for the eradication of lymphatic filariasis in the near future.

II

OBJECTIVES

2.1 General Objectives

To determine the human filarial infection and situation of MF (*Wuchereria bancrofti*) by means of sentinel survey to study risk factors in Kabhigoth VDC of Bara District of Nepal.

2.2 Specific Objectives

- To determine age and sex wise prevalence of filarial infection.
- To investigate knowledge, attitude and practices of people towards filariasis.
- To determine the filarial situation in relation to education and occupation.
- To determine symptomatic and asymptomatic filarial situation among the people of study area.
- To determine the age and sexwise endemicity rate of lymphatic filariasis.
- To bring awareness about filariasis among the community.

2.3 Objective Rational

It was hoped that the present study will give solution to eradicate filariasis problem and mosquito control problem, also it will help to aware the people against the diseases control. This will promote the country to plan for the health status to improve in the near future.

III

LITERATURE REVIEW

3.1 Diagnostic Aspect

3.1.1 Detection of Microfilariae

The standard tool for diagnosis of filarial infection remains detection of microfilariae in peripheral blood (Eberhard, 1991). For epidemiologic screening, 20-60 μ l of capillary (fingerstick) blood can be dried on a slide, stained and examined under a microscope. A more sensitive method is to filter at least 1 ml of venous blood through a 3-5 μ m Nuclepore filter (Eberhard, 1991; Dreyer, 1996), but collection of venous blood is generally less acceptable than fingerstick and the filters are relatively expensive. Advantages of using microfilaria detection in finger stick blood for initial assessment include low cost, the general availability of materials and trained staff in many filariasis endemic countries, and the fact that positive specimens are "Parasitologically confirmed". Disadvantages include the need to collect blood at night in most areas, because the microfilariae are present in the peripheral blood primarily at night in case of nocturnal periodic *W. bancrofti* packing between mid-night and 2 A.M. (Sasa, 1976; Dreyer, 1996, Simonsen, 1997), and the labour intensive nature of staining, preparing and examining slides. Estimated sensitivity of finger stick blood specimens depends on blood volume and microfilarial density, ranging from approximately 26-52% in patients with 1-30 microfilariae per ml venous blood (for 20 μ l and 60 μ l, respectively) to 100% for microfilarial densities of >100 per ml (Dreyer, 1996). With trained staff, specificity is essentially

100%. If lymphatic filariasis is suspected, urine can also be examined macroscopically for chyluria and then concentrated for microfilariae.

3.1.2 Antigen Detection Assay

A major recent advance in diagnosis of *Wuchereria bancrofti* infection has been the development of monoclonal antibody-based assays that detect circulating *W. bancrofti* antigens in peripheral blood. An enzyme-linked immunosorbent assay (ELISA) format is commercially available that utilizes the monoclonal antibody Og 4C3 (More and Copeman, 1990; Turner et al., 1993). In addition, a field-ready immunochromatographic test is now available for use with either whole blood or serum. This test is based on monoclonal antibody AD 12, which recognizes a 200 KDa antigen (Weil and Lillis, 1987; Weil et al., 1997).

The sensitivity of the test approaches 100% (Lammie et al., 1994; Chanteau, et al., 1994; Weil et al., 1997), although it may be as low as 72-75% in persons with ultra low microfilarial densities (Rocha et al., 1996; Chanteau et al., 1994). The assays have a high specificity, 98.6%-100% (Chanteau et al., 1994; Lammie et al., 1994; Rocha et al., 1996; More and Copeman, 1990). The major disadvantage is high cost, which may range from approximately US \$ 1 to US \$3 per test.

Wickremanayake et al., (2001) developed a dot-ELISA for detection of microfilariae of *W. bancrofti* in an endemic area. The test can differentiate the endemic normals from the microfilaremic asymptomatic individuals. Antigens of molecular weight 130 K Da and 50 K. Da of the cattle filarial worm *Setaria digitata* were used for this test. It was observed that these two antigens were also present in the serum of asymptomatic microfilaremic individuals.

Saverimutte et al., (2000) identified several antigens from the microfilarial stage *Wuchereria bancrofti* using immunoblots of microfilarial antigens and screening with immune sera and tropical pulmonary eosinophilia (TPE) sera. This analysis revealed an array of antigens with apparent molecular weights of 14 KDa, 35 KDa, 42 KDa, 63 KDa, 88 KDa and 200 KDa. Among these only the 14 KDa and 42 KDa antigens were recognized only TPE sera. Analysis of rabbit immune sera revealed that the 42 KDa antigen was shared by two development stages of *W. bancrofti*, viz., L3 and mf. This antigen could become a potential vaccine candidate. The 14 KDa antigen seems specific for the microfilarial stages and therefore could be a diagnostic mater for active infection. The 132 KDa antigen could aid in the diagnosis of TPE.

Omar et al., (2000) used two commercially available diagnostic tools (Trop-Bio ELISA and ICT cord test) to detect circulating filarial antigen of *W. bancrofti* infections among Indian expatriate workers in Saudi Arabia. Day time serum samples collected from 302 individuals (40 men and 92 women) were tested. A night blood survey for microfilaremia were restricted to overall prevalence of filarial antigenaemia was 10.6% (32 individuals) of these 32 antigen positive cases, microfilariae were found in 10 men (31%), with a mean microfilarial count of 105 mf/ml. No positive antigen results were found in control sera from 200 native healthy Saudia or from patients with helminthic infections (Schistosomiasis, Echinococcosis, Hookworm, ascariasis, and Trichuriasis). All 32 positive sera with the Trop-Bio ELISA showed a positive ICT card reaction (specificity and sensitivity 100%)

Sunish et al., (2001) examined percentage prevalences of microfilaroemia (PPMF) and antigenaemia (PPCFA) in 1999 in 3505

subjects from 3 villages in India. All microfilaraemics were positive for antigenaemia, and PPCFA was decreased steadily from 92% in the age group 2-5 years to 40% in the age group 21-30 years.

Schuetz *et al.*, (2000) used the ICT Filariasis test to assess residual antigen levels following antifilarial treatment. The results demonstrated that antigen levels persisted in microfilaria negative persons for up to three years after treatment.

Phantana *et al.*, (1999) found that ICT filariasis test had sensitivity (100%) specificity (96.57%), efficiency (96.70%) predictive value positive (PVP =70.70%) and predictive value negative (PVN=100%). When compared with 454 thick blood films (standard smear method)

Pani *et al.*, (2000) evaluated ICT-filariasis card test for its sensitivity and specificity in detecting microfilaria carriers among 189 individuals each in filariasis endemic and non-endemic areas in South India, and compared to both conventional night blood finger thick blood smear examination and venous blood membrane filtration. Through the specificity of the test was 100% against both techniques, but the negative predictive values were 99.5% against the finger prick blood smear 88.3% compared to the membrane filtration technique.

Bhumiratna *et al.*, (1999) assessed the efficiency of the ICT card test by using clinical and recall techniques and microscopy (thick and capillary tube technique) as reference in sera from 225 subjects living in *W. bancrofti* endemic villages of Tak province, Thailand, who were recruited during a cross sectional community survey. The ICT card test gave a 20% antigen positive rate, while other tests gave lower positive rates of the same 5.8% by clinical and recall techniques and thick smear, and 5.3% by capillary tube

technique, respectively. The ICT card test had a specificity of 100% when sera from microfilaraemic subjects were positive, as when sera from *W. bancrofti* non-endemic subjects either with *Brugia malayi* microfilaraemia or with other parasites, and those from normal controls were all negative by the test. When done in *W. bancrofti* microfilaraemia sera, the ICT card test had a sensitivity of 100% using a microscopy as reference, and 84.6% when using clinical and recall techniques. However, the ICT card test was more sensitive than the others when done in endemic normal sera (14% positive).

Chandrasena *et al.*, (2002) estimated the sensitivity, specificity and cost effectiveness of an immunochromatographic card test (ICT, AMRAD) for the diagnosis of bancroftian filariasis against two standard parasitological techniques: thick blood film (TBF) and nucleopore membrane filtration (NMF). Individuals were selected from endemic localities in the western province (n=213) of Sri-lanka. Blood was collected between 21.00 and mid night 60 μ l of non-heparinized blood and 1ml and 100 μ l of heparinized blood were used in TBF, NMF and ICT, respectively. NMF was positive in 31.5% (67/213) of the endemic groups, with a mean microfilaria (mf) count of 343/ml (range 8-1782, SD 422). All 67 were positive by ICT (sensitivity 100%), but only 63 were mf negative but antigen positive by ICT. There were, however, no false positive among the non-endemic controls, indicating the possibility that the ICT may in fact be more sensitive and 100% specific. Thus, ICT-filariasis test appears to be more effective (both sensitive and specific) than TBF or NMF in diagnosing infection in lymphatic filariasis.

Njenga *et al.*, (2001) validated the ICT-filariasis card test in the field in Kenya by comparing its sensitivity to the combined sensitivity of Knott's

concentration and counting chamber methods. A total of 102 (14.6%) and 117 (16.7%) persons were found to be microfilaraemic by Knott's concentration detected by both Knott's concentration and counting chamber methods were also antigen positive by ICT-filariasis card test (100% sensitivity). Further, of 97 parasitologically amicrofilaraemic persons, 24 (24.7%) were antigen positive by the ICT. The overall prevalence of antigenaemia was 37.3% of 100 non-endemic area control persons, none was found to be filarial antigen positive (100% specificity).

Simonsen et al., (1999) evaluated three new and commercially available tools for diagnosis of *Wuchereria bancrofti* infection of specific circulating antigens and compared them in the same groups of individuals from a highly endemic village in southern Ghana. The tests were: (1) the ICT card test for serum specimens; (2) the Trop-Bio ELISA test for serum specimens; and (3) the Trop-Bio ELISA test for filter paper specimens. A high degree of positive/negative response similarity was observed for the 3 tests, 100% for all tests. The antigen levels measured in the Trop-Bio serum test and the Trop-Bio filter paper test were statistically significantly correlated. Among antigen positive, the antigen levels in these two tests further more showed a positive association with the microfilarial intensity, but a statistically significant correlation was seen only for the filter paper version of the test.

3.2 Research on Filariasis in Different Countries

Lymphatic filariasis department of health and human services, Centers for Disease Control and Prevention (Feb.27, 2007) in U.S. gave the data of 80 countries but the infection of filariasis is disappeared in the 20th century in U.S.

Harnett, William and Harnett, (2006) showed about what causes lymphocyte hyporesponsiveness during filarial nematode infection.

Filarial nematodes persist in the parasitized host by modulating immune responsiveness. A feature of this that has been observed in a multitude of studies dating back several decades is an inability of lymphocytes to respond appropriately to filarial nematode antigen and, in some cases to other stimuli. The consistency of this observation, allied to the cease of measurement of lymphocyte hyporesponsiveness, has resulted in many attempts to understand its cause.

Dabir, et al., (2006) showed isolation and analysis of partial CDNA sequence coding for superoxide dimutase in *W. bancrofti*. Molecular characterization of *W. bancrofti* is essential to develop suitable antifilarial drugs and vaccines. They describe here isolation, sequence analysis and cloning of a partial CDNA of an enzyme superoxide dismutase from this parasite. The immunoscreening of a lambda Zap *W. bancrofti* microfilarial (mf) CDNA library with microfilaremic sera had resulted in the isolation of several sera reactive clones including wb SOD. This clone contained a 309 by insect and showed significant nucleotide and deduced amino acid sequence homologies to the super oxide dismutases of other nematode parasites. The antioxidant property of this enzyme may have important contribution in the defense mechanism of the parasite against host immune response.

Babu, et al., (2006) studied on regulatory networks induced by live parasites impair both Th 1 and Th 2 pathways in patient lymphatic filariasis: implication for parasite persistence-patient lymphatic filariasis is characterized by a profound down-regulation of immune responses with both

parasite Ag- specific tolerance and bystander suppression. Although this down-regulation is confined to the Th 1 arm of the immune system in response to parasite Ag, They hypothesized a more generalized suppression in response to live parasites. Indeed, when they examined the cytokine profile of a cohort of filarial-infected (n=10) and uninfected (n=10) individuals in response to live infective-stage larvae or microfilariae of *Brugia malayi*. They found that significant impartment of both Th 1 and Th 2 cytokines characterized by diminished production of IFN-r, TNF- α , IL-4, IL-5 and IL-10 in infected patients.

Rajkumar, et al., (2005) studied on *W.bancrofti* and *Onchocerca volvulus* co-infection in a refugee from Sierra Leone. A case of co-infection of *W. bancrofti* and *O. volvulus* that was diagnosed by direct blood smear (*W.bancrofti*) and serology (*O.volvulus*) in a native of Sierra Leone.

Fox, et al., (2005) studied on ultrasonographic, examination of Haitian children with lymphatic filariasis. A longitudinal assessment in the context of antifilarial drug treatment- To assess the clinical findings associated with detection of adult *W. bancrofti* worms on ultrasound, 186 school children in a filariasis-endemic area of Haiti underwent physical and ultrasonographic examinations. The filarial dence sign (FDS) of adult *W. bancrofti* was detected in the inguinal and crural lymphatics of 28 (15%) 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 lymphadenopathy on physical exam (p=0.01) 25 FDS positive children see examined after three annual cycles of mass treatment for lymphatic filariasis (Lf). The total number of adult worm nests detected by ultrasound decreased from 29 to 4 (P \leq 0.0001). FDS and lymphangiectasia

were detected in the intrascrotal (N=3) and inguinal (N=1) lymphatic vessels of the three post pubescent boys. This study demonstrates clinical and subclinical findings of Lf in FDS- positive children.

Kumar, et al., (2005) showed the impact of different housing structures on filarial transmission in rural areas of southern India to assess the filarial transmission levels in houses of different structure in rural areas of Andhra Pradesh, India. During this study ecologically similar households were selected for entomological study. The per-man hour density (PMDH) infection and infectivity rates were recorded in different ranges i.e. 16.1-77.6%, 0-31.2% and 0-5.6% respectively.

Jamail, et al., (2005) studied the field validation of sensitivity and specificity of rapid test for detection of *B. malayi* infection. Several endemic districts in state of Sarawak, Malaysia were used to determine the test sensitivity and determination of specificity was performed in another state in Malaysia which is non endemic for filariasis. In Sarawak both the rapid test and thick blood smears preparation were performed. Sensitivity of Brugia Rapid dipstick as compared with microscopy of thick blood smears was 87% (20/23; 95%ci:66.4-94.2) were as the specificity was 100% (512/512). The overall prevalence of brugian filariasis as determined by dipstick is 9.4 % (95% ci: 8.2-0.55) while the determined by microscopy is 0.90% (95% ci:0.5-1.3).

Hornberger, et al., (2005) studied the Idiopathic scrotal elephantiasis. He presented a unique case of 22 years old men with idiopathic lymphoedema isolated to the scrotum. After acquired causes of lymphoedema were ruled out, the patients were treated with scrotoectomy and re-construction.

Wongkamchai, et al., (2004) worked in the detection of antigens for diagnosing filariasis by examining the diagnostic potential of monoclonal antibodies (MAB) reactive to antigens of adult *Brugia malayi*, their microfilariae and antigen of *Dirofilaria immitis*. They tested patients with different clinical manifestation of brugian filariasis, i.e. microfilaremia (M), Lymphangitis (L) and elephantiasis (E), as well as non symptomatic inhabitants of a filariasis endemic area (NE) and compared them to the samples from non-symptomatic inhabitants of disease non endemic areas (NNE). They found that 22 of 31 (70.9%) of M, 7 of 13(53.8%) of L, 2 of 14 (14.2%) of E, 10 of 100(10%) of NE and none (0%) of NNE were positive for antigenaemia.

Koyadun et al., (2004) conducted a study on bancroftian clearance and Myanmar migrants after biannual mass treatment with DEC 300 mg oral dose FILDAC tablets in Southern Thailand. They found that out of 34 antigenaemic Myanmar index cases of varying initial CFA level who were initially screened out with ICT filariasis, 13 index cases were followed up, treated and monitored at the DEC post treatment 6,12 and 18 months. At 18 months, post treatment, residual antigenaemias (%) in 4 to 5 index cases (group 1) with high antigens titers $99.7-181.6 \times 10^3$ Au/ml) were 54.44%, 33.58%, 27.43% and 9.97 significant decrease of CFA levels in only 3 out of 5 index cases were affected by the response of DEC treatment ($P < 0.007$).

Jiang Jung, et al., (2004) found that 6 cases of filarial Chyluria were admitted to the hospital from November 2001 to June 2002. Of these cases, 4 were men and 2 were women with age of 32.52 years (mean 42 years). Operative time ranged from 69-120 minutes (mean 95 minutes). Interpretative blood loss was 50-80 ml (mean 85 ml) chyluria disappeared in

all patients immediately after operation. Mild haematuria occurred in 4 cases with in 12 hours and disappear at 24 hours.

Subramanian, et al., (2004) studied the dynamic of *W. bancrofti* infection: a model based analysis of longitudinal data from pondicherry, India. This paper presents a model based analysis of longitudinal data describing the impact of integrated vector management of the intensity of *W. bancrofti* infection in Pondicherry, India. The aims of this analysis were (1) to gain in sight into the dynamics of infection with emphasis on the possible role of immunity and (2) to develop a model that can be used to predict the effects of control using the LYMFASIM computer simulation program two models with different types of immunity. (anti – L3 larvae or antiadult worm fecundity) were compared with a model without immunity. Parameters were estimated by fitting the models to data from 5071 individuals with MF density measurement before and after cessation of a 5 year vector management programme. A good fit, in particular of the convex shape of the age-prevalence curve, required inclusion of anti-L3 or anti fecundity immunity in the model. An individuals immune –responsiveness were found to have in \$ 10 years after cessation of boosting. Explannation of the large variation in MF density required considerable variation between individuals in exposure and immune responsiveness. The mean life-span of the parasite was estimated at about 10 years. For the post-control period, the models predict a further decline in MF prevalence, which agrees well with observations made 3 and 6 years after cessation of the integrated vector management programme.

Tobian et al., (2003) studied the sensitivity and specificity of ultrasound detection and risk factors for filarial associated hydrocele for this

342 men above 15 years of age in endemic area in Papua New Guinea were evaluated. The observation suggested that filarial pathology of male genitalia is under reported when evaluated by physical examination alone. The duration and the intensity of infection are the risk factors for hydrocele.

Figueredo *et al.*, (2003) studied the histopathology of bancroftian filariasis and the role of adult worm in the lymphatic vessel with the clinical, ultrasonographic and surgical characteristics. The protean spectrum of alteration seen in the host's lymphatic vessel was discussed and the changes caused by live and dead worms are highlighted as independent events. Evidence of remodeling progress in which the lymphatic endothelial cells appeared to have a key role, was also provided for the first time.

Chadee *et al.*, (2003) studied on filariasis in George town of South America. They conducted a one year survey of febrile patients attending filariasis (Night) clinic. Out of 769 thick blood smears collected, 103 were positive for *W. bancrofti*, also the age group and sex of infected persons were described.

Kazura *et al.*, (2003) studied lymphatic filariasis in New Guinea. They found that filariasis is a major public health problem in Papua New Guinea; where the level of transmission by the mosquito vector human infection rates and clinical morbidity are highest among them in the world. Coordinated research affords with in the country involving the disciplines of epidemiology, vector biology, immunology and genetics have led to be in tight into the ecology and pathogenesis of human lymphatic filariasis. Research work should be helpful in assessing local and global strategies aimed at eliminating to bancroftian filariasis and in guiding research that will facilitate to achievement goal.

Gyapong J.O.D. et al., (2003) conducted study on geographical distribution of human infection with *W. bancrofti* in four west African countries (Benin, Burkina, Faso, Ghana and Togo) using the commercial immunochromatographic test for filarial antigens. Population (401) study was related randomly, the community exceeded 70% and that over large area of Burkina Faso community prevalence rate between 30% to 50%. Most of the Togo Southern Benin and much of the Southern Ghana appear completely free from the infection. Although these were foci on the Ghanaian coast with prevalence of 10% -30%. Such high prevalence did not extend into coastal Togo or coastal Benin.

Wongkamchai et al., (2003) the study was conducted for an antigen determining assay for diagnosing filariasis. They found that 22 of 31 (70.9%) microfilariaemia, 7 of 13 (53.8%) of lymphangitis, 2 of 100 (10.0%) of symptomatic inhabitants of filariasis in an endemic (NNE) area and none (0%) of the non symptomatic inhabitant of a filariasis in non endemic area were positive for antigenaemia. The assay was also positive in 14 of 15 (93.3%) blood samples from *B. malayi* microfilaraemic cats and in 7 of 7 (100%) blood sample of *Dirofilaria immitis* microfilaraemic dogs. So, the developed test has a high potential for routine diagnosis of active filariasis for epidemiological study in both humans and reservoir animals and for monitoring treatment efficacy.

Nuchprayoon et al., (2003) conducted study on comparative assessment of on Og 4 C3 ELISA and an ICT filariasis test of Myanmar migrants in Thailand. A total of 337 Myanmar participated in this study. The microfilaria rate was 3.3%. The Og4C3 ELISA could detect 19.1% of lymphatic filariasis while the ICT test detected 12.7%. Both antigens assay

could detect all microfilaremics. The Og4C3 ELISA detected 14.8% of microfilaremics while the ICT test identified 8.1%. Those who were positive by the Og4C3 ELISA. Those Og4C3 positive cases that were ICT negative (ICT-ve Og4C3 +ve) had statistically significant ($P < 0.05$) unpaired t-test lower Og4C3 antigen levels (409.5 units) range 117-2, 389 than those that were ICT positive (ICT positive/Og4C3+ve), 5, 2520 units range 130-28, 062). These antigens detection system are promising tools for the surveillance of bancroftian filariasis.

Weerasoriya *et al.*, (2001) reported 4.4% prevalence of microfilaraemia in three sub urban areas of Matara, in Sri-Lanka. Prevalence was significantly lower in female (4.2%) than in males (1.4%) and in males aged 20 years than in older males. Overall 9.5% of subjects had the clinical manifestation, 6.4% had filarial fever, 3.0% had elephantiasis and 6.2% had hydrocele. There was linear increase in prevalence after the age of 40 years.

Genga, N. *et al.*, (2001) conducted the study on ICT filariasis card test using whole capillary blood comparing with Knott's concentration counting chamber method in Kenya. A total of 102 (14.6%) and 117 (16.7%) person was found to be microfilaremic by Knott's concentration and counting chamber methods respectively. The geometric mean intensities (GMI) were 74.6% microfilarial and 256.5 mf/ml by Knott's concentration and counting chamber method respectively. An infected individuals detected by both Knott's concentration and counting chamber methods respectively were also antigen positive by the ICT filariasis card test (100% sensitivity). Further of 97 parasitologically a microfilaremic person 24 (24.7%) were antigen +ve by the ICT. Overall prevalence of antigenemia was 37.3% of 100

non endemic area control person. So, ICT card test is a simple sensitive specific and rapid test which convenient in field survey.

Jain *et al.*, (2001) studied on cytomorphology of filarisis revisited expansion of the morphologic spectrum and coexistence with other lesions of the filarial worm and associated tissue response on 33 cases. Twenty nine aspirate smears 28 patients were air dried and stained with May-Grunwold. Giemsa stain focur routine servical smears of urine were stained with Papani colour stain, MF along with adult gravid females were present in 25 and 4 cases respectively. In one case both the adult male and female worms with mf and eggs were seen. The diagnosis was based on the presence of eggs along in one case and fragments of female worms in two four of this cases were Neoplastic lesion and mf were found incidentally. In one cases of splenomegaly microfilarial were seen along with leishman Donovan bodies.

Ravindra *et al.*, (2001) studied on imflammation and immunological hyperactivity needed for filarial parasite development. The immune dependent growth and development of infection and pathogenesis of diseases are increasingly being recognized. It is proposed that the development of filarial larvae to adult stage parasites takes place in mammalian host and that susceptibility to filarial infection could be governed by the macrophase derived nitric oxide and host ability to produce anti bodies to independent carbohydrate antigens.

Schuetz *et al.*, (2001) studied on evaluation of the whole blood filariasis ICT test for short term monitering after antifilarial treatment. It was filariasis test which acts as rapid screening tools that will be useful for defining the prevalence and distribution of *W. bancrofti* as a part of the global programme to eliminate lymphatic filariasis. The result demonstrated

that antigens level persists in microfilarial negative persons for up to three years after treatment. Different strategies for monitoring control programmes may have to be considered.

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 age. Initial damage to lymphatic system by the parasites generally remains subclinical for many years or gives rise only to non-specific presentations of lymphadenopathy, however especially after poverty the characteristic clinical features of adult disease syndrome (Lymphoedema, Hydrocoele) manifest themselves.

Terhell *et al.*, (2001) studied the influence of the host age from length of exposure on acquisition of filarial infection. A total of 247 transmigrants were compared with those of 133 life long residents. In earlier studies antifilarial Ig G4 increased with age in the indigenous population, whose age was equivalent to length exposure. However by examining transmigrants it become clear that development of Ig G4 has influence by age, since level of this antibodies was consistently higher in transmigrants adults than in children despite on equal length of exposure of filarial infections.

Triteeraprapab *et al.*, (2000) conducted a study on transmission of nocturnal periodic strain of *W. bancrofti* by *Culex quinquefasciatus* in Thailand. The prevalence of *W. bancrofti* infection in immigrants (2.5%) prompted concern in public health community for re-emergence of lymphatic filariasis. The Myanmar immigrants were infected with the nocturnal periodic type *W. bancrofti* for which *Culex* serve as the main vector. The Thai strains of *Culex quinquefasciatus* have never been reported to transmit lymphatic filariasis. *W. bancrofti* with third stage larvae establish

the potential for establishing an urban cycle for transmission for transmission in Thailand.

Singh Sukhvir *et al.*, (2000) studied on filarial transmission in a non-endemic area of Pathankot (Punjab). The population study was 2,841, out of these blood samples 2136 blood smears were collected from migratory and local inhabitants. Microfilaria mf rate and mean (mf) density was 1.19 and 15.05 respectively. And the 20 mf carriers detected were known to hail from filarial endemic areas of different states. The Mf rate was highest in 20-49 years age groups whereas the mf density was high in younger age group. *W bancrofti* was the only infection encountered. A total of 339 female *culex quinquefasciatus* were dissected. No positive microfilaria were found in human, thus regarding any indigenous filariasis transmission in that town.

Phantana *et al.*, (2000) conducted the study on lymphatic filariasis through ICT test. Kit which is composed of specific polyclonal and monoclonal antibodies to *Wuchereria bancrofti* antigen chromatographic reaction with result. Plasma showed a result with in 5 minutes, when compared with 454 thick blood film (standard smear method) with in the same study. The ICT filariasis test had sensitivity = 100%, specificity = 96.37%, efficacy = 96.70% PVN = 99.50%, when the capillary tube technique (cap) with TBF (Total lymphatic filariasis) cap showed sensitivity = 85.4%, specificity = 100%, efficiency PVP = 100%, PVN = 98.60% with convenient high sensitivity efficiency, lack of cross reaction, no night blood collection, 20 night blood collection, single reagent and rapidity can be recommended for screening of lymphatic filariasis and is suitable for the conformation of suspected cases in the field when the microscopic diagnosis is not available.

Helmy *et al.*, (2000) studied on human IgG antibody responses to *W. bancrofti* infective third stage larvae L3 surface and somatic antigens by indirect influence of (IFA) and immunoblot with endemic Egypt area (n=115) with the aim of identifying target of protective immunity. The antibodies to L3 surface antigens were equally prevalent in uninfected children (75%) and adults (90%) but less prevalent in people with or without filarial antigenaemia 81%. IFA positive sera showed significantly enhanced recognition of antigens at 66, 40 and 14 KDa in immunoblots reactive to IFA negative sera, additional study were needed to further characterize antigens identified in that study and to established whether they were indeed targets of protective immunity in humans.

Swoboda Kopec *et al.*, (2000) studied on bacterial infection of skin and tissue in filariasis. Adenolymphangitis is a common occurrence in filarial lymphoedema. Damage due to the lymphatics and lymphnodes by *W. bancrofti* is followed by obliteration of lymph vessel and lymphatic obstruction of lymphatic vessels, prevents the bacteria peretrating the skin. Skin was evacuated with lymph stream to regional lymph nodes. Colonization of dermis, and lymphatics evokes the clinical symptoms of adenolymphangitis. The stain of bacteria is responsible for acute and chronic type of adenolymphangitis.

Thanomsub *et al.*, (2000) studied differential diagnosis of human lymphatic filariasis using PCR-RFLT. The most causative agent of human filariasis are *W. bancrofti* and *B. malayi*, the traditional method used to detect the filarial parasites in human, animals and vector population are tedious time consuming and little guarantee of sensitivity and species specificity. So they developed a rapid and specific method to detect filarial

parasites DNA's and mosquito sample using PCR technique. The primers used are Mf/f and Mf/R which simply a 1.5 kb glutathione peroxidase gene of filarial worms. Using the restriction fragments length polymorphism (RELP) technique. These PCR product will be further digested with restriction enzymes either Hpal, PstI, AluI or HinfI to differentiate the genus of filarial. PCR-RELP technique can be applied to used in diagnosis and differentiate between species of filariasis in human, the reservoir host and the mosquito vector in endemic areas.

Burah *et al.*, (2000) conducted study on the impact of housing structures in filarial infections, which was taken to correlate the impact of housing and pattern of house construction on the vector density and transmission of filariasis among the inhabitant of these houses. The different types of houses in ecologically similar hamlets of Hariharpur village in varanasi were selected for determining the density of *Culex quinquefasciatus*, the vector of *W. bancrofti* and its infectivity. The maximum prevalence per house density of the vector was recorded during March (31.66%, 4.33% and 41.33%), while the minimum was recorded in June (1.3,2.6 and 0.53%) in all the three types of houses. During infection rate in vector collected from the poorly constructed houses was observed during April, May, October and January of the following year whereas the moderately constructed houses infections was observed only in September and in the well constructed houses dissections results did not reveal any infections during the month of the study.

Massaga *et al.*, (2000) reported the prevalence of *W. bancrofti* in 31.8% of 1025 inhabitants (32.1%) of female and 31.5% of male. In Hale area in Northeast Tanzania, clinically 6-9% of examined individuals had

elephantiasis and 28.5% males aged 15 years and above the 15 years of age had hydrococele. Both the clinical manifestation and microfilaria prevalence increased with age.

Sharma *et al.*, (1999) studied on detection of *W. bancrofti* circulation antigens in patients with *S. digitataglucan* combination directed filarial out bodies. The study was carried out to determine the optional adjuvant dose of β . 1-3 glucan for immunization against filariasis in habitat were immunized with filarial antigen (*S. digitata*) and β_1 glucan used as an immunological at different doses. The 40 mg/kg body weight of glucan was found as optimum dose for successful immunization of experimental animal with *S. digitata* antigen expressed by elicited human and cellular immune responses. These antibodies were used to detect filarial parasite in patients by sand witch ELISA. A total of 85 sera samples were used in four different groups of clinical manifestation. The sera sample of microfilaraemic patients showed 87% positivity for circulating antigen with mean titre of 1:21582, whereas 63% positivity in chronic filarial samples with mean titre of 1:13689 was observed. Only 26.6% positivity was detected and endemic control with two titre values as compared to mf +ve and Chronic cases. The result showed a significant correlation in active infection with circulating antigens.

WHO, (1997) carried out a study to detect filariasis in India. At present, about 428 million people with 28 million microfilaria carriers and 21 million clinical cases were spread in 13 states and 5 union territories. India contributed about 74% of disease burden in the region. *W. bancrofti* did the most predominant infection comprise 99.4% of the problem in the country while *B. malayi* was confined to the western coast of Kerala and in other six states. Both the infections were nocturnally periodic. In the Nicobar

group of Island, diurnally sub periodic infections are transmitted by *Aedes nigves* group was detected about three decades back.

WHO (1997) carried out a study in Northern Ghana in Rural Community where filariasis is highly endemic (41% of the population aged over 10 years in microfilaraemic with *W. bancrofti* and 3% has chronic disease), showed that lymphatic filariasis can be a major social and economic burden on poor communities, disability and indirect economic loss associated with adenolymphagitic (ADL).

Research on Filariasis in Nepal

Jones et al., (1970) reported about three cases of bancroftian filariasis in Kathmandu valley, of them one died due to heavy infection of microfilariae.

Rana (1973) collected the data regarding the occurrence of filariasis disease from hospital attendance in Kathmandu, Birgunj, Hetauda, Biratnagar and Nepalgunj. He observed that the prevalence of filariasis was more common in genital region than in lower limb in case of male, while only in the lower limb in case of female.

Mishra (1987) presented a paper entitled “Ecological Management of Parasites in Nepal” in which he mentioned about the mosquito borne diseases including filariasis (*W. bancrofti*).

Bhusal et al., (2000) studied the prevalence of *W. bancrofti* infections in Tokha-Chandeshwori VDC, Kathmandu, Nepal in 1998. A survey of 978 nocturnal blood film in the VDC indicated an overall prevalence of 5.8% for microfilaraemia and the crude disease rate of *Wuchereria bancrofti* was recorded to be 13.0% in the study area. The highest microfilaraemia

infection rate was recorded as 11.8% among age group of 40-49. And the highest crude disease rate was recorded 36.4% in the age group of 70 and above.

Jung, (1973) reported the prevalence of lymphatic filariasis in Nepal to be about 25% and detected *Culex quinquefasciatus* as a vector in all the surveyed areas. He reported the disease from 300 feet above sea level in the plain terai ecological zone to 5900 feet above sea level in high hill area.

He studied all together 9 sites which showed 4.99% to 6.15% *W. bancrofti* in all age groups and both the sexes in the urban population 6.6% to 10.3% in the semi urban population and 1.2% to 17.8% in the rural population. Similarly 7.1% to 9.16% microfilariae rate was found in the urban population 10.03 to 11.3% in the semi urban population and 0.8% to 17.69% in the rural population.

Pradhan et al., (1997) carried out an epidemiological study on lymphatic filariasis in Gokarna VDC of Kathmandu valley. Study reported 24.6% endemicity rate with the overall, 12.75% microfilarial infection (15.09% in male and 8.9% in female) and 11.95% crude disease rate (8.49% in male and 16.59% in female) of *Wuchereria bancrofti*. On the contrary, the microfilaria rate among the females was lower than the males (microfilaria rate: 15.09% in males and 8.97% in females). The study showed 7.4% (n=135) infection rate in vector *Culex quinquefasciatus*. The study identified 12 species of Mosquitoes in Gokarna (*Anopheles nigerrimus*, *Anopheles vagus*, *Anopheles willmori*, *Anopheles kessele*, *Culex fuscocephala*, *Culex gelidus*, *Culex pseudovishnui*, *Culex quinquefasciatus*, *Culex sinensis*, *Culex vishnui*, *Culex whitmori* and *Culex tritaeniorhynchus*) Among these species *Culex quinquefasciatus* was found to be predominant.

Sherchand (2001) conducted an epidemiological survey to determine the prevalence of disease due to lymphatic filariasis in Magaragadi VDC, Bardia district of Nepal. The study population were selected above the 15 years of age group and the immunochromatographic test (ICT-Filariasis) was used to screen for circulating filarial antigens (CFA). The prevalence of lymphatic filariasis from 500 sample population was 141 (28.2%). Among 214 female mosquitoes caught, 2 were infective larvae of *Wuchereria bancrofti*, found from *Culex quinquefasciatus*.

Tuladhar and Sherchand (2001) conducted an epidemiological study in three different geographical regions, viz., terai (Sipawa VDC of Rupandehi district), and inner-terai (Dovan VDC of Palpa district) and hill (Katunje, Golmandi, Itachhen and Byasi of Bhaktapur district) of Nepal. A total of 53 blood samples (10.35%) were found ICT filariasis positive in the study. 39 blood smears from buffy coat out of a total of 410 blood samples and 27 thick blood smears were found positive in Bhaktapur. Among the three different methods in detection of microfilaria, smear from buffy coat was found the best. ICT card technique in antigen detection was still better for field survey in diagnosis of filariasis of all techniques.

Manandhar (2001) conducted an epidemiological study of microfilaria in three geographical regions of Nepal. The study reported 19.9% crude disease rate with highest rate of crude disease infection (38%) in 70 and above age group.

Bista et al., (2000) reported the cases of filariasis in out patient clinics of different health institutions and reported through the HMIS during the fiscal year 1995/1996 to 1998/1999.

Sherchand *et al.*, (2003) studied the prevalence of *W. bancrofti* filarial infection in 37 districts of Nepal. The study populations were selected above 15 years age of respondents and the immunochromatographic test (ICT-filarial test) was used to screen for circulating filarial antigen (CFA). The overall prevalence of lymphatic filariasis from 4,488 sample population was 13% and 33/37 district were found to be endemic for lymphatic filariasis. On the basis of geographical data, the highest numbers of cases were found at altitudes of 500-700 meter. However a substantial number of infected individuals were found in the highly populated Kathmandu valley at altitude between 900-1500m, of the 33 districts, 11 districts were having above 20% prevalence (with the highest rate of 40%), 15 districts between 6-19% and in 7 districts between 0.1-5%.

Ghimire *et al.*, (2003) studied the prevalence of lymphatic filariasis in an endemic areas, Mahendranagar and Nagrain VDC's of Dhanusa district in the terai plain region of Nepal. It was found that the prevalence of microfilaraemia is higher in Mahendranagar than in Nagrain VDC. Out of total 1085 finger prick thick blood smear samples collected, 25/468 (5.3%) of Mahendranagar and 14/617 (2.3%) from Nagrain VDC were found positive for *W. bancrofti*. The prevalence was found to be higher in females, although the participation of both sexes was almost equal.

Chhetri (2005) studied in Bishnupar VDC of Rupandehi in which out of 530 samples, 40 (7.54%) samples were found to be positive for LF with age range 2-85 years. The highest distribution of LF was found in 41-50 years age group (20.68%) and lowest in 61-70 and >70 years (3.33%).

Koju, (2006), studied Lf in Dhading, Benighat VDC. It was found that out of 541 total samples, 4(0.76%) MF, +ve, Maximum prevalence

was seen in age group 41-50 years (2.78%) and least was found in 21-30 years (0.90%) age group CDR was reported to be 0.96% and total endemicity rate was 1.73%.

The following table gives an out look of lymphatic filariasis situation in National and region wise. (DOHS, MOH and HMG (NG) Nepal from 1995/96 to 2005/06.)

**Table 1: National and Region Wise Number of Filarial Cases of Nepal
FY 1995/96 to 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	2,964	257	981	736	302	418
1997/98	2,384	328	605	976	317	155
1998/99	2044	165	671	913	281	14
1999/00	1797	209	718	632	195	9
2000/01	1632	262	546	692	123	56
2001/02	1183	142	173	733	79	46
2002/03	809	63	302	334	64	16
2003/04	550	47	246	221	20	20
2004/05	549	25	274	180	50	3
2005/06	542	82	193	175	89	3
Total	17,291	2,073	5,558	6,381	2,182	1097

Source : EDCD, 2006.

IV

MATERIALS AND METHODS

4.1 Study Area

Nepal is located from 80⁰4' East to 88⁰12' East longitude and from 26⁰22' North to 30⁰27' North latitude. It is administratively divided into 5 development regions, 14 zones and 75 districts, 20 districts are in the Terai region, 38 are hill districts and the remaining 17 districts are in the mountainous region. The present study was carried out in Bara district. It is situated in terai belt of Narayani zone and included in the Central Development Region. Bara is situated over nearly 30 km air distance to the South of Kathmandu. Its latitude is 26⁰51' 25.2" North to 27⁰21' 57" North of the Equator, while longitude is 84⁰51' East to 85⁰16' 12" East of the prime meridian. Area of Bara is about 1,190 square Kilometre. Geographically it is mid Terai region. Which is nearly below 400m mark from mean sea level (MSL). It is surround in the East by Sarlahi district, in the North by Makawanpur district, in the west by Parsa district and in the South by India (Uttar Pradesh State of India).

The average annual rainfall in this district ranges from 100cm to above 250cm and the temperature ranges from 10-45⁰C. According to "The Population and Socio-economic Atlas of Nepal Survey Department and Central Bureau of Statistics" HMG, Census, (2001), the population of Bara district is 5,591,135 (Male 2,89,397 and female 2,69,738). 2.42% of the Nepal's total population. Population density is 470 per square kilometer (CBOS, HMG, 2001).

The administrative division include 98 Village Development Committees (VDC's), 1 Municipality, Area numbers 15 and altogether 4 election areas. The health facilities include 99 health centres in curing population density per health centre 5,648. The educational status include 341 schools, 86,883 students and 1,584 teachers.

The district consists mainly 5 ethnic groups like Mushlims (13.43%), Tharu (11.31%), Yadav (10.43%), Brahman (5.29%) and Kanu (4.34%) Mother languages spoken in percentage of population are Bhojpuri (76.09%), Nepali (11.10%), Tharu (7.17%), Tamang (3.19) and Magar (0.55%).

Peoples of different religions (in %) seen are Hindu (81.94%), Mushlim (13.42%), Buddha (4.48%), Christian (0.09%), Kirata (0.02%) and others (0.05%). Economically active population (above 10 years of age) is 47.42%, female 22.96% and male 69.95% main occupation is farming and agriculture (59.93%) and others (40.07%).

The study site Kabahigoth VDC lies in the southern part of Bara district. Longitude of Kabahigoth is 85⁰0'58.6" East to 85⁰2'37.1" East, and its latitude is 26⁰54'57.4" North to 26⁰53'28.7" North. Kabahigoth is surrounded by Bagahi in the East, Parsurampur in the North Badkaphulbariya in the West and India in the South. It consists of all together 9 wards.

Households operating small scale non agricultural economic activity by types of activity for Kabahigoth VDC according to Central Bureau of Statistics (2001) shows total households number 781, out of them having economic activities are 36 households and not having economic activities are 745 households. Out of 36 households of having economic activity 4



Source: Census 2002, National Bureau of Statistics

Map prepared by the Department of Census, National Bureau of Statistics, P.O. Box 102, Kathmandu, Nepal

BARA DISTRICT

ZONE NARAYANI

District Code : 35

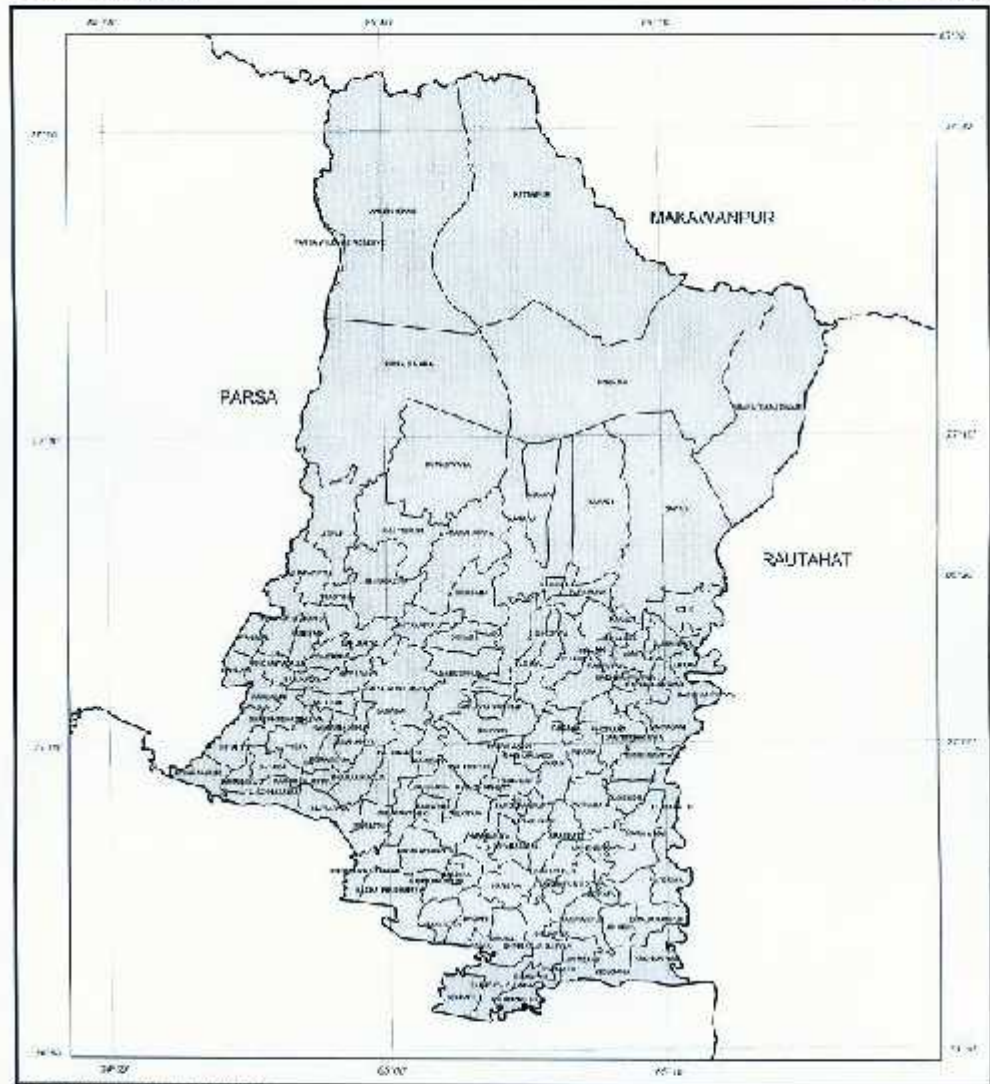


Figure 1: Administrative map of Bara District

SCALE 1 : 325000

LEGEND	
	District Boundary
	VDC Boundary
VORANG	District Name
	VDC Name



HORIZONTAL DATUM

Spheroid: Everest, 1830
 Projection: MGRS
 Origin: Longitude 84° E, Latitude 27° N
 False Easting of origin: 500 000 m, False Northing: 0 m
 Spheroid's Central Meridian: 85° 30'

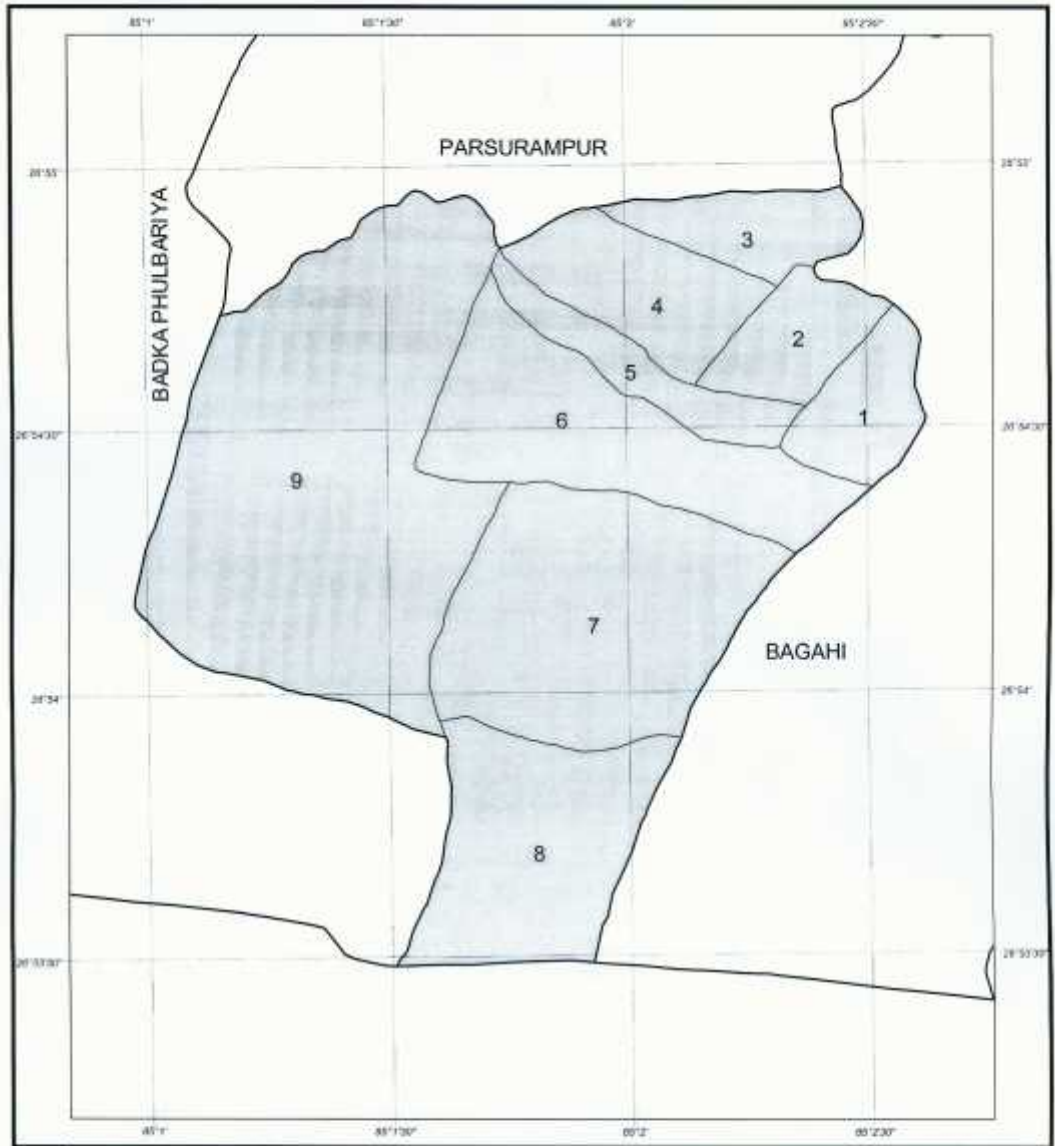
LOCATION MAP



KABAHIGOTH VDC

DISTRICT : BARA

VDC Code : 33047



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SCALE 1 : 20000

LEGEND	
	VDC Boundary
	Ward Boundary
BUKHEL	VDC Name
5	Ward Number

0 400 800 Meters

HORIZONTAL DATUM
 Spheroid Everest 1830
 Projection MUTM
 Origin Longitude 84° E., Latitude 0° N.
 False coordinates of origin 500 000 m. Easting, 0 m. Northing
 Scale Factor at Central Meridian 0.9999

BARA DISTRICT
 VDC Location Map



Map compiled from National Topographic Database of Nepal

households are manufacturing, 21 households are engaged in business, 1 household in transport, 5 households in services and 5 households have other occupation.

Total population of Kabahigoth VDC is 5291, among which 2768 are male and 2523 are females. Out of 5291 total population, native born are 4877, born in same district are 4839 while born in other district are 38, but foreign born population is 414. Out of total population of Kabahigoth 5269 are born in Nepal while 22 are born in India and other countries 3.

In Kabahigoth VDC literate population is 4389, out of which 2336 are male and 2053 are female.

4.2 Survey

4.2.1 Preliminary Field Survey

The study was conducted on 15 December 2006 to know about the lymphatic filarial situation in Kabahigoth VDC of Bara District. The site was selected as by the recommendation of the DPHO. The main aim of selecting Kabahigoth VDC was to focus on the study of mf in unsuspected and suspected filarial patients to know the actual situation of filariasis in Kabahigoth.

4.2.2 Primary Data Collection

The study was conducted from 15 January 2006 to 15 February 2006. During the blood sampling in the field, sentinel sampling was chosen without missing any house (each and every house) till the target samples were achieved. Before the blood-sample collection, the respondents were gathered and were given orientation about filariasis and the aim of the study, advantage and disadvantage was also explained. The blood samples were

taken from those respondents who were at home during the collection period.

4.2.3 Secondary Data Collection

Secondary data were taken from Central Bureau of Statistics, Ministry of Health, EDCD and journals.

4.3 Study Design

Cross-sectional epidemiological sentinel survey design was applied as the research tool in this study.

4.4. Instrumentation

Different tools used in this study were as follows:

- **Questionnaire**

The questionnaire structured containing Name, Age, Sex, Occupation, Education, Marital status, Relationship of the respondents with the family head, surrounding environment and its effects against the disease, Respondents current residential status, knowledge about mf, their views about the disease, their current health status, clinical symptoms of filariasis, was administered to the respondents.

- **Night Blood Sample Collection**

The sample was collected after 10 pm at night.

4.4.1 Laboratory tools for preparation of blood smear

- Materials slides, sterile lancets, tooth pick, cotton wool, gloves, measuring cylinder, slide box, compound microscope, mask, dropper, slide box etc.

- Reagents

Methanol, Distilled water, Giemsa stain 5%.

4.4.2 Procedure

Sampling Technique and Sample Size

A total of 506 ear prick human blood sample were taken as the sample from the community people of Kabahigoth VDC of Bara district. During the survey the blood samples were collected at night from 12 A.M. to 2.30 A.M. and blood smears were prepared in the field. The samples were taken to laboratory for microscopic examination.

Questionnaires were filled at the spot to record the clinical history of the respondents during the sampling period. To ensure the better condition the following precautions were taken.

- During the study, questionnaires were filled up by interviewing the respondents to record their family background, knowledge about the disease and the clinical history of patients.
- The sample slides were properly cleaned and dried without using antiseptic. The lancets were handled carefully and disposed after use. Once used lancets were not used for other patients.
- Each sampling slides were labled with code number HH1/A, HH2/B etc. which were coded according to the questionnaires.
- The blood samples were collected from the ear lobe by adding three drops of blood on slide at three different spots. After pricking the ear lobe of the respondents, three thick blood smears were prepared in each slide with the help of toothpick or another slide prepared blood

smears were left unstained for drying about 8 to 10 minutes and kept them properly in the slide box.

After collection of blood samples, these were stained with Gimsa stain. The staining process was done by following methods.

a) Fixing of blood smear:

The collected blood smears were fixed in methanol by dipping for about 5 seconds in order to fix the nematodes. The fixed blood smears were dried at room temperature.

b) Dehaemoglobinisation of thick blood smear:

The fixed blood smears were dehaemoglobinised by using distilled water and dried at room temperature.

c) Staining of blood smears:

The dehaemoglobinised blood smears were stained in Gimsa stain at 1:10 dilution for 30 minutes. They were washed with distilled water and then dried.

d) Observation

The stained blood smears were examined in compound microscope under 5x, 10x, 40x and 100x objective lenses. Taxonomic keys of microfilariae (WHO, 2002) was used by monitoring and epidemiological assessment of the program to eliminate lymphatic filariasis. The same key was used in the present context for the identification of *Wuchereria bancrofti* on the basis of the following characters:-

- i) Discrete nuclei
- ii) Sheath stained

- iii) Empty space between the nuclei and body wall
- iv) Cephalic tip of tail
- v) Tip of tail may be bent back under-neath the body.

4.4.3 Diagnosis of elephantiasis by cross sectional sampling technique for the detection of microfilaria.

This technique was structured to indicate the presence or absence of microfilaria in blood samples in relation to age group, sex, occupation, education, use of bed nets and surrounding environmental condition.

4.5 Data processing and analysis

- **Data editing** : Data were edited as soon as possible to detect errors, misspelled words and to make sure that the data was accurate, uniformed and well arranged.
- **Coding** : Information were coded so that they were easily classified and tabulated.
- **Classification and tabulation**: All the data were classified according to the need of the objective and tabulation was done for summarizing the data and displaying statistically.
- **Data analysis** : Data analysed by means of table, bar-diagram and pie-charts.

4.6 Validity and reliability of the study

- Quality control on specimen collection, processing and conformation of *W. bancrofti*.
- The study was properly instructed and guided by the supervisors.
- Questionnaires were filled by the respondents in the trend of the investigator.
- All reagents, equipments and laboratory methods were standardized.

RESULTS

The study was carried out among the filarial suspected and unsuspected people by using cross-sectional sampling method. All together 506 blood samples were collected from the Kabahigoth VDC of Bara District. The blood samples were collected from the respondents above 2 years of age. The study was divided into two parts i.e. questionnaire survey and microscopical examination of blood samples. The economical and demographical characteristics of the study population were also studied to know the back ground of respondents in the study area.

5.1 Results of Questionnaire Survey Analysis and Microscopic Results

5.1.1 General Prevalence of Microfilariaemia

Among the 506 blood samples collected, 1 sample (0.2%) was found to be infected with microfilaria.

Crude disease rate: suspected and symptoms of LF like elephantiasis, hydrocoel, breasts welling, chiluria and lymphatic swelling was found in 31 samples (6.13%)

5.1.2 Sex wise Prevalence of Microfilaria:

Out of 506 blood samples, 251 (49.60%) were males and 255(50.39%) were females. Among 251 males, not any infection of Mf was seen. While infection rate in females was found to be 0.39% (Table 2).

A degree of freedom χ_1^2 value corresponding to probability 0.05 is 3.84 calculated value 0.986 is lower, hence no significant at 5 % level. Observed differences in sexwise prevalence of Mf is due to chance. Thus severity of the disease filariasis does not affect either male or female particularly.

Table 2: Sex wise Prevalence of MF.

Sex group	Result		Total sample
	Mf. +ve (%)	Mf. -ve (%)	No (%)
Males	0(0%)	251 (100%)	251(49.60%)
Females	1(0.39%)	254 (99.60%)	255(50.39%)
Total	1(0.20%)	505(99.80%)	506(100%)

5.1.3 Age-wise Prevalence of Microfilaria

Out of 506 total samples the only one found positive lies in the age group 41-50 years where the total sample examined is 59. So the % of prevalence to this group is only 1.69%. The highest samples collected was under 10 years while the lowest above 70 years i.e.

Refering to the χ^2_7 table, the value at 5% level at 7 degrees of freedom, i.e., χ^2 is 14.07 which far exceeds the observed χ^2 value, 7.575. Thus the observed distribution fits to the normal distribution. The variation observed is only by chance; i.e. the mf. positive observed is just an accidental.

Table 3: Age-wise Prevalence of Microfilaria

Age group	Sample result		Total
	Microfilaria mf. +ve (%)	Microfilaria mf-ve (%)	Samples
<10	0	14	14
11-20	0	92	92
21-30	0	82	82
31-40	0	67	67
41-50	1(1.69%)	58(98.30%)	59
51-60	0	45	45
61-70	0	18	18
>70	0	2	2
Total	1	505	506

Calculated $\chi^2 = 7.575$, tabulated $\chi^2 = 14.07$ (P<0.05)

5.1.4 Sexwise Endemicity Rate of Lymphatic Filariasis

The study showed that the total ER was 32(6.32%). The highest ER 23(9.16%) was found among the 251 males. The ER among the 255 females was 9(3.52%). Statistically there was significant difference of infection in both sexes.

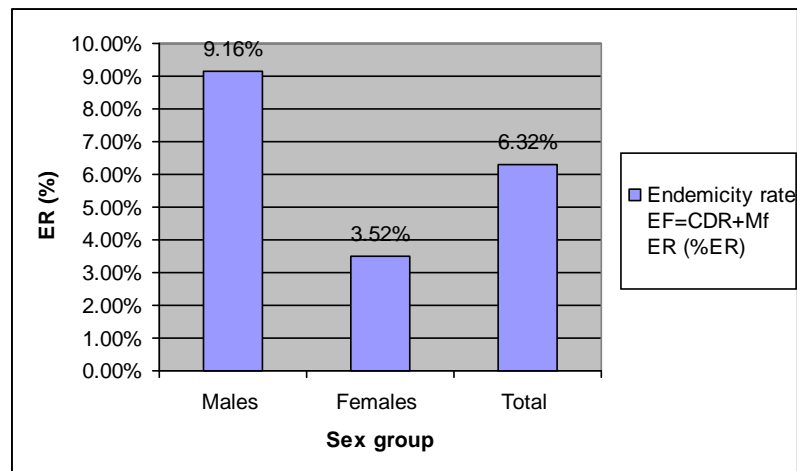
At one degree of freedom, at 5% level the value of $\chi_1^2=3.84$. the calculated value of χ_1^2 is 6.598, hence it is significant. The inequality is due to crude disease where the microfilaria may be found dead in lymphnodes and show only symptoms but filaria could not be found in blood stream. So it is not a chance but it may be actual.

Table 4: Sex wise Endemicity Rate of Filariasis

Sexes	Crude disease rate CDR	Microfilaria mf. +ve	Endemicity rate ER=CDR+Mf ER (%ER)	Total number of respondents
Males	23	0	23(9.16%)	251
Females	8	1	9(3.52%)	255
Total	31(6.13%)	1(0.198%)	32(6.32%)	506

Calculated $\chi_1^2= 6.598$; Tabulated, $\chi_1^2=3.84$ (P<0.05)

Fig. 1: Sex wise Endemicity Rate of Lymphatic Filariasis



5.1.5 Age wise Endemicity Rate of Lymphatic Filariasis

At 7 degrees of freedom, χ_7^2 value 32.748; being much higher than the table value of 14.07 ($P < 0.05$) is highly significant. Null hypothesis of no association of age-wise endemicity with lymphatic filariasis is rejected, and increase in endemicity of flariasis with increase in age is proved statistically.

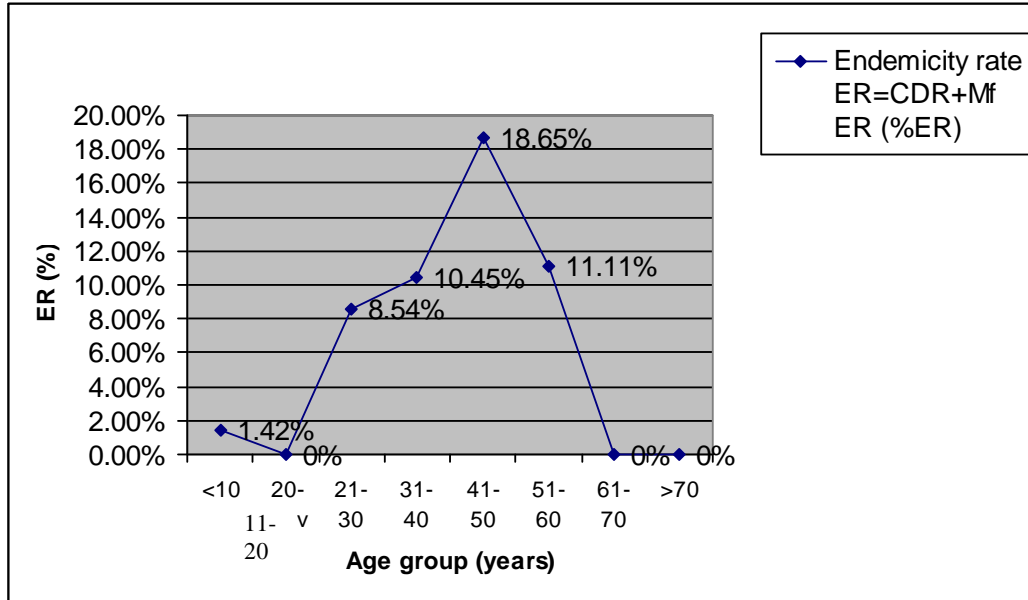
Table 5 shows the age wise endemicity rate of filariasis among the respondents from Kabahigoth VDC of Bara. The highest endemicity rate was found in the age group 41-50. Out of 59 respondents from the age group 41-50 years, 11(19.65%) were recorded as highly endemic. The lowest acdemicity rate was found in the age group 11-20; 61-70; >70 years age group where the endemicity rate being zero. Statistically there was significant difference of infection between the different age groups.

Table 5: Age wise Endemicity Rate of Lymphatic Filariasis

Sexes	Crude disease rate CDR	Microfilaria mf. +ve	Endemicity rate ER=CDR+Mf ER (%ER)	Nonendemicity rate NER	Total number of respondents
<10	2	0	2(1.42%)	139	141
11-20	0	0	0(0%)	92	92
21-30	7	0	7(8.54%)	75	82
31-40	7	0	7(10.45%)	60	67
41-50	10	1	11(18.65%)	48	59
51-60	5	0	5(11.11%)	40	45
61-70	0	0	0(0%)	18	18
>70	0	0	0(0%)	2	2
Total	31	1	32(6.33%)	474	506

Calculated $\chi_7^2 = 32.748$; Tabulated, $\chi_7^2 = 14.07$ ($P < 0.05$)

Fig. 2: Age wise Endemicity Rate of Lymphatic Filariasis



In the curve graph age group of 41-50 yrs, the endemicity rate (18.65%) is highest, at this age people work in fields and thus are more expose to mosquito.

5.1.6 Education wise Distribution of Microfilaria

The study showed that total number of 1(0.198%) person was Mf positive among 506 respondents. Maximum rate 0.36% of infection was found among the respondents with illiterate level (out of 281 respondents, 1 was mf. +ve) and minimum rate of 0% was found among the literate and other educated people.

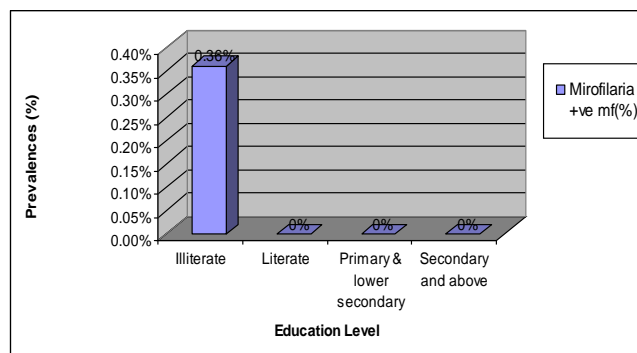
At 3 degrees of freedom and 5% level of significance tabulated value of $\chi_3^2 = 7.82$ ($P < 0.05$), but calculated value of χ_3^2 is 0.80 is much lower, hence insignificant. Thus the observed difference in distribution of microfilaria in different educational level is by chance.

Table 3: Education wise distribution of Microfilaria

Educational level	Mirofilaria +ve mf(%)	Microfilaria -ve	Total samples examined
Illiterate	1(0.36%)	280	281(55.53%)
Literate	0(0%)	17	17(3.36%)
Primary & lower secondary	0(0%)	141	141(27.86%)
Secondary and above	0(0%)	67	67(13.24%)
Total	1(0.198%)	505	506

Calculated $\chi_3^2 = 0.80$; Tabulated, $\chi_3^2 = 7.82$ ($P < 0.05$)

Fig. 3: Education wise Prevalence of Mf.



5.1.7 Occupation wise Distribution of Microfilaria

Out of total samples of 506, the maximum number of respondents were farmer (229) and the lowest number of respondents were labours. One (1.266%) positive slide was found in house wife among the 79 housewife respondents.

Table 7: Occupation Wise Distribution of Microfilaria

Occupation	Microfilaria mf+ve	Microfilaria mf-ve	Total samples examined
Farmer	0(0%)	229	229(45.25%)
Student	0(0%)	118	118(23.32%)
Labour	0(0%)	3	3(0.59%)
Housewife	1(1.266%)	78	79(15.61%)
Businessman	0(0%)	8	8(1.58%)
Unemployed	0(0%)	14	14(2.76%)
Teacher	0(0%)	4	4(0.79%)
Others	0(0%)	3	3(0.59%)
Child	0(0%)	48	48(9.48%)
Total	1(0.198%)	505	506(100%)

Calculated $\chi_8^2 = 5.41$; Tabulated, $\chi_8^2 = 15.51$ ($P < 0.05$)

5.1.8 Prevalence of Filariases in Relation to the use of Mosquito Precautions.

Out of 506 samples, 281 were illiterate among which 272 (96.79%) used mosquito nets and 9(3.2%) used smokes. Total mosquito net user were 478(94.47%). Primary and lower secondary were 141 (27.87%) among which 132(93.61%) used mosquito nets. Secondary and higher secondary were 67 (13.24%) among which 58(86.56) used mosquito nets. Only 1(0.36%) positive is found out of 506 respondents and it lies in illiterate column among 272(96.79%) (Table-8).

Table 8: Prevalence of Filariasis in Relation to the use of Mosquito Precautions

Education	Precautions used				Total no. of respondents	Mf. +ve
	Mosquito nets	Mosquito mats	Smoke	Ointment of mosquito		
Illiterate	272(96.79%)	0	9(3.2%)	0	281(55.53%)	1(0.36%)
Literate	16(94.11%)	0	1(5.8%)	0	17(3.36%)	0(0%)
Primary and lower secondary	132(93.61%)	0	9(6.3%)	0	141(27.87%)	0(0%)
Secondary and higher	58(86.56%)	0	9(13.4%)	0	67(13.24%)	0(0%)
Total	478(94.47%)	0	28(5.53%)	0	506(100%)	1(1.98%)

5.1.9 Prevalence of Filariasis in Relation to the Knowledge about Lymphatic Filariasis (LF).

Out of 506 samples, 20(3.95%) had knowledge about lymphatic filariasis, and 486(96.05%) did not have knowledge about LF. Among 20 respondents who had knowledge about Lf, 4(1.42%) were illiterate out of which 1 was Mf +ve. Among the respondents without knowledge there was no +ve slide was found (Table 9).

Table 9: Prevalence of Filariasis in Relation to the Knowledge about Lymphatic Filariasis, LF.

Education	Knowledge about LF	Donot having knowledge about LF	Total respondents	Microfilaria Mf. +ve
Illiterate	4(1.42%)	277 (98.5%)	281	1
Literate	0(0%)	17 (100%)	17	0
Primary and lower secondary	5(3.54%)	136 (96.4%)	141	0
Secondary and higher	11(16.41%)	56 (83.5%)	67	0
Total	20(3.95%)	486(96.05%)	506(100%)	1(0.198%)

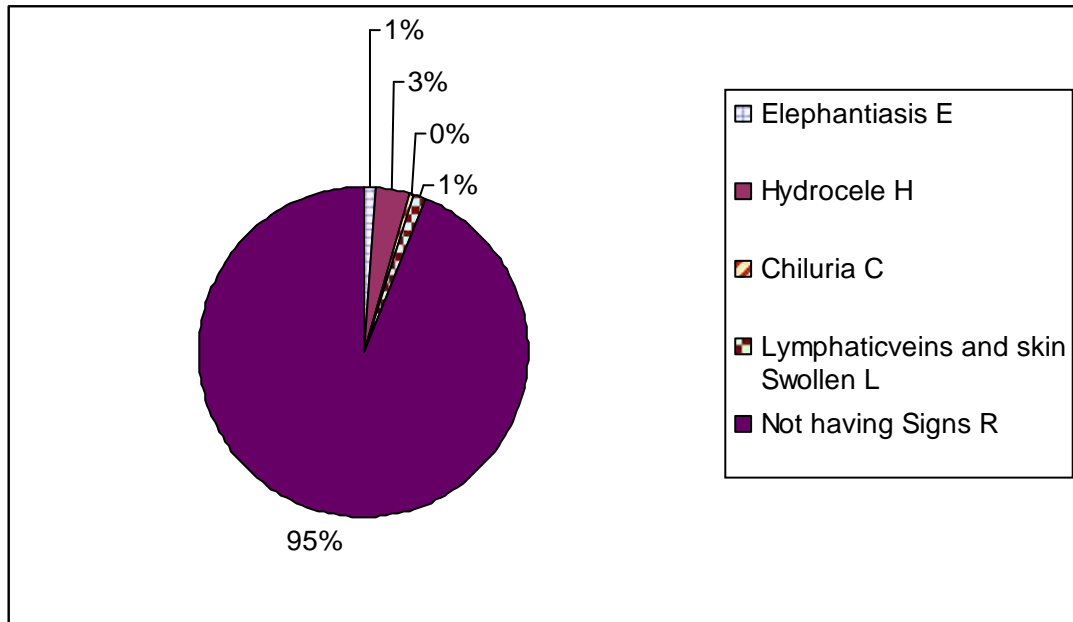
5.1.10 Sexwise Distribution of Signs and Symptoms of Lymphatic Filariasis

Out of 50 samples, 31 samples (24 males and 7 females) were with sign and symptoms of Lf. The crude disease rate (CDR) was recorded to be 31(6.13%) with 24(9.56%) males and 7(2.75%) females. Among 506 total samples 6(1.19%) was infected with elephantiasis, 17(3.36%) with hydrocele, 2(0.40%) with chiluria and 6(1.19%) with lymphoedema. Among 251 males, 3(1.20%) had elephantiasis, 17(6.77%) hydrocele and 4(1.59%) with lymphatic veins and skin swollen. Among 255 females, 3(1.18%) were infected with elephantiasis, 2(0.79%) with chiluria and 2(0.79%) with veins and skin swollen. Swollen breast and thick skin was not found (Table 10, Fig. 4).

Table 10: Sexwise Distribution of Signs and Symptoms of Lf

Sex	Total samples	Total signs and symptoms of LF						Total CDR samples	
		Elephantiasis	Hydrocele	Breast swelling	Chiluria	Lymphatic veins and skins swollen	Thick skin only	CDR No.	CDR%
Male	251(49.60%)	3(1.20%)	17(6.77%)	0	0	4(1.59%)	0	24	9.56%
Female	255(50.40%)	3(1.18%)	0	0	2(0.79%)	2(0.79%)	0	7	2.75%
Total samples	506(100%)	6(1.19%)	17(3.36%)	0	2(0.4%)	6(1.19%)	0	31	6.13%

Fig. 4: Total Distribution of Signs and Symptoms of LF.



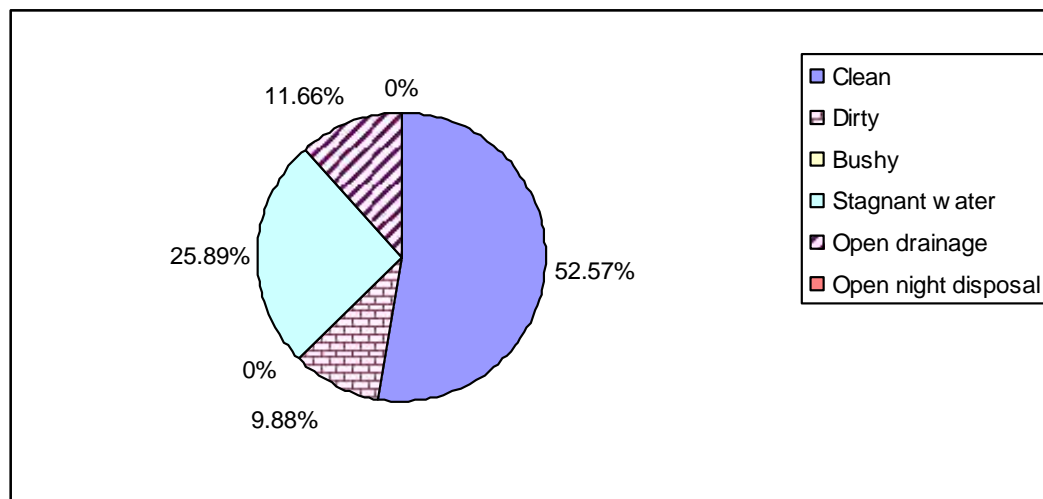
5.1.11 Environmental Status of Household

Out of 506 samples taken from Kabahigoth area, 266 (52.57%) samples had clean surroundings, 50(9.88%) had dirty surroundings 131(25.89%) had near by stagnant water and 59(11.66%) samples had open drainage environment. No bushy area nor open night-soil disposal was found in the study area.

Table 11: Environmental status of Household

Environmental state	Total no. of samples	Percentage of samples
Clean (garbage not found)	266	52.57%
Dirty (garbage found)	50	9.88%
Bushy	0	0%
Stagnant water	131	25.89%
Open drainage	59	11.66%
Open night disposal	0	0%
Total	506	100%

Fig. 5: Environmental Status of Household



5.1.12 Demographic Status of the Samples Collected

Out of 506 total samples, the maximum respondents were the childrens <10 years, in which males were 68(48.23%) and females were 73(51.77%) among the total of 141(27.87). While the least respondents were seen in age group >70, in which altogether 2(0.40%) respondents were seen among which 1 was male and 1 was female.

Table 12: Distribution of Demographic Status of the Samples Collected

Age groups (years)	Male samples	Female samples	Total no. of samples
<10	68(48.23%)	73(51.77%)	141(27.87%)
11-20	50(54.35%)	42(45.64%)	92(18.18%)
21-30	35(42.68%)	47(57.32%)	82(16.21%)
31-40	37(55.22%)	30(44.78%)	67(13.24%)
41-50	28(47.46%)	31(52.54%)	59(11.66%)
51-60	22(48.89%)	23(51.11%)	45(8.89%)
61-70	10(55.56%)	8(44.45%)	18(3.56%)
>70	1(50%)	1(50%)	2(0.40%)
Total	251(49.605%)	255(50.395%)	506(100%)

VI

DISCUSSION AND CONCLUSION

Filariasis is a group of infectious disease caused by the group of microfilariae with adult worms living in lymphatic system including *W.bancrofti*, *B. malayi*, *B. timori* termed as filarial worm. They are human and animal parasites. Filariasis is a mosquito born diseases responsible for great problem in human-beings causing elephantiasis in leg, hands, enlargement of scrotum, labia, clitoris and vulva, breast, forearm, lymphnodes of the inguinal regions with lymph, scrotum and lymphoedema scrotal lymphangiovarices showing vesicle chylocele and lymph varix of the spermatic cord, chyle varices of the posterior abdominal wall (rupture of those vesicles in the urinary tract causes chyluria, chylothorax, chylous ascites and chylous diarrhoea, Lymphatic filariasis has been estimated as endemic in some 80 countries including 120 millions of people (WHO, 2000).

Lymphatic nematohelminthic disease also known as lymphatic filariasis is worldwide in distribution mainly in Asia, Africa, Central and South America, Eastern Mediterranean and the Islands of Oceania and Pacific covering about 70 countries. But seriously endemic areas are Asia and Africa. Nocturnal periodic *W. bancrofti* is wide spread almost all the endemic areas of the world except Islands of New Polynesia as well as Nicobar Islands of India, where diurnal subperiodic *W. bancrofti* is endemic. No natural reservoir of *W. bancrofti* has been found. Nocturnal subperiodic *B. malayi* is only distributed in swamp forest area of Malaysia, Indonesia and Thailand. Monkeys and cats are its animal reservoirs.

According to the fifth report of WHO expert committee on filariasis, issued in 1991 almost 751 million people were living in endemic areas and were at risk of these 72.8 millions were infected with *W. bancrofti* and 5.8 millions with *B. malayi* and *B. tomori*.

At present 1.1 billions people (20% of the world's population) in some 80 endemic countries located in the tropical areas of the world are at risk of infection by *W. bancrofti* and *B. malayi* (WHO, 2000).

Filariasis has been known to be endemic in Nepal since longtime (EDCD, 2000). It has been reported from Nepal from different areas. The present surveillance conducted in Kabahigoth VDC of Bara district shows the total endemicity rate of 6.33% with overall microfilariaemia of 0.2% and crude disease of 31(6.13%) out of 506 total respondents.

Jung (1973) gave first report from central Nepal. He reported 4.99% to 6.15% crude disease rate in all age groups and both the sexes in the urban population, 6.6% to 10.3% in the semi urban population. Similarly, the study showed 7.1% to 9.16% microfilariaemia in the urban population, 10.03% to 11.3% in the semi urban population and 0.8% to 17.69% in the rural population. Pradhan *et al.*, (1997) studied in Gokarna VDC of Kathmandu valley, reported 24.6% endemicity rate with the overall 11.95% microfilariaemia and 12.64% crude disease. Bhusal *et al.*, (2000) reported overall 5.8% prevalence of microfilariaemia and 13.0% crude disease rate in the study of population in Tokha-chandeshwari VDC. Manandhar (2001) reported 19.9% of crude disease from sipawa, Dovan and Bhaktapur.

The results found out by Jung (1973) Pradhan *et al.*, (1997) and Bhusal *et al.*, (2000) are higher than that of the present study in comparison to the endemicity rate, microfilariaemia and crude disease. This may be due

to increasing awareness of people towards disease and availability of medicine. Sherchand *et al.*, (2002) surveyed on 37 districts of Nepal and reported 13% prevalence of microfilariae among the different districts.

The present study showed, out of 255 female samples, 1 female (0.39%) with positive microfilaria. Male 251 samples were totally negative. Weerasooriya *et al.*, (2001) Sri-lanka delivered the fact that males usually are infected more due to their outdoor sleep habit without using mosquito nets, dressing naked only vest and half pants favours for mosquito bite. Lymphatic filariasis is a disease of poor environmental and poor hygienic condition with Low Socio-economic Status, low literacy rate and high percentage of illiterate situation (WHO, 1997).

Present study revealed that maximum endemicity rate was recorded from those people whose socio-economic status and literacy rate was very poor. Filarial disease are associated with Low Socio-economic status, illiteracy, poor health and hygiene and lack of knowledge about the filarial diseases.

All age groups are susceptible to filariasis. The present study showed high endemicity rate of infection in the age group 41-50 years i.e. 11/59 (18.65%) while least in the age group 11-20, 61-70 and >70 i.e. 0%. High endemicity rate in the age group 41-50 years suggest that for appearance of crude diseases takes time. Age wise distribution of filariasis is equivalent to the length of exposure, which is supported by WHO (2001).

Most of the people 484 out of 506 do not have knowledge about (96.05%). Only 20(3.95%) had knowledge about Lf. This also favours the vector borne disease to spread.

The environmental status of household also plays role in infection by filariasis. Stagnant water 131(25.89%), open drainage 59 (11.66%) and dirty 50(9.88%) favours for the breeding of mosquitoes and thus help to increase the population of filariasis.

Low prevalence of mf in health post area is due to facility of medicine. But high CDR rate shows the boarder of India, the Kabahigoth of Bara may have imported the disease from highly endemic area of Bihar.

VIII

RECOMMENDATIONS

Based on the present study conducted in Kabahigoth VDC of Bara district the following recommendation for effective control of Lf should be put forward.

- Local peoples should be made aware of the causes and effect of filariasis and early symptoms through mass gathering and training programme, through television, FM radios using local languages.
- Preventive measure should be given by the government. Like distributing mosquito nets, mats, spraying etc.
- Boarder areas of India should be proper quarantined.
- The biological control of mosquito should be done by larvivorous fishes in small ponds.
- Ecology and behaviour of local vectors should be studied to eradicate breeding sites of mosquito.
- Proper management of waste products, open drainage, surrounding environments, marshy lands and bushy areas.
- Regular health check up and mass treatment of drugs/medicines.

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ANNEX – I

Process of Making Dilute Giemsa Solution

Giemsa Stain:

Giemsa stain is an alcohol based Romanowsky's stain, it is highly flammable with flash point 12⁰c, that requires dilution in PH 7.1-7.2 buffered water before use. It gives best staining of microfilarial parasites in thick and thin blood films, if the concentration of the stain is low, the staining time is long. Care must be taken to prevent water from entering the stock stain.

Making a Giemsa 10% Working Solution:

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

Preparation of reagents for Giemsa stain:

To make about 500ml of Giemsa stain we use:

Giemsa 3.8 grams.

Glycerol (Glycerine) 250 ml

Methanol (Methyl alcohol) 250ml

1. Giemsa is weighted on a piece of clean paper (Pre weighted) and transferred to a dry bowl of 500ml capacity that contains a few glass beads.
2. Using the same cylinder, the glycerol is also measured, and added to the stain, then mixed well.
3. The bottle of stain is placed in water bath at 50-60⁰C or up to 2 hours at 37⁰C that will help the stain to dissolve and also at intervals the stain should be mixed well.

4. Thus prepared stain is poured in a clean bottle, labeled and marked in flammable and toxic. It should be stored at room temperature in the dark, if kept well stoppered, the stain is stable for several months. For use: filter a small amount of stain into a dry-dispensing container.

Cautions:

1. Giemsa stain will be spoiled if water enters the stock solution during its preparation or storage.
2. Methanol is toxic and highly flammable, therefore it should be handled with great care and kept away from open flame.

Controlling Stains and Reagents:

Giemsa stain is used mainly for staining microfilaria, malarial parasites, trypanosomes and leishmanial parasites.

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 from entering the stain.
3. For routine use, a small amount of the 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 fresh blood, dried and folded individually in paper, sealed in a plastic bag and stored in a freezer at 20°C.

Characteristics of Giemsa Stain:

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

Chromatin of parasite	Dark red
Cytoplasm of Parasite	Blue
Schuffner's dots	Red
Maurer's dots (clefts)	Red maure
Red cells	Grey to pale maure
Reticulocytes	Grey blue
Nuclei of Netrophils	Maure purple
Granules of eosinophils	Red
Cytoplasm of mononuclear cells	Blue grey

Precautions and Warnings:

Optional results will be obtained by strict adherence to this protocol. Reagents must be added carefully to maintain precision and accuracy. Once pricked or used lancet should never be reused. Biological contamination of dispensing equipment, containers or reagents can leads to false results. Precaution against microbiological and serological hazards in specimen handling, disposal and throughout all procedures should be taken with great care. Date expired components should never be used and the blood samples collected should be stored only in dry boxes.

ANNEX – II

Questionnaires for Filariasis Cross Sectional Survey in Kabahigoth VDC of Bara District of Nepal

Date:

S.N.:

1) Name of the Respondent :

Address : District :

Ward No./Tole/Block No.

2) Age/sex

3) Education:

- | | | | | | |
|---------------------|--------------------------|----------------|--------------------------|------------------|--------------------------|
| i) Literate | <input type="checkbox"/> | ii) Illiterate | <input type="checkbox"/> | iii) Primary | <input type="checkbox"/> |
| iv) Lower secondary | <input type="checkbox"/> | v) secondary | <input type="checkbox"/> | vi) Intermediate | <input type="checkbox"/> |
| vii) Bachelor | <input type="checkbox"/> | viii) Masters | <input type="checkbox"/> | ix) Others | <input type="checkbox"/> |

4) Occupation:

- | | | | | | |
|-----------------|--------------------------|--------------|--------------------------|---------------|--------------------------|
| i) Farming | <input type="checkbox"/> | ii) Labour | <input type="checkbox"/> | iii) Business | <input type="checkbox"/> |
| iv) Student | <input type="checkbox"/> | v) Housewife | <input type="checkbox"/> | vi) Teaching | <input type="checkbox"/> |
| vii) Unemployed | <input type="checkbox"/> | viii) Others | <input type="checkbox"/> | | |

5) Marital Status:

- | | | | |
|-------------------|--------------------------|---------------------------|--------------------------|
| i) single/Married | <input type="checkbox"/> | ii) Widow/Widower/Divorce | <input type="checkbox"/> |
|-------------------|--------------------------|---------------------------|--------------------------|

6) Relationship with the head of the family/family size

7) Respondents current residence status:

- | | | | | | |
|----------------|--------------------------|-------------|--------------------------|----------------|--------------------------|
| i) Birth place | <input type="checkbox"/> | ii) Migrate | <input type="checkbox"/> | iii) Temporary | <input type="checkbox"/> |
|----------------|--------------------------|-------------|--------------------------|----------------|--------------------------|

(How long have you been staying here? years/months.

8) Surrounding environmental condition:

- i) Clean ii) Dirty iii) Bushy
iv) Open drainage

9) Use of the any means for the protection against mosquito bite:

- i) Yes ii) No

If yes, which one of the following:

- i) Mosquito net ii) Antimosquito cream iii) Smoke
iv) Spraying insecticides v) By burning mosquito coils

10) Do you have knowledge about the disease filariasis (elephantiasis)?

- i) Yes ii) No

If yes, how is it transmitted?

- i) By mosquito biting ii) Contact with diseased person
iii) Mother to foetus

11) Respondent current health status:

- i) Healthy ii) Unhealthy

If unhealthy since when Years months

12) Do you have any symptoms?

- i) Yes ii) No

If yes, which of the following

- i) Fever ii) Effect on genital iii) Headache
iv) Swollen lymphnodes v) Hydrocele
vi) Swollen hand or limb
vii) Skin thick, red and swollen blood vessels

- viii) Chyluria ix) Abscess formation x) Nausea
xi) Epigastric pain xii) Weakness xiii) Lazyness

If yes have you used any medicine?

- i) Yes ii) No

If yes, which of the following?

- i) Ayurvedic ii) Allopathic iii) Herbal

13) According to your knowledge, is the disease is more in parent's time or now?

- i) Parent's time ii) Now iii) Don't known

14) Have you seen any persons suffering from this disease?

- i) Yes ii) No

If yes, how many

15) Is there any person suffering from this disease in your family or relatives?

- i) Yes ii) No

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

Thank you very much for your valuable time.

Result of the test: Positive Negative

If positive : number of microfilaria per 20 ml.



Microscopic Photographs of : *Wuchereria bancrofti*



Microscopic Photographs of : *Wuchereria bancrofti*

Photo Page 4: Photos of Microfilaria



Going to field



District Public Health Office - Bara



Health post - Kabahigoth



Field surrounding environment

Photo Page 1: Field area concerning photos



Elephantiasis in women's right leg



Secondary infection in left leg



Hydrocoel in man Symptoms of filariasis

Photo Page 2: Appeared symptoms of filariasis



Staining in Lab



Observing on binocular microscope

Photo Page 3: Working in lab

ANNEX - III

General Structure and life cycle of *Wuchereria bancrofti*

Adult Worms

These are long hair like transparent nematodes often creamy white in colour, filliform in shape and both ends are tapering, the head end terminating in a slightly rounded swelling. The male measures 2.5 to 4 cm in length by 0.1 mm in breadth or thickness. Its tail end is curved ventrally and contains two spicules of unequal length. The female measures 8-10 cm in length by 0.2-0.3 mm in thickness. Narrow and abruptly pointed tail end females are more numerous than male. Life span of Adult worm is long usually 5-10 years.

Embryos (Microfilaria)

They pass through the lymph nodes. When unstained they appear colourless and transparent bodies with blunt head and pointed tails. Embryo measures about 290µm in length when dead and stained with Romanowsky's or Giemsa stain the embryo shows following morphological characteristics.

i) A hyaline Sheath

This is a structureless sac which is best seen beyond extremities of the embryo. The sheath is much longer (359µm) than the larval body so that the larva can move forwards and backwards within it. The sheath represents the chorionic envelope of the egg, it remains as an investing membrane round the larva.

Microfilariae of *Wuchereria bancrofti*

Wuchereria bancrofti

ii) Cuticula is lined by subcuticular cells and is seen only with the vital stains.

iii) Somatic cells or nuclei

These appear as granules, do not extend up to the tip of the tail (terminal 5%) and serve as a distinguishing feature of mf (*W. bancrofti*). At the anterior end there is a space, also devoid of granules, called the cephalic space which is long as it is broad with in vital stains, the presence of a style is seen.

➤ The granules are broken at definite places serving as the landmarks for identifications of the species. This includes the following

a) Nerve ring as oblique space

b) Anterior V-spot, represent the rudimentary excretory system and

c) Posterior v-spot or tail spot represents the terminal part of the alimentary canal (anus or cloaca).

➤ A few G-cells (the so called genital cells) posteriorly while G-cells 2,3 and 4 are just in front of the anal pore, G-cells 1 is situated further in front.

➤ Innerkorper of filariform or central (internal) body of manson extends from the anterior v-spot to the G-cell 1. It represents the rudimentary alimentary canal.

The larval form do not undergo any further development in the human body unless they are taken up by their appropriate intermediate host (Mosquitoes). If these microfilaria are not sucked up by the mosquito, they die in course of time. The life span of microfilaria in the human body has been found to be as long as 70 days (Rao, 1933).

- Somatic Cell or nuclei: These appear as granules.

Life Cycle, Pathogenicity and Transmission of MF

The life cycle of *Wuchereria bancrofti* is digenetic. Man is the primary or definitive host where the adult worms are harboured in the lymphatic system. Live embryos (mf) are discharged which find their way into the blood stream. They are then taken up by culicine mosquito, or some times *Aedes*, *Anopheles*, *Mansonia*, *Psorophora* which act as secondary host or intermediate host. They (mf) undergo further development in mosquito.

Infection by Mf and Life Cycle in Man and Development into Adult Worm

When the infected mosquito bites a human being, the 3rd stage larva (L₃) are not directly injected into the blood stream like malarial parasite but are deposited on the skin near the right of puncture. The size is 1400-2000 µm in length by 18-23 µm in diameter and have three sub terminal caudal papillae. They enter into the puncture wound or penetrate through the skin on their own. After that they enter into lymphatics and settle down in lymph nodes where they develop into adult worms after moulting twice. They are thin, long thread like and remain coiled at the place of their infection. The anterior end of the body tapering with a slightly swollen front end, there are no lips, the pharynx is muscular anteriorly and glandular posteriorly. Sexes are separate with distinct sexual dimorphism. In course of time, probably 5-18 months they become sexually mature. The female is longer and broader (80-100mm in length by 0.24-0.3 mm in diameter) than the male (25-40 mm in length by 0.1mm in diameter). The tail of the male remains coiled like a tendril and has a pair of unequal spicules in the cloaca. The male worm, unlike female also has a number of copulatory papilla near the cloacal aperture. Sexually mature worms reside in the lymphatic, which provide nutrition to them (Arora and Arora, 2001). The mature worms can live for many years in their host depending on the host's immune response. Their mean life span is 4-6 years but they can survive up to 15 years or more (Cheesbrough, 1998).

The females are ovoviviparous. Mature female after copulation becomes gravid with large number of fertilized eggs and give rise to large number of sheathed Mf the 1st stage larva (L₁) measuring 127-320 μm in length by 7.5-10μm in diameter. The body surface is covered by thin layer of flattened epidermal cells; a conspicuous cord of cytoplasm containing nuclei extends the length of the body and represents the anlagen of the alimentary canal. In addition to this cord, there are: (a) a dash at the anterior tip of the body that is claimed by most to represent the beginning of the mouth. (b) developing nerve ring (c) a v-shaped invagination that represent the future excretory pore (d) a renette cell or the so called G-cells, which eventually developed into the portion of the gut and (e) a tail spot in the posterior region that represents the developing anus. There are no nuclei at the tip of the tail. Body is slightly curved and the sheath stains pink with Giemsa stains. Through lymphatic, they find their way into general circulation. The appearance of Mf in the peripheral blood showed marked periodicity. Nocturnal periodicity that is occurrence of Mf in the peripheral circulations at night mostly after two hours at rest.

Several hypothesis have been contributed to explain nocturnal periodicity.

- i) There is chemostatic attraction between the microfilaria and the saliva of the mosquito hosts (vectors) which are more plentiful at night.
- ii) The relaxation of the host during sleep induces the microfilaria to migrate into the peripheral circulation.
- iii) The migration results from a response to oxygen and carbondioxide supply.
- iv) The microfilaria survive for only short period and it is during the nocturnal period that they are most abundant.

None of these hypothesis is completely satisfactory. The microfilaria are most abundant in the peripheral circulation between 10.0 pm to 2.0 am. (Cheng, 1999)

The microfilariae are chiefly in the capillaries and in the lungs when not circulating in the peripheral blood (Haslett *et al.*, 1999).

The different periodicities of MF correspond with the biting habits of their principle vector. For eg:- The nocturnal periodic Mf are transmitted by night biting habits of mosquitoes. This adaptation enhances their chance of onward transmission. The periodicity is due to a biological rhythm inherent in the microfilariae but influenced by the circadian rhythm of the host (cook, 1996).

Infection by Mf in Mosquito and Development in Mosquito

When an appropriate female mosquito vector bites an infected human host during blood meal at the appropriate time, microfilariae in the peripheral circulation are ingested. 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, they become shortened into short, sausage-shaped bodies measuring 127-250 μm in length by 10-17 μm in diameter. At this stage of development the first moult occurs in 3-7 days after which the tail portion atrophies and the intestinal tract becomes well defined. These second stage larvae (L_2) measure 225-300 μm in length by 15-30 μm in diameter. A second moult follows on the 10th or 11th day, and the resulting filiform 3rd stage (L_3) migrate anteriorly into the proboscis sheath of the mosquito's mouth parts. When an infected mosquito feeds again the infective (L_3) larvae can enter the human host (Cheng, 1999).

Clinical Features of Lymphatic Filariasis

It may be acute or chronic in nature.

Lymphatic Filariasis

Symptoms predominantly result from the presence of adult worms residing in the lymphatic system. Symptoms include fever, inguinal or axillary lymphadenopathy, testicular or inguinal pain, skin exfoliation and limb or genital swelling. Microfilaremia is generally considered to be asymptomatic, though subjects with heavy microfilarial loads may develop acute or chronic inflammatory granulomas secondary to splenic destruction. Passage of cloudy, milk-like urine may denote chyluria.

Acute manifestations of lymphatic filariasis usually are referred to as adenolymphangitis (ADL). ADL is characterized by episodic attacks of fever associated with inflammation of the inguinal lymph nodes, testes, spermatic cords,

lymphoedema, or a combination of these. Skin exfoliation of the affected body part usually occurs with resolution of an episode.

- Repeated episodes of inflammation and lymphoedema lead to lymphatic damage, chronic swelling and elephantiasis of the legs, arms, scrotum, vulva and breasts. Elephantiasis is seen as coarse thickening, hardening and cracking of the skin overlying enlarged fibrosed tissues. Secondary bacterial and fungal infections of the skin can occur. Hydrocele is the commonest manifestation of chronic *W. bancrofti* infection in males in endemic areas. Chyluria also may be present in individuals who are chronically infected.

Tropical pulmonary Eosinophilia (TPE)

TPE is a form of occult lymphatic filariasis. Presenting symptoms include a paroxysmal dry cough, wheezing dyspnoea, anorexia, malaise and weight loss.

Scattered wheezes and crackles are heard in both lung fields. Lymphadenopathy and hepatomegaly may be present.

Non-Filarial Elephantiasis

In tropical countries, cause of elephantiasis other than filarial worms include tuberculosis and siliceous deposits. Endemic elephantiasis of the lower legs associated with siliceous deposits have been reported from the highlands of Kenya, Tanzania, Ethiopia, Rwanda, Burundi, Western Sudan, Cape Verde islands, Cameroon and Rajasthan in India. Damage to local lymphatics with obstruction occurs when silica from the soil is absorbed through bare feet (Cheesbrough, 1998).

Control and Transmission of Filariasis

If transmission can be reduced and ultimately interrupted new infection will stop. Interruption can be achieved by treating the affected population to eliminate the reservoirs of *Mf* by removing human vector contact or both. Control measure

can be varied depending on the parasite vector situation, existing health care services and infrastructure, the availability of funds and local culture.

Even when the microfilaria is eliminated from an individual, the secondary infection caused by microbial pathogens is seen to induce lymphatic pathology. Lymphoedematous lymph are particularly susceptible to bacterial super infection. Attention to the problem of clinical disease can alleviate suffering and limit disability in infected person while control of transmission is being established. Experiment has shown that these efforts help considerably in enlisting the full cooperation of the public in filariasis control campaigns (WHO, 1997).