INTRODUCTION

Lymphatic filariasis is one of the most prevalent vector born tropical disease, which causes a dramatically disability, disfiguring disease usually affecting one or both legs, hydrocele, swollen in the breast, enlargement of clitoris and vulval, grotesque enlargement of the male scrotum and penis. Infection also causes acute fever, inflammation of lymphatic system and branchial asthmatic condition known as "Pulmonary Tropical eosinophilia" (WHO Geneva, 1995).

The massive swelling of entire legs, scrotum or genitalia, hardening and thickening of skin is commonly known by the name elephantiasis. It is caused by filarial nematode, the adult worms of which are found in the lymphatic vessels, lymphnodes as well as in connective tissues, sub-cutaneous tissues and body cavity of the host. While, the microfilariae are found in the peripheral blood of vertebrate host and abdominal region of invertebrate vector mosquitoes where cyclo-development takes place when they reach mosquito vectors.

Altogether there are eight main species of filarial nematodes that infect human beings namely *Wuchereria bancrofti, Brugia malayi, Brugia timori, Onchocerea volvulus, Dipetalonema perstans, Dipetalonema streptocerreum, Mansonella ozzardi* and *Loa loa*. Among all mentioned above species, the first three, *Wuchereria bancrofti, Brugia malayi* and *Brugia timori* cause lymphatic filariasis and are responsible for disfigure

and morbidity. *W. bancrofti* is the main cause of lymphatic filariasis in Nepal. The remaining five species cause non-lymphatic filariasis.
Unsystematic and sprouting of urban cities has created innumerable breeding sites for the mosquitoes which are the main vector or biological agent that provides cyclo-development site and transmission of disease. Both sexes are equally prone to infection especially the poor community of the society.

Lymphatic filariasis is well established disease in tropical and sub-tropical regions of the world. The enormous and unplanned increased in growth of cities has created numerous breeding sites for the mosquitoes which are the main vectors that transmit the disease. It is a significant cause of acute and chronic illness in both sexes mainly affecting the poorer community of the society. Lymphatic filariasis is endemic in 80 countries and more than 1.1 billion people worldwide are estimated to be at the risk (WHO Geneva, 2000). Approximately 120 million people in tropical and sub-tropical regions of the world are infected, of these 90% are caused by *W. bancrofti* and 10% by *Brugia malayi*, limited to Asia and some parts of Pacific (WHO, 1997). Almost 27 million men suffer from genital disease (hydrocele), more than 15 million suffer from lymphoedoma or elephantiasis of leg. 83 million people from lymphatic functional disability and 30 million from renal pathology (WHO, 1997). It has been a major socio-economic burden globally in tropics and sub-tropics. Approximately one third of the population at risk live in the Indian sub-continent with an estimated 45 million infected individuals. In South-East Asian region, about 600 million people live in the endemic areas constituting about 60% of the global burden.

Emphasis on the vector control measures is being given for controlling the transmission of the disease. Diethyl Carbamazine Citrate (DEC) is being used from more than 40 years for the treatment of filariasis as a chemotherapic means. Repeated treatment with DEC results significant reduction in the incidence of acute and chronic attacks and the risk of developing chronic disease. Control with DEC involves either mass treatment of all population of endemic community or selective treatment of those who are recognized as microfilarial positive during night blood surveys. Nowadays treatment with single dose of DEC along with Albendezole is of great practical interest. By the end of 2001, a total of 25,479,136 people had received mass drug administration in 22 countries participating in the Programme to Eliminate Lymphatic Filariasis (PELF). This is marked increased in comparison to the year 2000 when only 20 countries participated and 3 million people at risk were covered (WHO, 1999).

In most of the world's endemic areas, there are no effective filarial control measures. This is mainly because filariasis has not been regarded as a major public health problem and also most of the control strategies are too complicated and expensive to be sustained. In the past, parasitic control on transmission have eradicated filariasis in several endemic areas, however eradication is very difficult to achieve in most places. It will be appropriate to focus first on the development and implementation of simple, cost effective and sustainable, strategies for morbidity control (Ramachandran, 1993).

Thus, control of lymphatic filariasis for its elimination can be done only by recognizing the main vector of lymphatic filariasis and application of vector control measures.

Lymphatic filariasis is a public health problem in Nepal. The disease is wide spread throughout the country except mountainous and hilly areas. According to the recent LF mapping, it has been estimated that 60 of 75 districts are endemic for lymphatic filariasis with about 24 million people living in those districts are at risk out of total population (23.2 million approximately, CBS 2058). Here, a very few surveys on lymphatic filariasis have been under taken so far. Jung (1973), in a cross-sectional survey, found the prevalence ranging from 0-17.8% in nine different sites of Central Nepal. Pradhan *et al.*, (1988), in a study in Gokarna VDC of Kathmandu, found an endemicity rate of 24.6%. Bista *et al.*, in a situation analysis study during 1995-1999, recorded lymphatic filariasis at 13.2-23.4% in different regions of Nepal. Recently, Sherchand (2001/02), on epidemiological mapping of Lymphatic filariasis in 37 districts, found 11 districts above 20%, 15 districts with 6-19% and seven districts with 1-5% antigenaemia. The epidemiological mapping for filarial infection by ICT (Immunochromatography) card test carried out in 2001 in 37 districts revealed 33 districts (89%) were endemic. Among these 33 endemic districts, 11 districts have prevalence rate of over 20%, in 15 districts between 6-9% and in 7 districts the prevalence rate is found between 1-5% (EDCD, 2062/063).

Wuchereria bancrofti is the only recorded parasite in Nepal. The mosquito, *Culex quinquefasciatus*, an efficient vector of the disease has been recorded in all the endemic areas of the country. With WHO's global strategy to eliminate lymphatic filariasis as a public health problem by

2020 and the government's political commitment, the Epidemiology and Disease Control Division, Department of Health Services, Ministry of Health and Population has formulated a National Plan of Action (2003-2018A.D.) for elimination of lymphatic filariasis in Nepal. Government of Nepal, Ministry of Health and Population has also established a National Task Force for the Elimination of Lymphatic Filariasis (NTF-ELF) leaded by Director General of the Department of Health Services.
To action this, and to prioritize the districts for the implementation of LFE, it becomes crucial to know that exact prevalence of LF for the entire country through WHO recommended standard techniques.

On the other hand the prevalence of vector of LF which is mainly mosquito of genus *Culex quinquefasciatus* has not been done in Nepal except Peters and Dewar, (1956) who reported this genus from Koshi, Sunsari, Dharan, Kathmandu, Makwanpur, Narayani and Hetauda, as a serious domestic pest, found in vast numbers in exposed ground pools, drainages, pots and domestic grease traps.

The biology and distribution of *Culex quinquefasciatus* and the first report from Nepal was given by (Joshi *et al.*, 1965 and Shrestha, 1966). The species is the principal vector of *Wuchereria bancrofti* in Nepal, which is within the endemic zones of filariasis (Jung 1973).

Significance of the Study

The prevalence of lymphatic filariasis is seen in many places of Nepal, mainly in Terai region where the mosquito completes its life cycle easily. But, according to the sentinel survey taken under the initiation of PARASED, Nepal hilly region is also equally prone to infection by

lymphatic filariasis. The main reason behind it may be due to geographical and climatic variation. The second reason may be due to global warming and favourable breeding habitats created because of poor sanitary condition and ignorance among the people of hilly region about the mode of transmission of filariasis. In view of this, LF is highly pertinent to enquire into the status and situation of filariasis. So, as to deal more efficiently with the problem to establish the epidemiology of the disease in all regions of Nepal and to find prevalence of the infection on the basis of age, sex, ethnic groups, knowledge and attitude and illiteracy rate of the people regarding this view the present study has been under taken. This may be coin assistance to WHO and other concerned organizations for the elimination of LF from the world.

The principal strategy for this disease in achieving the goals is to prevent transmission of parasite through the vector, mosquito by eliminating the infections MF from the infected person through one year single doze treatment with combination of two drugs (DEC and Albendazole) of entire population at risk for LF, such treatment should be continued for four to six years the estimated reproductive life span of filarial adult stage, Ottensen (2002).

Recently, the initiatives is taken by "PARASED" Nepal, which is financially supported by joint collaboration of WHO, EDCD and Government of Nepal. The prevalence rate of LF and its vector recorded in Daraunepokhari, Dapcha VDC of Kabre district may be helpful for the implementation of the lymphatic filariasis eradication programme which may be a part for the global elimination of LF as called by WHO.

The identification & prevalence of vector also gives a wink assistance to know about its prevalence, probability and degree of transmission of this vector born disease.

II

OBJECTIVES

General Objective:

To determine the filarial situation in human and prevalence of vector of *W. bancrofti* in Daraunepokhari, Dapcha VDC of Kabre District, Nepal and to provide the collected data essential to the planning, implementation and evaluation of services for the prevention, control and treatment of lymphatic filariasis.

Specific Objectives:

- To determine the age and sex-wise prevalence of filariasis.
- To determine the Endemicity Rate (ER) of the disease.
- To study the knowledge, attitude and practices of people towards filariasis in relation to education
- To determine the asymptomatic and symptomatic filarial cases among the people of survey area.
- To determine the filarial prevalence in relation to education and occupation.
- To determine filarial prevalence in relation to sanitation of their residential areas.
- To determine the prevalence of *Culex quinquefasciatus* vector of *W. bancrofti*.

Objective Rational:

The present study is expected to facilitate solution to the filariasis problem, mosquito vector control problem, awareness against the disease filariasis, protection from the bites of mosquito as well as formulation of comprehensive countrywide programme for controlling and eradicating this parasitic disease in the near future as per the aim of WHO through determination of its vector prevalence.

III

LITERATURE REVIEW

3.1 Research in filarial cases and its vector in Global Context

The symptoms of Bancroftian filariasis is also known by name "elephantiasis arabicum" in the ancient Hindu literature, viz: Susratha (600 B.C.). The term "Malabar leg" was applied to the condition by Clark in Cochin in 1709 A.D. Our present knowledge of filariasis owes much to the investigation carried out towards the end of nineteen and beginning of the twentieth centuries.

Microfilariae (First stage larvae) were first demonstrated by Demarquay in 1863 in the hydrocele fluid of a patient from Cuba. In 1868 Otto Wucherer in Brazil found microfilariae in the urine of patient with haematochyluria. Lewis (1872) in India demonstrated the same in the peripheral blood. Adult worms were found by Bancroft in Brisborne in 1876 and *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 *W. bancrofti* in the mosquito *Culex quinquefasciatus* and established the essential role of vector. This was the first demonstration that female *Culex* mosquito could harbour an infective agent of a parasite. A year earlier he confirmed that these nematodes were the cause of elephantiasis (Cheng, 1986). In 1881, he described nocturnal periodicity of *W. bancrofti*, the microfilariae being present in the greatest number in the peripheral blood during night hours.

The mosquitoes were reported by the Japanese before World War II which include *Anopheles sinensis, Culex quinquefasciatus, Culex mimeticus, Armigeres subalbatus, Aedes albopictus, Ae. aegypti* and *Ae. togoi* (Anonymous, 1931).

The immatures of *Culex quinquefasciatus* and *Cx. halifaxii* were collected from artificial containers. They were predaceous on the larvae of other mosquitoes as well as chironomid larvae. This species is also reported from U.S.S.R, China, Korea, the Oriental region (including Taiwan), Nepal and Some of the Pacific Islands. (Wiedemann, 1820) and was published in Japan on Mosquito Systematic Vol. 18(1), 1986.

Say (1823) studied *Culex quinquefasciatus* for the first time in the world. This species is very common throughout the Archipelago. The larvae were commonly preyed upon by those of *Culex halifaxii* and *Cx. fuscanus*. This is the prevalent domestic mosquito, biting humans at night. Adults were collected throughout the year by light traps and few fed and gravid females were found even in the winter season. There were clearly two population peaks of adults during the year, one in May and the second in October (Toma *et al.*, 1978b). This species is reported from Palearctic Japan and is cosmotropical (Takako Toma and Ichiro Miyagi, 1986).

Culex quinquefasciatus is the most and worldwide mosquito in the Archipelago and the only domestic species biting at night. Filariasis due to *Wuchereria bancrofti* had been a serious health problem until 15 years ago in the Archipelago. However, owing to improvements of general sanitary conditions, the disease has disappeared in the Archipelago (Sasa, 1976, Sasa *et al.*, 1977).

The occurance of *Cx. quinquefasciatus* throughout the Oriental and Palearetic Japan is 28 (39.4%) but in Oriental region 56 spp. (78.9%) were found (Tanaka *et al.,* 1979).

3.2 Recent Study on Lymphatic Filariasis and its Vector

Bregami *et al.,* (2005) studied the effects of thiabendazole in *Mansonella perstans* filariasis and found out that the parasitic density, eosinophilia and symptoms significantly reduced after one and two step therapy in most patients.

Gupta *et al.,* (2005) studied on biochemical targets on filarial worms for selective antifilarial drug design. They highlighted the research and developed the rational antifilarial agents and discuss the pitfalls since the discovery of Diethylcarbamzine, the only drug of choice for controlling filariasis despite of its adverse effect.

Mathieu *et al.,* (2005) while studying on the factors associated with participation in the campaign of mass drug treatment against lymphatic filariasis in Leogane district, Haiti, found that absenteeism during the drug distribution (12%), use of contraceptives (12%) and pregnancy (11%) to be the primary factors for failing to take drugs while people who knew filariasis to be the mosquito transmitted disease and having learnt about the mass drug administration through posters and banners were found to be positively associated with taking the drugs.

Michael *et al.,* (2005), studied about the patterns of vector abundance and transmission in two East African communities with different levels of endemicity. Vector, *Culex quinquefasciatus* were collected in light traps and dissected to check filarial larvae. The overall vector density and transmission intensities were found significantly higher in Kingwede

which was about 3.7 times and annual transmission potential by 14.6 times. The variation in the vector abundance, vector composition and transmission intensity were discussed in respect to its cause and its significance to control bancroftain filariasis were also attempt.

Onapa *et al.,* (2005) conducted a study on rapid assessment of the geographical distribution of lymphatic filariasis in Uganda by screening of school children for circulating filarial antigens (CFA), by using the process called rapid immuno-chromatographic card test which reveals that CFA prevalence generally decreases with increasing altitude and no CFA positive cases were found at sites that were >1300m above sea level.

Rajkumar *et al*., (2005) conducted a study on *Wuchereria bancrofti* and *Onchocerca volvulus* co-infection in a refugee from Sierra Leone. The diagnosis was done by using direct blood smear (*W. bancrofti*) and Serology (*O. volvulus*). They have commented briefly on the therapeutic implication of the co-infection.

Anosika *et al.,* (2004) conducted a study on human filariasis in Dass local government area of Bauchi State, Nigeria. The infection rates, intensity and clinical manifestations of human filarial infections were studied. 215 (20.3%) of 1059 males and 99 (19.1%) of 569 females examined were infected. Microfilariae of *Onchocerca volvulus, Wuchereria bancrofti, Mansonella streptocerca, Loa loa* and *Mansonella perstans* were encountered. Sex wise, age wise, community wise and occupation wise ($P<0.05$) prevalence of the parasites were also studied.

Bora *et al.,* (2004) studied about Bancroftian filariasis in Bagdora town, Darjeeling to know about filarial endemicity that gradually increasing over years in Bagdora town (West Bengal). Out of 511 night blood

smears, 35 were found positive for *W. bancrofti* (Mf rate 2.32%). The microfilaria (Mf) rates for males and females were 2.48% and 1.79% respectively. The age of these positives ranged from 5-45 years and highest (4.45%) was found in age group of 20-29 years. Vector density was found to be 30-35 out of 49 female *Culex quinquefasciatus* and when dissected to find the presence of filarial infection, non was found positive. But, during 1976 survey in the same town Mf rate in *Culex quinquefasciatus* was found, Mf rate 1.8%.

Brakye *et al.*, (2004) conducted the study in Ghana regarding the ability of vector mosquitoes to transmit microfilariae (Mf) of *Wuchereria bancrofti* when the levels of microfilaraemia in human in which vectors feeding were very low to understand transmission dynamics of lymphatic filariasis. When 662 *Culex* were collected hourly during night and dissected, one *Culex* mosquito was found to harbour microfilaria.

Yahathugodia *et al.*, (2004) while studying on the knowledge about lymphatic filariasis in two communities (Unawatuna, a coastal community and Baddegama, an inland community) of the Galle district, reported that the people of Unawatuna had greater awareness towards clinical and parasitological features of the disease (P=0.0003) and drug treatment (P=0.00380) than that of Buddegama.

Koyadun *et al.*, (2004) conducted a study on bancroftian antigaemia clearance and Myanmar migrants after biannual mass treatment with DEC 300mg oral dose FILDEC tablets in Southern Thailand. They found that out of 34 antigaemic 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 with high antigens titers (99.7-181.6×10^3 Au/ml) and found 54.44%, 33.58% and 9.97 significant decrease of the CFA levels ($P<0.007$).

Keylem *et al.,* (2004) determined the impact of long-term Ivermectin on *Wuchereria bancrofti* and *Mansonella perstans* infection. The study was conducted in 11 communities of Burkin Faso and the drug was given under community directed treatment strategies. The implication of this study were discussed in relation to the old Onchocerciasis Control Programme (OCP) and to the ongoing African Program for Onchocerciasis (APOC).

Jiang Jung *et al.,* (2004) found six cases of filarial chyluria in the hospital admitted 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 50-80ml (mean 65ml). Chyluria disappeared in all patients immediately after operation. Mild haematuria occurred in 4 cases within 12 hours and disappeared at 24 hours.

Alves *et al.,* (2003) studied on immunocytochemical localization of antigens recognized by tropical pulmonary eosinophilia and individuals with intestinal helminthes antisera in microfilaria of *Wuchereria bancrofti* which suggest that sera from people of non-endemic area for filariasis harboring intestinal helminthes also share antifilarial antibodies that recognize antigens of micro filarial of *W. bancrofti*.

Chadee *et al.,* (2003) studied on filariasis in Georgetown, South America. They conducted 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.

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

Hammad *et al.*, (2003) studied the impact of DEC on vector competence of *Culex pipiens L.* to *Wuchereria bancrofti* Cobbold and found out that an annual single doze of DEC has greater potential to mediate sustained microfilaria reductions thereby reducing but not eliminating transmission and killing the filarial parasites with the mosquito.

Pacella, *et al.*, (2003) studied a case report of a 23 years old man immigrant from Sri Lanka suffering from an acute painful volume increase of the right scrotum without fever. Clinical examination suggested a diagnosis of testes torsion. An inflammatory spermatic cord and epididymis with a purple nodule of the middle portion were found which was diagnosed as filarial infection.

Rajendran *et al.*, (2003) studied on the influence of the mass drug administration of diethylcarbamazine alone or with albendazole on the prevalence of filarial antigaemia; which concluded that the use of DEC alone produced a slightly greater reduction in the prevalence of antigaemia than the use of both DEC and albendazole and to maximize

the benefits of Mass Drug Administration (MDA), greater efforts should be made to increase treatment coverage among young children.

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 areas in Papua New Guinea were evaluated. The observation suggested that filarial pathology of the male genitalia is under reported when evaluated by physical examination alone. The duration and the intensity of infection are the risk factors for hydrocele.

Chandrasena *et al.,* (2002) estimated the sensitivity, specificity and cost effectiveness of an immunochromatographic test (ICT, AMRAO) for the diagnosis of lymphatic filariasis against two standard parasitological techniques; thick blood film (TBF) and Nucleopore Membrane Filtration (NMF) for which blood was collected from the individual of endemic areas in the western part of Sri Lanka, which shows the ICT to be more effective than TBF or NMF in diagnosing infection in lymphatic filariasis.

Das *et al.,* (2002a) conducted study on entomological monitoring of annual mass drug administration for the control and elimination of lymphatic filariasis by antifilarial drug treatments. Female mosquitoes were caught manually and also by light traps from 100 households in each study village and were dissected to find rate of infection. The study was conducted by using PCR too in order to promise and establish a route part of control programme.

Das *et al.,* (2002b) studied about modeling the epidemiology, transmission and control of lymphatic filariasis. *W. bancrofti* transmitted

by *Culex quinquefasciatus* accounted >90% of the global burden of lymphatic filariasis (LF). In order to control the problem created by LF, they also conducted study on population biology of the parasites.

Singh *et al.*, (2001) conducted study of filarial transmission in a non-endemic area of Pathankot (Punjab) on total population of 28,041; 2136 blood smears were collected from migratory and local inhabitants where Mf rate and mean density were 1.19 and 15.05 respectively. Disease rate was nil. Mf rate was highest in 20-49 years age group, where as Mf density was high in young age group. A total of 399 female *Culex quinquefasciatus* were dissected. Non was found positive for human microfilariae parasite. Thus negating any indigenous filariasis transmission in this town. It was found that non-endemic areas were found to be non endemic for filariasis despite considerable increase in Mf rate among migratory population and vector density.

Weerasoriya *et al.*, (2001) reported 4.4% prevalence of micro filaraemia in three suburban area of Matara, in Sri Lanka. Prevalence was significantly lower in female than in male and in males aged < 20 years than in older males. Overall 9.5% of the 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.

Wickremanayake *et al.*, (2001) developed a dot – ELISA for detection of microfilaria of *W. bancrofti* in an endemic area. The test can differentiate the endemic normals from the microfilaraemic symptomatic individuals. Antigens of molecular weight 130kDa and 50kDa 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 microfilaraemic individuals.

Bockaire *et al.,* (2000) used an application of PCR – ELISA to detect *W. bancrofti* in pools of wild, caught *Culex quinquefasciatus*, the main vector of lymphatic filariasis in Papua New Guinea. Using traditional dissection techniques 35 batches were dissected out of 633 mosquitoes, they found six batches contained *W.bancrofti* infected mosquitoes giving minimum infection rate (0.9%). This was not different than actual rate of infection 9 (1.4%) of 633 mosquitoes (P = 0.48). a total of 621 mosquitoes were processed for PCR – ELISA where 486 caught by human bait and 135 by light traps. It was found that infection rate was identical to that obtained by dissection of individual mosquitoes (1.4%). The minimum infection rate for light trap was 2.7%. They found that PCR-ELISA techniques was comparable to traditional dissection techniques for monitoring intensity of transmission of LF in endemic areas.

Massage *et al.,* (2000) reported the prevalence of *W. bancrofti* in 31.8% of 1025 inhabitants with 32.1% infection in female and 31.5% in male; studied in the Hale area of Northeast Tanzania. Clinically 6-9% of examined individuals had elephantiasis and 28.5% males of age 15 years and above had hydrocele. Both the clinical manifestation and microfilaria prevalence were found to increase with age.

Tritee-raprapab *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 the immigrants (2.5%) promoted concern in the public health community for the re-emergence of Lymphatic filariasis. It was concluded that *W. bancrofti*

infective with third stage larva got the potential for establishing an urban cycle of transmission in Thailand.

Bhumiratna *et al.*, (1999) assessed the efficiency of the ICT card test by using clinical recall techniques and microscopy (Thick smear and capillary tube technique) in the Sera of 225 subjects living in *W. bancrofti* endemic village of Tak province, Thailand. The ICT card test gave a 20% antigen positive rate of the same, 5.8% by 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. When it was done in *W. bancrofti* micro filaraemia sera, the ICT card test had a sensitivity of 100% using microscopy as reference and 84.6% when using clinical and recall technique. However the card test was more positive than the other when done in endemic normal sera (14% positive).

WHO (1997a) carried out a study in Northern Ghana in a rural community, where filariasis is highly endemic (14% of the population age over 10 years were microfilaraemic with *W. bancrofti* and 3% was chronic disease), showed that Lymphatic filariasis can be a major social and economic burden on poor communities and also indirect economic loss associated with adenolymphagitis (ADL). Another study carried out in rural community in Southern India showed that the productivity by male weavers with chronic lymphatic filariasis was reduced by an average of 27.4% in comparison with matched controls.

WHO (1997b) conducted a study in India to determine the prevalence of filariasis. In India filariasis is major public health problem next to Malaria. At present WHO estimates about 428 million people with 28 million of Mf carriers and 21 million clinical cases that spread in 13 states and five union territories. India contributed about 74% of endemic

population and 81% of disease burden in the region. *W. bancrofti* was the most predominant infection comprising 99.4% of the problem in the country, while *Brugia malayi* was confined to the western coast of Kerala and a few areas of the six other states. Both the infections were nocturnally periodic. In the Nicobar group of Island, diurnally sub-periodic infections were transmitted by *Aedes niveus* group was detected about three decades back.

3.3 Study of Filariasis and its Vector in Nepal

3.3.1 Research on Vectors in Nepal

The study of distribution and prevalence of the vector of lymphatic filariasis was conducted by Peters and Dewar in 1956 and was the first record of the presence of *Culex quinquefasciatus* in Nepal. They reported *C. quinquefasciatus* from Bagmati, Koshi, Narayani and Janakpur districts where they conducted survey for the first time.

Jung *et al.*, 1965 and Shrestha, 1966 also reported the same species from the same places where it was reported earlier by Dewar and Peters in 1956. Sirivanakarn in 1976, Reisen and Boreham in 1979 reported that they feed on man which is their preferred host at night in Indo-Nepal subcontinent.

In Nepal 3 mosquito-borne diseases are prevalent and cause morbidity and mortality. Besides malaria and JE, filariasis (Kessel 1966, Jung 1973) is one of the most disabling and disfiguring disease.

Puri (1955) and Peter *et al.*, (1955) were the first to report certain culicine species occurring in Nepal.

Joshi *et al.*, (1965) made major contribution by reporting 59 species of culicine including 28 new countries records. An extensive review of the mosquito fauna of Nepal was published by Shrestha (1966). He reported 97 species, including 36 anophelines and 61 culicines. Similar records of Nepal anophelines were included by Romachandra Rao (1981).

Pradhan and Darsie, 1989 made 13 new country records. In 1988, 7 more species were recorded by Pradhan *et al.,* 1990.

3.3.2 Research on Disease Filariasis in Nepal

Gupta, R. (2007) submitted a report on surveillance of Lymphatic Filariasis in seven districts of Nepal to Filariasis Elimination Program WHO.

Gupta et al., (2006) conducted study about the microfilarial infection in eight districts of Nepal. Out of 958 individuals studied 45 (4.70%) were found to be positive for filarial antigenaemia, in which two people had elephantiasis and two people had microfilariaemia. Overall CDR was 103 (10.75%) and endemicity rate was found to be 154 (16.08%).

Gupta *et al.,* (2006) conducted base line sentinal survey in five districts of Nepal.

Maharjan *et al.,* (2005) submitted a report on Epidemiological surveillance of Lymphatic Filariasis in four districts of Nepal to Filariasis Elimination Program, EDCD, Nepal Government.

Ghimire *et al.*, (2003) conducted a survey to study the prevalence of lymphatic filariasis in the endemic areas, Mahendranagar and Nagrain VDCs of Dhannusa district in Terai plain region of Nepal, from June –

July 2002. The result showed that the prevalence of microfilaraemia in Mahendranagar was higher than in Nagrain VDC. A total of 1085 finger prick thick blood smear samples were collected from volunteers at two sentinel sites, 468 from Mahendranagar and 612 from Nagrain VDC, from 22:00 – 2:00hr. 25/468 (5.3%) of Mahendranagar and 14/617 (2.3%) from Nagrain VDC were found to be positive for *W. bancrofti*. The prevalence was found to be higher in female although the participation of both the sexes was almost equal.

Sherchand *et al.,* (2003) studied the prevalence of infection by *W. bancrofti* in 37 districts of Nepal from July to December (2001). The study populations were selected above 15 years age of respondents and the immunochromatographic test (ICT – filariasis test) was used to screen for circulating filarial antigen (CFA). The overall prevalence of lymphatic filariasis from a 4,488 sample population was 13% and 33/37 districts were found to be endemic. On the basis of geographical data, the highest numbers of cases were found at altitudes between 500-700 meters however a substaintial no. of infected individuals were found in the highly populated Kathmandu valley at altitudes between 900 – 1500m. Prevalence rates above 20% were found in 11 districts (with the highest rate of 40%), 6 – 9% in 15 districts and 0.1 – 5% in 7 districts.

Sherchand (2002) conducted an epidemiological survey to determine the prevalence of disease due to lymphatic filariasis in Magaragadi VDC, Bardia district of Nepal. The study population selected was above 15 years of age and the process used was Immunochromatographic card test (ICT – test) to screen the circulating filarial antigens (CFA). Out of 500 samples collected 141 were infected with larvae of *W. bancrofti*. The

vector for this parasite was found to be the mosquito of genus *Culex quinquefasciatus*.

Manandhar (2001) conducted an epidemiological study of microfilaria in three different 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.

Tuladhar and Sherchand (2001) conducted and epidemiological study in three different geographical regions, viz; Terai (Sipwa VDC of Rupendehi district), in Terai (Dovan VDC of Palpa district) and Hill (Katunje, Golmadi, Ittachen and Byasi of Bhaktapur district) of Nepal. A total of 53 blood samples (10.35%) was found ICT – filariasis positive in the study, 39 blood smears from buffy coat. Out of a total 410 blood samples, 27 thick blood smears were found positive in Bhaktapur. Among three different methods in the detection of Mf, smear from buffy coat was found best. ICT card technique in antigen detection was still better for field survey in diagnosis of filariasis of all techniques.

Bhusal *et al.,* (2000) studied the prevalence of *W. bancrofti* infections in Tokha Chandeswori VDC of Kathmandu in 1998. A survey of 978 nocturnal blood samples were collected in the VDC which indicated on overall prevalence of 5.8% for microfilaraemia and the crude disease rate of *W. bancrofti* was recorded to be 13%. The highest microfilaraemia infection rate was recorded as 11.8% among the group of 40-49 years and the highest crude disease rate was recorded as 36.4% in the age group of 70 and above.

Bista *et al.,* (2000) which studying the situation analysis during 1995-1999 recorded the prevalence of lymphatic filariasis at 13.2 – 23.4% in

different regions of Nepal, in out patient clinics of different health institutions and through the HMIS during the fiscal year 1995/96 to 1998/99.

Pradhan *et al.,* (1997) reported 24.6% endemicity rate, 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* in Gokarna VDC of Kathmandu valley and identified 12 sps of mosquitoes (*Anopheles nigerrimus, Anopheles vagus, Anopheles willmori, Anopheles kessele, Culex fescocophela, Culex gelidus, Culex pseudovishni, Culex whitmori* and *Culex tritaeniorhynchus*) from the study area. Among these species *Culex quinquefasciatus* was found to be more prominent.

Pradhan *et al.,* (1990) found that *Culex quinquefasciatus* and other culicine mosquitoes transmit disease like JE and filariasis, although anophelines do play a secondary role in this spread.

Jung *et al.,* (1973) studied all together 9 sites which showed 4.99% to 6.15% *W. bancrofti* in all age group and both sexes in the urban population, 6.6% - 10.3% in the semi urban population and 1.2% - 17.8% in the rural population. Similarly 7.1% - 9.16% microfilariae rate was found in the urban population, 10.03% - 11.3% in the semi urban population and 0.8% - 17.69% in the rural population.

The following table provides information about National and Region – wise distribution of lymphatic filariasis, which is based on the annual reports DoHs, MoHs and HMG, Nepal from 1995/96 to 2005/06

Table 1: National and Region-wise Number of Filarial cases in Nepal Fiscal Year 1995/96 to 2005/06

Year	National	Eastern Region	Central Region	Western Region	Mid-Western Region	Far-Western Region
1995/96	3100	493	849	789	662	317
1996/97	2694	257	981	736	303	418
1997/98	2371	328	605	976	317	155
1998/99	1744	165	671	913	281	14
1999/00	1797	209	718	632	195	43
2000/01	1632	262	546	692	123	09
2001/02	1183	142	173	733	79	56
2002/03	809	63	302	334	64	46
2003/04	550	47	246	221	20	16
20004/05	549	25	274	180	50	20
2005/06	542	82	193	175	89	3

Table 2: Distribution of filarial cases according to the geography of Nepal of the Fiscal Year 2005/06

Year	Total cases	Mountain	Hill	Terai
2005/06	542	1	176	365

IV

MATERIALS AND METHODS

4.1 Study Area

Nepal, a developing landlocked country is surrounded by China at the North and India at the East, the West and the South. It lies in the Northern hemisphere in between 80°4' to 88°12' east longitude and 26°22' to 30°27' north latitude respectively. In order to administer and have equal development, it is divided into five development regions, 14 zones and 75 districts. Among 75 districts 20 lie in Terai region, 38 in hilly region and the remaining 17 in the mountainous region. The Kabre district which was chosen for the base line survey and study was carried out, is a district of hilly region in Bagmati Zone and falls in the Central Development region.

Kabre is situated in the South East of Kathmandu valley, at the height of 500m to 2000m from the sea level. This district is surrounded by Sindupalchowk in the North, Ramechhap and Dolakha in the East, Sindhuli in the South, Makawanpur in the Southwest, Lalitpur in the West and Kathmandu and Bhaktapur in the Northwest. The total area occupied by this district is 1396 square kilometer approximately (Central Bureau of Statistics, HMG, 2001) with 87 VDCs and 3 municipalities. Dhulikehl is the headquarter of this district. The total population of this district is 385672 (male =188947 and female =196624) i.e. 1.67% of total country's population with the sex ratio of 17.05 and annual growth rate of 1.76% (CBS, 2058 B.S.)

There are 70,509 households with the average household size of 5.47. The ethnic groups of this district include Chhetri, Brahmin, Magar,

Thapa, Tharu, Tamang, Newar, Sanyasi, Chepang, Kami, Shah, Kuwor, Rana and others there are 94 healthposts in this district. 64.45% of the total people living there are dependent upon agriculture as their main occupation while the rest 35.55% are involved in other works rather than the agricultural activities. Among all the districts of Central Development Region, this district has highest agricultural land 37404.7 hectares. According to the annual report published by Epidemiological Department and Central Division, HMG, 2005/06, the total number of lymphatic filarial cases reported in outpatient Department in the health institutions of Kabre District during the fiscal year 2005/06 was reported to be seven but morbidity rate was reported to be zero.

The single VDC, Daraune Pokhari, Dapcha, situated in the middle of the district, was selected for the purpose of study. It is a small plateau area on the top of sloppy hill with a lake of about 500 square meters from which the VDC got its name. The total area of the VDC is 8 square kilometer and is at the height of 1610m from the sea level. The actual location of this VDC in the map of the world is 85^{o}38' east longitude and 27^{o}33' north latitude. It has no direct touch with any rivers and is surrounded by Mathurapati Phulbari VDC in the North, Khanalthok VDC in the East, Shikhar Ambote VDC in the South and Chhatrebanjh VDC in the West. It has total population of 3,286 (male = 1,538 and female = 1,748) with 620 number of households with the average of 4.03 households size. The VDC is dominated by Tamang, Newar, Chhetri, Brahmin, Rana, Magar, Bika and others.

People are mostly farmers and are also involved in business activities. Human habitations were surrounded by poor sanitary conditions. In each households they have cattle sheds in the ground floor which has created

unhygienic condition for human settlement and have provided favourable indoor resting sites for the vectors. Besides this accumulation of cattle dung outside their houses have aggravated the condition. The soil is clayey which is the most favourable breeding place for the vector during Monsoon where puddles and ditches resist maximum water for a longer period of time. Not only this, the main breeding place of *Culex* mosquito, the vector of lymphatic filariasis are unhygienic, uncovered water tanks, reservoirs and container with water collected and stored for their cattles. Though, there are facilities like electricity, drinking water supply, communications but still there are no facilities for irrigation and transportations to all parts of this VDC. So, it is suffice that, yet people of this VDC are still away from all the development facilities and education.

4.2. Population Surveyed

After counselling with Dhulikehl Hospital Personnels, the population from the areas highly affected by lymphatic filariasis (Ward no. 2, 4, 5, 7, 8 and 9) of Daraune Pokhari, Dapcha VDC were choosen for the sentinel survey. The collection of blood samples from the study population were done in all age groups above two years. The main focus of the study was not only to find CDR, but also to find mf rate in unsuspected filarial patients, along with the prevalence of vectors in the study area.

4.3. Study Design

Epidemiological cross sectional sentinel survey method was applied as a research design in the study of LF cases.

4.4 Laboratory Materials and Chemicals

4.4.1 Laboratory Materials

slides	entomological pins
sterile lancets	ivory paper
cotton	black chart paper
gloves	hand lens
mask and apron	aspirator and suction pump
measuring cylinder	beaker
dropper	sampling vials (paper cup)
slide box	cellotape
compound microscope	thermocol
toothpick	insect fixing box

4.4.2. Chemicals:

methanol	giemsa stain 10%
camphor	colourless nail polish
fevicol	chloroform
distilled water	

4.5. Sampling Techniques and Sample Size

A total of **500 blood samples** collection and questionnaire filling of the same population were conducted from the community of Daraune Pokhari, Dapcha VDC at ward no. 2, 4, 5, 7, 8 and 9 of Kabre district. Questionnaires were filled in the late afternoon with the help of the team of sub-health post personnels and the blood samples were collected at night from 10 p.m. to 2:30 a.m. when people were at full rest for at least 2 hours in their beds. Inorder to ensure better condition, the following precautions were taken:-

1. During the study, questionnaire were filled by interviewer themselves by direct interview method with the help of health post personnels after giving training to them.
2. The sample slides, lancets were properly cleaned and dried but no antiseptic was used.
3. Each sampling slides were labelled with code number as per the questionnaire.
4. About 60ml of blood was collected from each individuals dividing into three different films, each containing about 20ml. as soon as the blood was taken out it was made into thick smear and was dried before collecting in the slide box.

A total of **500 mosquitoes** were collected by manual (hand) collection in order to find the prevalence of vectors in the VDC. The method applied for the collection of vectors performed in survey area was random selection method.

4.5.1 Mass Orientation Programme

Inorder to motivate the people and make them to participate in the programme, mass orientation programme was launched before the collection of blood was done in the study area. The programme was not only to motivate but also to inform all the respondents about the disease filariasis and its hazadous effect. Each individual was informed about the purpose of the study. They were informed that blood samples will be collected from all the members of the family above 2 years of age and will be collected during night hour. During sampling if any respondents would be absent from their house, they were not included in the target sample collection. Only those respondents who could be available in their houses during the collection period were included.

4.5.2 Questionnaire Filling

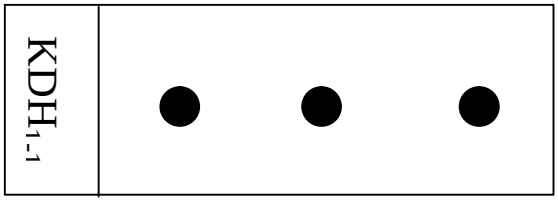
The questionnaire (Annex 1) contained name, age, sex, occupation, marital status, education, relationship with the head of the family, surrounding environment, their probable effects against disease, their current health status, clinical symptoms, the preventive measures they apply against the bite of vector, mosquito, the medicines applied against the disease, Lf. A structured questionnaire was prepared, pre-tested and piloted before administrating in the community in survey area.

4.5.3 Human Blood Sampling

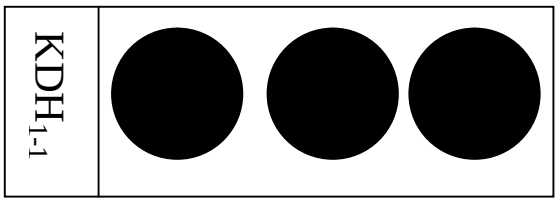
The ear lobes were pricked in order to obtain human blood samples.

4.5.3.1 Procedure for Blood Sample Collection

After pricking the earlobe of the respondents, three thick blood smears were prepared with the help of toothpick. After allowing the smears to dry for about 8-10 minutes, they were kept properly inside the slide box during the collection period and labelled them according to the questionnaire filled. Each smear of blood contains approximately 20ml of blood.



Blood drops on the slide.



Thick blood smears.

4.5.3.2 The Blood Slide Preparation

After the blood smears were brought to lab, they were stained with Giemsa stain.

The preparation of stained blood smears for microscopical observation was as follows:-

i. **Dehaemoglobination of thick blood smear**:- The thick blood smears were dehaemoglobinised using distilled water and dried at room temperature.
ii. **Fixing of blood smear:-** Blood smears were fixed in methanol by just dipping for about 5 seconds and dried at room temperature. The reagent methanol helps to fix the nematodes if present in the blood smears.
iii. **Staining of blood smear:-** The dehaemogobinised blood smears were stained in Giemsa stain at 1:10 dilution for 30 minutes and dried in air at room temperature.
iv. **Observation:-** The stained blood smears were examined under 5×, 10×, 40× and 100× objective lenses using oil immersion for 100× of the compound microscope inorder to confirm the specimen observed. The microfilariae were identified as *Wuchereria bancrofti* on the basis of following characters:-

Stained sheath, discrete nuclei, empty space between the nuclei and body wall, cephalic space, absence of nuclei at the tip of tail, bent tail tip underneath the body.

4.5.4 Collection, Killing, Fixing and Preservation of Vector

4.5.4.1Collection

Indoor hand collection of adult vectors of lymphatic filariasis were done during the morning from 6 a.m. to 10 a.m. and during the evening from 6 p.m. to 10 p.m. from bedrooms, toilets, and cattle sheds. Altogether six house holds were selected for collection of vectors. The indoor hand collection was done for 15 minutes from each locations.

Similarly outdoor hand collections of adult mosquitoes were done for half an hour from each site. The collection was done from water storage containers, reservoirs, bushes and other surroundings of the six house holds selected for the collection. Altogether 500 samples of mosquitoes were collected.

4.5.4.2 Killing

As soon as the mosquitoes were collected they were kept in paper cup, covered by mosquito net. In order to avoid the specimens from being discoloured the cotton soaked in chloroform was kept at the opening made in the net covering the cup and was covered by hand to fumigate cup to kill the mosquitoes.

4.5.4.3 Fixing

Dry preservation is the best method of fixing the vector of lymphatic filariasis collected as sample specimens. The samples were kept in separate paper cups keeping 10 to 20 samples in a paper cup.

A triangle card point was cut out of ivory paper and a small drop of colourless nail polish was placed at the apex of the triangle. Each specimen was fixed with the help of colourless nail polish. The tip of the card point was bent down at a right angle, so when the insect (mosquito) was in upright position the bent tip of the point fitted against the right under side of the thorax attached to (right) pleuron. This method of fixing the vectors of lymphatic filariasis i.e. mosquito is known as triangle carding and is the best way of mounting the smallest dry specimens.

Now after fixing with nail polish in the way as mentioned above, the other end of ivory paper was pricked with entomological pins and after

keeping complete information about collected locality, time and site below each specimens they were fixed in the thermocol kept in the box.

Care was taken inorder to prevent them from damage of their legs, wings, antennae, maxillary palpi, proboscis and abdomens.

4.5.4.4 Preservation

In order to preserve the specimens from attack of ants and other insects camphor was kept inside the reel box making holes with a needle.

4.5.4.5 Observation

In order to avoid over burden only female *Culex* were fixed in fixing box by observing carefully by naked eye, hand lenses and for further identification of vector *Culex quinquefasciatus*. Key of Pradhan (1996) was used.

The most identifying characters of adult vector species are:-

a) The antennae of female mosquitoes contain less hairs comparing to male which is bushy.
b) i) Maxillary pulpus is shorter than the proboscis.
   ii) Pulvilli present; tarsal claws unusually small.
   iii) Pleuron with distinct scale patches at least on upper and lower mesokatepisternum and anterior mesepimeron ……*Culex.*
c) One or two lower mesepimeral setae present; proboscis without distinct pale-scaled band; tarsomeres without pale bands at joints…….……. *Culex.*

d) i) Anterior surface of midfemur without median longitudinal pale-scaled stripe.

   ii) Abdominal terga with basal transverse pale-scale bands and pale integumental stripes.

e) Integument of thoracic pleuron without dark stripe; scutal integument yellowish or pale brown……*quinquefasciatus*.

4.6 Data Processing and Analysis

The collected raw data were firstly edited to detect errors and omissions and to make them accurate, uniform and well arranged. Then, they were coded for easy classification and tabulation. Thus, classified and tabulated data were analysed by means of table and bar diagrams.

4.7 Validity and Reliability of the Study

- Standardization of all the reagents, equipments and laboratory methods were done.
- Quality control on sample collection, processing and confirmation of the parasite *Wuchereria bancofti* and its vector *Culex quinquefasciatus* were maintained throughout the test and observations.
- As per the suggestion of the supervisor, the questionnaires were filled.
- The proper guidance and instructions were provided by the supervisor during the study.

V

RESULTS

The study was carried out among the filarial suspected and unsuspected people of ward no. 2, 4, 5, 7, 8 and 9 of Daraune Pokhari, Dapcha VDC of Kabre District. Altogether 500 blood samples from 135 households were taken as the sample size. The information regarding the participants was collected with the questionnaire survey and blood samples were collected from the sample population. Blood samples from both sexes and from different age groups, above 2 years were collected and subjected for the microscopical examination to detect the human filarial infection. In order to find the prevalence of vector and its identification a total of 500 mosquitoes were collected from the area and taken to the expert for the identification.

Different results obtained from microscopical examination and questionnaire analysis can be categorized as follows:

5.1 General Prevalence of Microfilariaemia

The microscopical observations revealed that out of 500 respondents, 52 (10.4%) were infected with the microfilarial parasites. (Table 3)

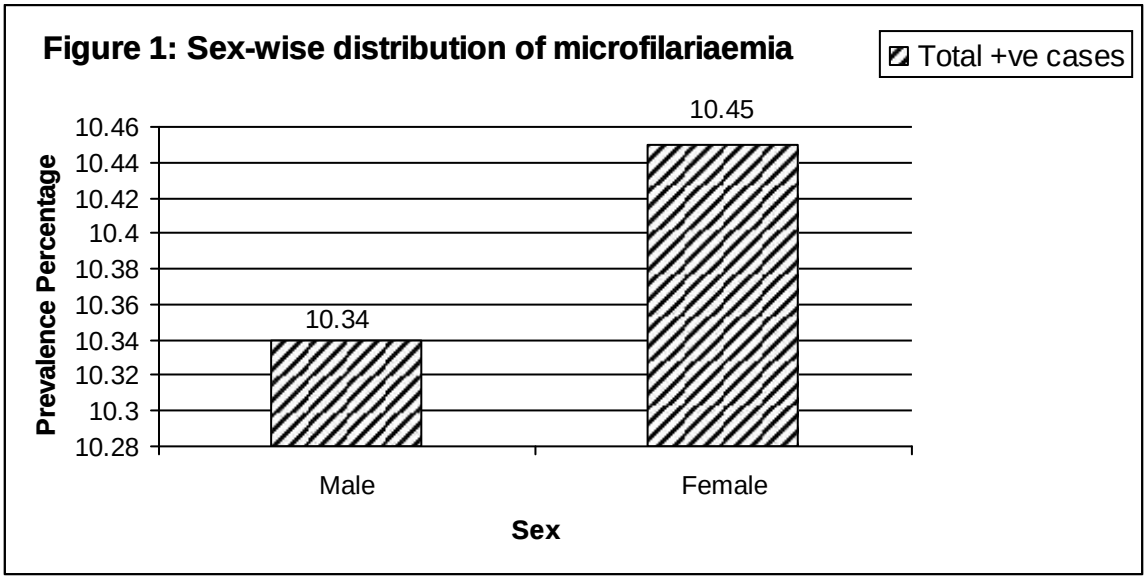
5.2 Sex-wise Prevalence of Microfilariaemia

Table 3 shows that total number of respondents included in the study and the distribution of microfilariaemia in relation to sex, 46.4% of males and 53.6% of females were included in the survey, out of which 10.34% of males and 10.45% of females were infected with microfilarial parasites which showed that females were more infected than males. A total of 10.4% were the microfilarial positive cases.

Statistically, the difference between male and female prevalence rate of microfilariaemia was found to be insignificant. (χ^2 =0.153, P<0.05, 3 d.f)

Table 3: Sex-wise distribution of microfilariaemia

Sex	Examined Samples		Positive samples	
	No.	%	No.	%
Male	232	46.4	24	10.34
Female	268	53.6	28	10.45
Total	500	100.00	52	10.4



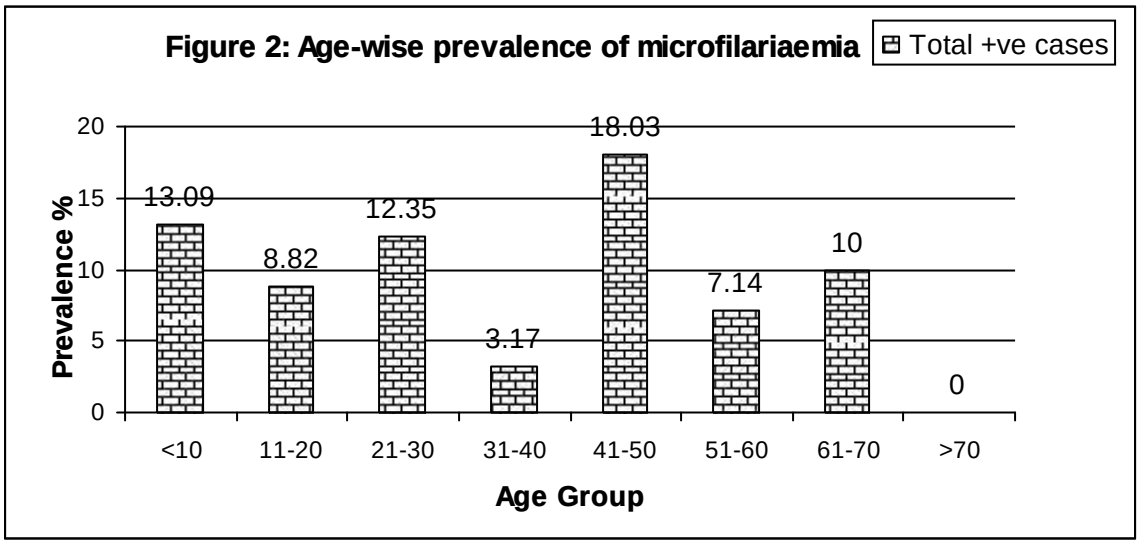
5.3 Age-wise Prevalence of Microfilariaemia

Table 4 shows that the maximum and minimum number of respondents included in the survey were from the age group 11-20 years (27.2%) and >70 years (1.00%) respectively and also shows the age wise prevalence of filarial parasites among the population studied. High prevalence was recorded in the age group 41-50 years (18.03%) while least in the age group 31-40 years (3.17%). Similarly infection rate of 13.09%, 12.35%, 10.00%, 8.82% and 7.14% were recorded in the age groups ≤10years, 21-30 years, 61-70 years, 11-20 years and 51-60 years respectively and was found no prevalence of mf in age group above >70 years.

Statistically, the difference between age-wise prevalence of MF was found to be insignificant (χ^2 =9.27, P<0.05, 15 d.f)

Table 4: Age-wise prevalence of microfilariaemia

Age group	Examined samples		Positive cases	
	No.	%	No.	%
≤10	84	16.8	11	13.09
11-20	136	27.2	12	8.82
21-30	89	17.8	11	12.35
31-40	63	12.6	2	3.17
41-50	61	12.2	11	18.03
51-60	42	8.4	3	7.14
61-70	20	4.0	2	10.00
>70	5	1.0	00	00
Total	500	100.00	52	10.4

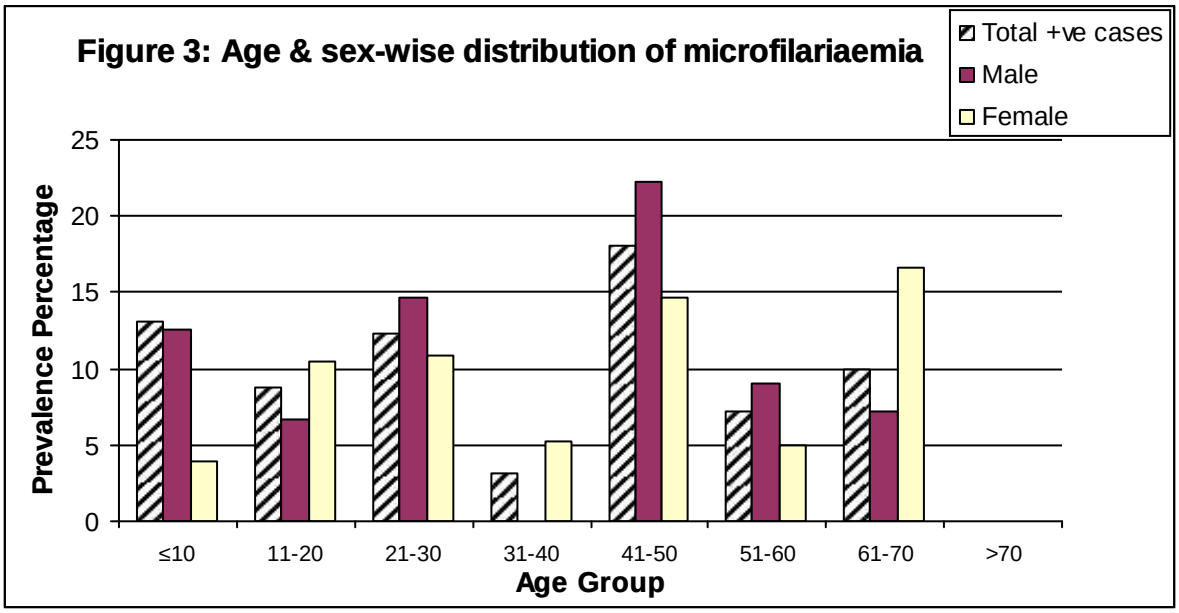


5.4 Age and Sex-wise Distribution of Microfilariaemia

The highest percentage of infection was found among the age group of 41-50 years (22.22%) in males and 61-70 years (16.66%) in females, while no infection was recorded among the age group of >70 years in both sexes and in the age group of 31-40 years in males. The maximum number of samples were collected from the age group of 11-20 years from both the sexes, while infection rate was not found to be maximum among this age group. But was recorded quite high rate of infection among the age group ≥10 years (males=12.5%, females = 3.88%).

Table 5: Age and Sex-wise Distribution of Microfilariaemia

Age group Year	Total Samples	Total +ve Cases	Total %	Male			Female		
				Total Samples	+ve Cases	%	Total Samples	+ve Cases	%
≤10	84	11	13.09	48	6	12.5	36	5	3.88
11-20	136	12	8.82	60	4	6.66	76	8	10.52
21-30	89	11	12.35	34	5	14.7	55	6	10.90
31-40	63	2	3.17	25	00	00	38	2	5.26
41-50	61	11	18.03	27	6	22.22	34	5	14.70
51-60	42	3	7.00	22	2	9.09	20	1	5.00
61-70	20	2	10.00	14	1	7.14	6	1	16.66
>70	5	00	00	2	00	00	3	00	00
Total	500	52	10.4	232	24	10.34	268	28	10.45



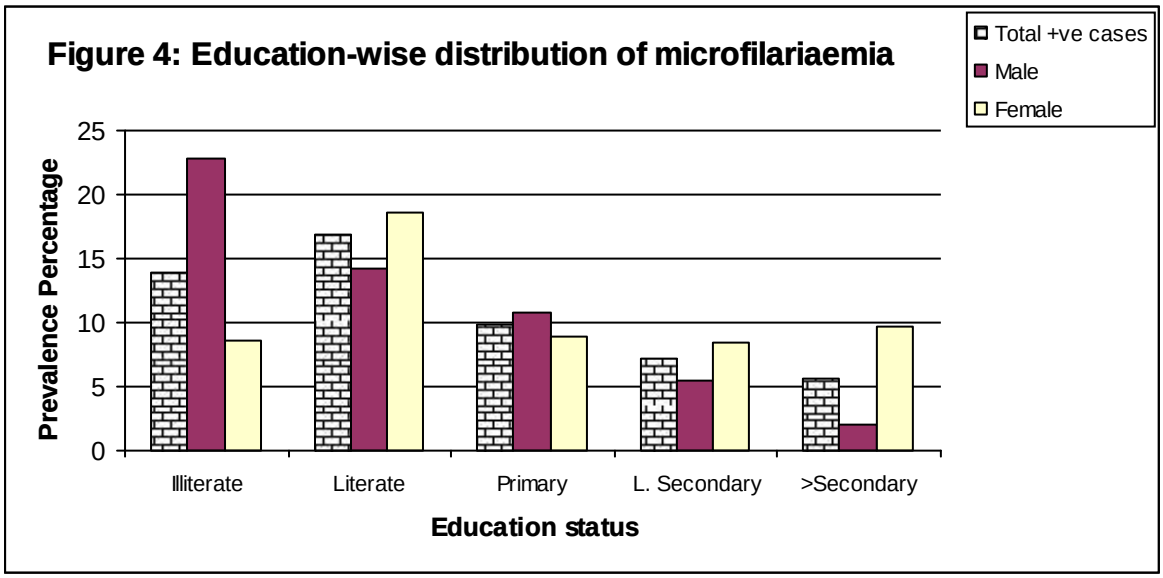
5.5 Education-wise Distribution of Microfilariaemia

The distribution of microfilarial parasites in the study population in relation to education is indicated by the table 6. The maximum prevalence was observed in the respondents who were just literate (16.90%) and illiterate (13.98%) and minimum in those respondents with the education level of ≥secondary (5.56%). In relation to sex-wise distribution, maximum infection with microfilariaemia was observed in the illiterate males (22.86%) and literate females (18.64%) while the minimum infection rate was found in education level of ≥secondary in males (2.04) whereas almost in average rate of infection (about 8%) in illiterate, primary and lower secondary levels of education in females.

Statistically, the difference between education wise prevalence of microfilarial parasites were found to be insignificant (χ^2 =7.77, P<0.05, 9 d.f).

Table 6: Education-wise distribution of microfilariaemia

Education level	Total Samples	+ve samples		Males			Females		
		No.	%	Total Samples	+ve samples		Total Samples	+ve samples	
					No.	%		No.	%
Illiterate	93	13	13.98	35	8	22.86	58	5	8.62
Literate	71	12	16.90	28	4	14.29	43	8	18.64
Primary	162	16	9.88	83	9	10.84	79	7	8.86
L. Secondary	84	6	7.14	37	2	5.41	47	4	8.51
≥Secondary	90	5	5.56	49	1	2.04	41	4	9.76
Total	500	52	10.40	232	24	10.34	268	28	10.45



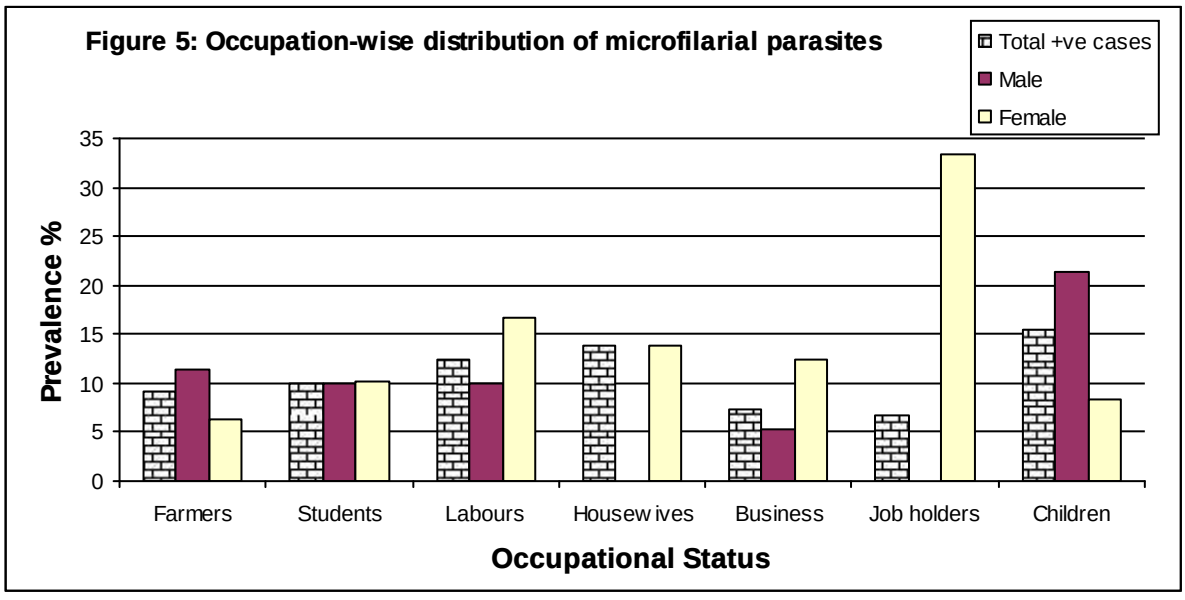
5.6 Occupation-wise Distribution of Microfilariaemia

The highest rate of infection according to the occupational status in the study population was recorded among the children (15.38%) and second highest rate of infection was recorded in house wives (13.89%). While the minimum rate of infection was found among job holders (6.67%). According to sex wise distribution regarding occupation, the maximum prevalence was recorded in job holder females (33.33%) and among children in males (21.43%) while no infection at all was recorded among job holder males.

Statistically, the difference between occupation wise prevalence was found to be insignificant. (χ^2 =3.10, P<0.05, d.f=13).

Table 7: Occupation wise distribution of microfilariaemia

Occupation	Total Samples	+ve samples		Males			Females		
		No.	%	Total Samples	+ve samples		Total Samples	+ve samples	
					No.	%		No.	%
Farmers	175	16	9.14	96	11	11.46	79	5	6.33
Students	169	17	10.06	81	8	9.88	88	9	10.23
Labours	16	2	12.50	10	1	10.00	6	1	16.67
Housewives	72	10	13.89	00	00	00	72	10	13.89
Business	27	2	7.41	19	1	5.26	8	1	12.50
Job holders	15	1	6.67	12	00	00	3	1	33.33
Children	26	4	15.38	14	3	21.43	12	1	8.33
Total	500	52	10.40	232	24	10.34	268	28	10.45



5.7 Clinically Manifested Cases in Relation to Age and Sex

Altogether the clinical manifestations found in the study population were 12 elephantiasis cases, 8 chylurial cases, 2 hydrocele cases and 11 fever and headache cases. Hydrocele cases were more prevalent in age group 31-40 (4.00%), chylurial cases were equal in age group 31-40 years and 41-50 years (4.00%) while not found in other age groups in males. But maximum chylurial cases were found in age group >70 years (33.33%) in females. Higher prevalence of leg elephantiasis were recorded in females (3.73%) than in males (0.86%). Besides above mentioned clinical cases, total fever and headache cases were recorded to be 1.29% and 2.99% in males and females respectively.

Table 8: Clinical features in relation to age and sex

Age group (Years)	**Total samples**	**Male**					**Female**			
		Total	**Fever & headache**	**Hydrocele**	**Chyluria**	**Elephentiasis**	**Total**	**Fever & headache**	**Chyluria**	**Elephentiasis**
≤10	84	48	00	00	00	00	36	00	00	00
11-20	136	60	00	00	00	00	76	1(1.32%)	00	00
21-30	89	34	00	00	00	00	55	00	00	1(1.82%)
31-40	63	25	1(4.00%)	1(4.00%)	1(4.00%)	1(4.00%)	38	3(5.89%)	2(5.26%)	3(7.89%)
41-50	61	27	1(3.70%)	1(3.70%)	1(4.00%)	1(4.00%)	34	2(5.88%)	3(8.82%)	3(8.82%)
51-60	42	22	1(4.54%)	00	00	00	20	1(5.0%)	00	2(5.0%)
61-70	20	14	00	00	00	00	6	00	00	00
>10	5	2	00	00	00	00	3	1(33.33%)	1(33.33%)	1(33.33%)
Total	500	232	3(1.29%)	2(0.86%)	2(0.86%)	2(0.86%)	268	8(2.99%)	6(2.24%)	10(3.73%)

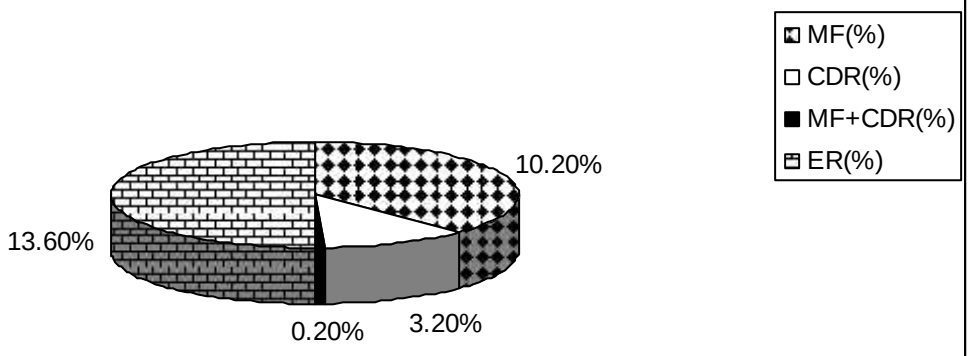
5.8 Total Endemicity Rate of Lymphatic Filariasis

The total endemicity rate of filariasis in the study population was found to be 13.60% with 10.4% microfilariaemia (MF) and 3.20% crude disease rate (CDR). Out of 10.40% of microfilarial infection, 10.20% were found with the presence of microfilarial parasites without any clinical manifestations whereas 0.20% showed microfilarial parasite with filarial symptoms.

Table 9: Total endemicity rate of lymphatic filariasis

Total Samples	MF(%)	CDR(%)	MF+CDR(%)	ER(%)
500	51(10.20%)	16(3.20%)	1(0.20%)	68(13.60%)

Figure 6: Total endemicity rate of lymphatic filariasis



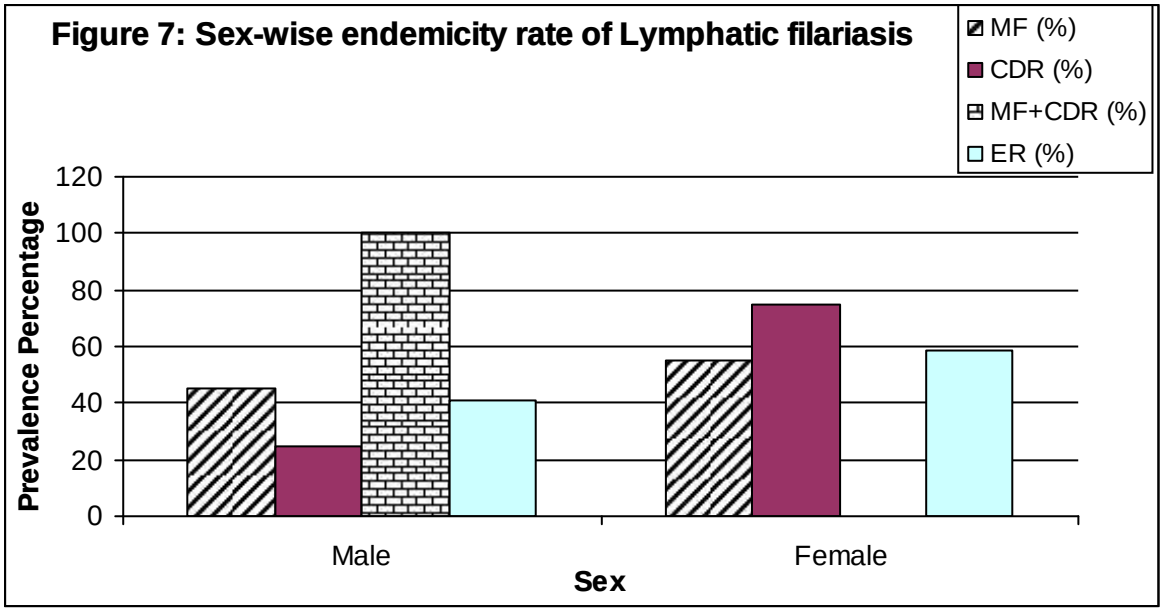
5.9 Sex-wise Endemicity Rate of Filariasis

Table 10 indicates the endemicity rate of lymphatic filariasis in relation to sex which was found to be highest in females i.e. 58.82% than in males, 41.18%.

However the difference between sex-wise endemicity rate was found to be statistically insignificant ($\chi^2 = 0.87$, $p<0.05$, 3 d.f)

Table 10: Sex-wise endemicity rate of lymphatic filariasis

Sex	MF		CDR		MF+CDR		ER	
	No.	%	No.	%	No.	%	No.	%
Male	23	45.10	4	25.00	1	100	28	41.18
Female	28	54.90	12	75.00	00	00	40	58.82
Total	51	10.20	16	3.20	1	0.20	68	13.60



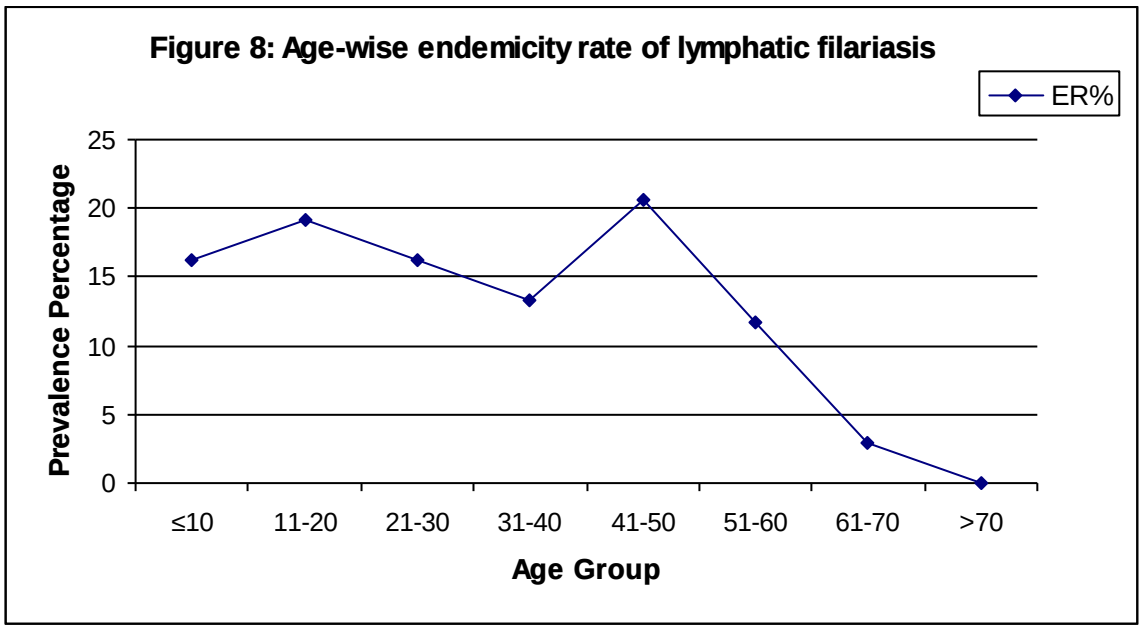
5.10 Age-wise Endemicity rate of Lymphatic Filariasis

Age-wise endemicity rate of lymphatic filariasis is illustrated by table 11. The highest endemicity rate was seen in the age group 41-50 years i.e. with 20.59% while low in age group 61-70 (i.e. 2.94%) and no any endemicity rate at all in age group >70years. The endemicity rate of 19.12%, 16.18%, 13.24% and 11.76% were found in age group 11-20 years, ≥10years and 21-30 years, 31-40 years and 51-60 years respectively.

The different between age wise endemicity rate was found to be statistically insignificant. (χ^2=9.38, p<0.05, 15d.f)

Table 11: Age-wise endemicity rate of lymphatic filariasis

Age group	Total samples	MF (No.)	CDR (No.)	MF+CDR (No.)	ER%	
					No.	%
≤10	84	11	00	00	11	16.18
11-20	136	12	1	00	13	19.12
21-30	89	11	00	00	11	16.18
31-40	63	2	7	00	9	13.24
41-50	61	11	3	00	14	20.59
51-60	42	2	5	1	8	11.76
61-70	20	2	00	00	2	2.94
>70	5	00	00	00	00	00
Total	500	52	16	1	68	13.60



5.11 Practice of Respondents towards Filariasis and Prevalence of Microfilarial Parasites

Practice of respondents towards the use of bed nets who occasionally use it was found to be 69% and who never use it was found to be 16.8% and who use bed nets always was recorded to be 14.2%. The prevalence of microfilariae in the respondents who never use bed nets was 4.76%, who occasionally use was found to be 13.91% while no positive cases were found among those respondents who always use bed nets.

Regarding sleeping habits, the respondents who sleep on ground floor and upper floor were recorded as 50.2% and 49.8% respectively and the prevalence of microfilariae were 10.76% and 10.04% respectively.

The study showed that out of 500 respondents, the practice of keeping animal husbandry away from their houses, nearby their houses and sharing the same houses were found as 10%, 7.4% and 82.6% respectively. The prevalence of filarial parasites in those respondents were 10%, 21.62% and 9.44% respectively. Among them, the highest prevalence was found in those respondents who keep their cattle nearby their houses (21.62%). It is illustrated by the table 12.

Table 12: Practice of respondents towards filariasis and prevalence of microfilarial parasites

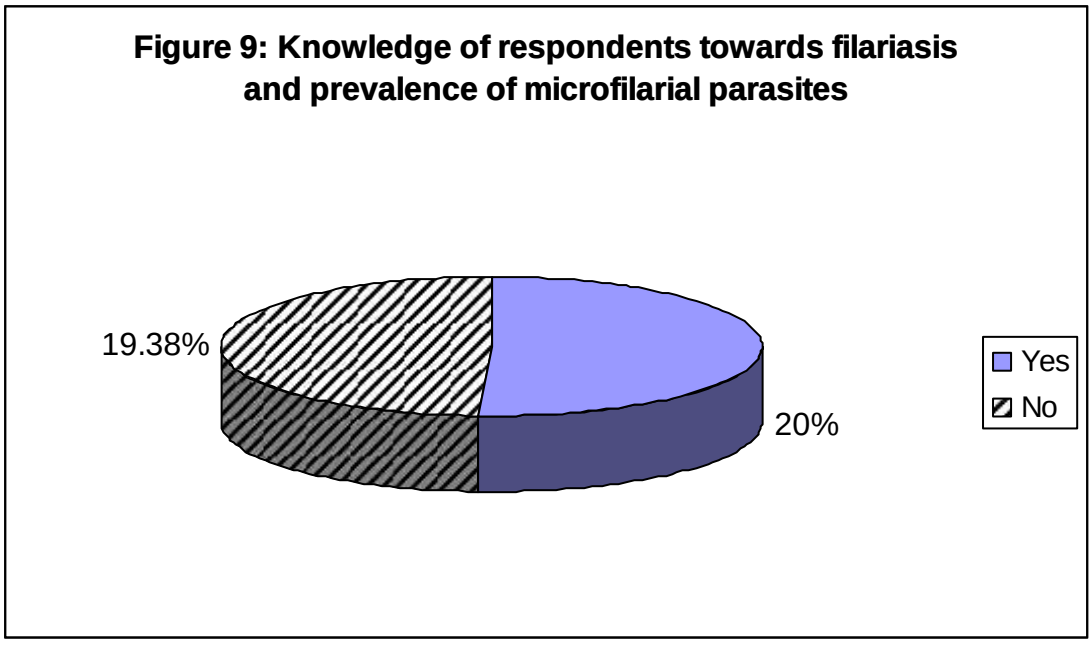
Practice of respondents		No.	%	+ve Samples	
1.	**Use of bed nets**			**No.**	**%**
	a) Never	84	16.8	4	4.76
	b) Always	71	14.2	00	00
	c) Occasionally	345	69	48	13.91
	Total	500	100	52	10.40
2.	**Sleeping habits**				
	a) Ground floor (indoor and outdoor)	251	50.2	27	10.76
	b) Upper floor	249	49.8	25	10.04
	Total	500	100	52	10.40
3.	**Animal Husbandry Practice (Keeping cattle)**				
	a) Away from house	50	10	5	10
	b) Nearby house	37	7.4	8	21.62
	c) Sharing the same house	413	82.6	39	9.44
	Total	500	100	52	10.40

5.12 Knowledge of Respondents towards Filariasis and Prevalence

The study conducted showed that, 97% of the respondents didn't have any knowledge about the disease while only 3% of the respondents know about it. It was found that 20% of the respondents who had knowledge about filariasis were found positive while 19.38% of the people without any knowledge about it were found to be infected with microfilarial parasites (Table 13).

Table 13: Knowledge of respondents towards filariasis and prevalence of microfilarial parasites

Knowledge status		Total Samples		Positive Samples	
		No.	%	No.	%
1.	Yes	15	3	3	20
2.	No	485	97	49	19.38
	Total	500	100	52	10.40



5.13 Prevalence of Vectors from the Study Site

Out of 500 specimens of vectors collected by manual collection from the field visited, 278 were male mosquitoes and the remaining 222 females of different species including *Culex quinquesfaciatus*. As male mosquitoes do not suck blood they have no role in the transmission of diseases. Hence, they were not considered. But, for species identification and to indicate prevalence photographs have been taken. Of the total female mosquitoes 67 (30.18%) *Culex* species were recorded. Amongst 67 samples of *Culex* species 7 (10.45%) were identified as *Culex quinquefasciatus* and of the total specimens, the prevalence of the vector of microfilarial parasite, *Wuchereria bancrofti* recorded after proper identification were 7 (1.40%).

Table No. 14: Prevalence of Vectors from the Study Site

Mosquito samples collected		No. of *Culex* species	No. of female *Culex quinquefasciatus*	Prevalence (%)
Male	Female	Female		
278	222	67 (30.18%)	7	1.40
500				

VI

DISCUSSION AND CONCLUSION

Lymphatic filariasis has been a public health problem of not only for Nepal but in all over the world in tropical and sub-tropical regions. It is endemic in some 80 countries including 1.2 billions of people (i.e. 20% of the world's population) (WHO, 2000). This disease is widely spread throughout the country. According to Lf mapping, it has been estimated that 60 of 75 districts are endemic for Lf (EDCD, 2062/2063). The epidemiological mapping for filarial infection by ICT card test carried out in 2001 in 37 districts revealed that 33 districts were endemic. Among these 33 endemic districts, 11 districts have the prevalence rate of over 20%, in 15 districts between 6-9% and in 7 districts the prevalence rate is found between 1 – 5% (EDCD, 2005/06). The present surveillance conducted in Daraune Pokhari, Dapcha VDC of Kabre district shows the endemicity rate of 13.60% with overall microfilariaemia of 10.20% and crude disease rate of 3.20%. Jung (1973) was the first to report crude disease rate of 4.99% to 6.15% in all age groups and both the sexes in the urban population, 6.6% to 10.3% in semi-urban population and 1.2% to 17.8% in the rural population from Central Nepal. The present study was conducted in rural population where the result is similar to Jung (1973). Similarly, the study indicated 7.1% to 9.16% microfilariaemia in the urban population and 0.8 - 17.69% in rural population in Central Nepal. Pradhan *et al.*, (1997) in Gokarna VDC of Kathmandu Valley, reported 24.6% endemicity rate with the overall 11.95% microfilariaemia and 12.64% crude disease which is similar to the present study. Bhusal *et al.*, (2000) reported overall 5.8% prevalence of microfilariaemia and 13.0% crude disease rate in the Tokha Chandeshwori VDC which is

comparatively smaller rate in Mf and higher CDR than the present study. Manandhar (2001) reported 19.9% of crude disease from Sipwa, Dhovan and Bhaktapur which is higher than the present study. Sherchand *et al.*, (2000) surveyed 37 districts of Nepal and reported 13.0% prevalence of microfilaria. This result is quite higher than the prevalence rate of Dapcha VDC.

Among 52 microfilarial cases 24/232 (10.34%) were of males and 28/268 (10.45%) were of females. Males and females were infected in the ratio of 6:7 i.e. 86 males were susceptible to Mf out of 100 females. This result is comparatively contrary to the result obtained by Weerasooriya *et al.*, (2001) Sri Lanka. This is perhaps due to the fact that the population of female was more than the male. During the optimum breeding seasons of the vectors, the females are engaged in both indoors as well as outdoor activities, in morning as well as in the evening to the late night and sleep without mosquito nets due to poverty and illiteracy which increases susceptibility of mosquito bite.

All the age groups of the people are susceptible to filariasis. The study conducted at present indicates that high rate of infection in the age group 41 – 50 years 11/61 (18.03%) while least in the age group 31 – 40years (3.17%) and no prevalence rate in age group > 70 years. The highest prevalence of Mf in the age group may be due to high exposure to outside environment, lack of awareness, poverty and carelessness towards using personal protection methods (mosquito nets, mosquito coils, mosquito mats etc) and about health and hygiene while no prevalence of Mf among age group >70 may be due to less number of respondents participated in Survey 5/500 (1.00%) and indoor resting habit due to their

age factors. Age wise distribution of filariasis is equivalent to the length of exposure, which is supported by WHO (2001).

According to Witt (2001) although LF is first acquired in childhood, clinical features occur only after puberty and hence increases with age. Message *et al.,* (2000) also supported that clinical manifestations and microfilaria prevalence increases with age.

The maximum prevalence rate was reported in the people who were just literate 12/72 (16.90%) and illiterate people 13/93 (13.98%). This may be due to ignorance against causative agent, vector of Mf and its mode of transmission. This result is different from the result obtained by Byanju (2006), Kaju (2006) and Chhetri (2005). This may be, because the total number of respondents participated in the survey, high illiteracy rate and poverty too. Regarding the knowledge about this vector born global problems 97% of the people were still unaware about the disease and only 3% knew about the vector and the disease but the Mf rate of infection was found higher in them. This may be due to their carelessness about the preventive measures.

Females, students and people of the age group 11 – 20 years were found to be relatively higher in the study. It is found that, most of the males have gone to Kathmandu valley as it is near to VDC surveyed and overseas for better income and employment opportunity. Thus, greater number of females and school children participated in the survey. The field survey conducted on Dapcha VDC revealed that the in-sanitary environmental condition, type of their houses and the habit of keeping their cattle along with themselves in their houses has aggravated the

condition for spreading Mf. The present study was similar to the result obtained by Byanju (2006), Kaju (2006), Chhetri (2005) and Jha (2003). Pradhan *et al.*, (1997) obtained that clayey soil which retains water for a long time in the ditches and ponds available during monsoon is the most favourable breeding site for the vectors of filariasis. Such type of soil texture is prevalent in the VDC surveyed. Not only this, bushy habitat, a large Pokhari and their ventilationless dark houses supports largely by providing enough hiding and breeding places and also provides opportunity to the vector for transmission of the disease. As the vector *Culex quinquefasciatus* is the efficient indoor biter throughout the night. The other practices of the respondents towards filariasis are their sleeping habits, occupations where most of them were farmer (9.14%), labour (12.50%) and housewife (13.89%) in which almost all of them are illiterate. That is why, there is increased in the risk for acquiring the vector born disease filariasis. The habit of keeping cattles along with them in their houses and nearby their houses should divert the mosquitoes from human beings to cattle for their blood meal. But, to the vector of filariasis *Culex quinquefasciatus* preferred host is not cattle, but the man (Sirivanakarn, 1976, Reisen and Boreham, 1979).

The prevalence of vector of Lymphatic filariasis is recorded in all endemic regions of Nepal (EDCD, 2062/063). This is similar to the present study where prevalence rate was found to be 7/500 (1.4%). This result matches with the research conducted by Peters and Dewar (1956) where they recorded this species for the first time from the districts of Bagmati, Koshi, Narayani and Janakpur zones. Hence extensive study and research is the prime requisite to determine epidemiological and etiological factors as well as the prevalence of the vector that has become one of the national and global burden for its eradication.

VII

RECOMMENDATIONS

Minimization of filarial cases and prevalence of vectors of Lymphatic filariasis can be achieved in Daraune Pokhari, Dapcha, VDC of Kabre district by taking concern on following recommendations:

- The mass awareness programme is the most recommended expectation to make people aware about the disease lymphatic filariasis. Thus, to make them familiar about filariasis, awareness activities should be spread through mass media, radio, television, distributing posters and pamphlets, organizing different programmes etc. to improve health status and to maintain environmental sanitations.
- To keep themselves away from the bite of vector mosquitoes, they should be conscious of using personal protection method such as the use of mosquito nets, mosquito repellents (mosquito coils, mosquito mats, fumigants, ointments etc.)
- The success of elimination programme can only be assisted by regularizing mass drug administration and control programme along with monitoring the same study population.
- Public health education should necessarily be included in all levels of educations. The teachers and health post personnels should be properly trained about the control measures of filarial diseases. Different extra curricular activities should be oriented in school levels that will provide great assistance to bring awareness in the society and community level, since school going children are the pioneer of the society and the country.

- The concerned authority who is taking initiative in controlling vectors should strictly introduce suitable control measures as per the location and possibility.
- Still almost all people of the VDC are unaware about the vector of the disease LF. They should be made aware about the vector, its time of bite and mode of transmission.
- The concern organization and the interested researchers should take prompt initiative to study about the biology, prevalence, density and diversity of the vectors. So that it would assist in elimination programme.

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ANNEX - I

Questionnaires for Filariasis Sentinel Survey in Daraune Pokhari, Dapcha VDC of Kabre District of Nepal

Date:
S.N. .....

1. Name of the respondent : .....
Address: District: .....
Ward No./Tole/Block No.....
2. Age/Sex .....
3. Education:

Literate	
Illiterate	

If literate,

1. Primary		5. Intermediate	
2. Lower Secondary		6. Bachelor	
3. Secondary		7. Master	
4. S.L.C.		8. Others	

4. Occupation

1. Farming		5. Housewife	
2. Labour		6. Teaching	
3. Business		7. Unemployed	
4. Student		8. Others	

5. Marital Status

Single/Married	
Widow/Widower/Divorce	

6. Relationship with the head of the family/family size .....
7. Respondent's current residence status:

Birth place	
Migrate	
Temporary	

(How long have you been staying here?) ..... years/months
8. Surrounding environmental condition:

1. Clean		3. Bushy	
2. Dirty		4. Open Drainage	

9. Sleeping habits

Ground floor (indoor & outdoor)	
Upper floor	

10. Animal husbandry practice (keeping cattle)

Away from house	
Nearby house	
Sharing the same house	

11. Use of the any means of the protection against mosquito bite:

Yes	
No	

If yes, which one of the following:

1. Mosquito net		4. Spraying insecticides	
2. Anti mosquito cream		5. By burning mosquito coils	
3. Smoke			

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

Yes	
No	

If yes, how is it transmitted?

By mosquito biting	
Contact with disease person	
Mother to foetus	

13. Respondent current health status:

Healthy	
Unhealthy	

If unhealthy since when ..... yrs ..... months

14. Do you have any symptoms?

Yes	
No	

If yes, which of the following

1. Fever		8. Chyluria	
2. Effect on genital organ or breast		9. Abscess Formation	
3. Headache		10. Nausea	
4. Swallon Lymphnode		11. Epigastric Pain	
5. Hydrocele		12. Weakness	
6. Swollen hand or limb		13. Lazyness	
7. Skin thick, red and swollen blood vessels			

If yes, have you used any medicine?

Yes	
No	

If yes, which of the following?

Ayurvedic	
Allopathic	
Herbal	

15. According to your knowledge is the disease is more in parent's time or now?

Parent's Time	
Now	
Don't know	

16. Have you seen any person suffering from this disease?

Yes	
No	

If yes, how many .....

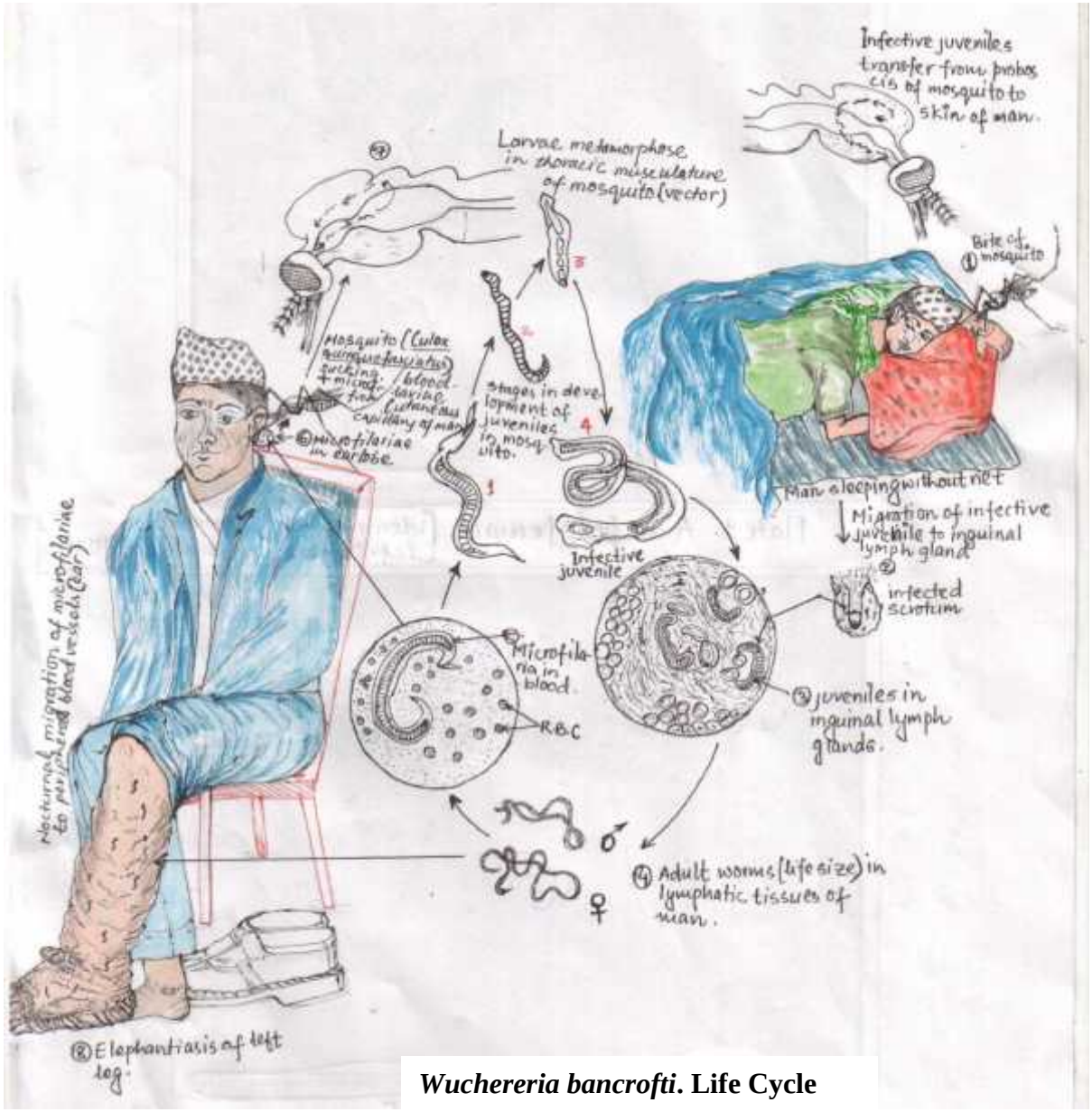
17. Is there any person suffering from this disease in your family or relatives?

Yes	
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 20ml .....

ANNEX - II



***Wuchereria bancrofti*. Life Cycle**

By: Bhim Bdr. Dahal

ANNEX - III

Structure of Microfilaria and its Vector Mosquito

Adult Worms

These are long hair like transparent nematodes often creamy white in colour, filariform in shape and both ends tapering, the head end terminating in a slightly rounded swelling. The male measures 2.5 – 4cm in length by 0.1mm in breadth. Its tail end is curved ventrally and contains two spicules of unequal length. The female measures 8 – 10cm in length by 0.2 - 0.3 mm in thickness. Narrow and abruptly pointed tail end, mainly viviparous. Male and female remain coiled together and can only be separated with difficulty. But females are usually more numerous than males and the latter are difficult to find. Life span of the adult worm is long probably last for about 5-10 years.

Adult vector (viz. *Culex quinquefaciatus*, female)

General Background of Vector

Mosquito constitute one of the largest dipteran family culicidae and are of major significance both vectors of several tropical diseases such as malaria, filariasis and numerous viral diseases. In temperate climate they are more important as nuisance pest than as vectors. There are about 3000 species of mosquito of which 140 are found in Nepal included in 14 genera (Pradhan *et al.*, 1996).

Structure of adult vector (*Culex quinquefasciatus*)

Mosquitoes like other insects have three body parts: a head, a thorax and a ten segmented abdomen with a short movable neck that joins head with thorax. They possess three pairs of long legs where prothoracic leg is the shortest and metathoracic is longest amongst three pairs. The main distinguishing features of the limbs from other mosquito in the vector are absence of pale bands at the joints of tarsomeres and no median longitudinal pale scaled stripe at the anterior surface of midfemur, pulvilli present and tarsal claws unusually small.

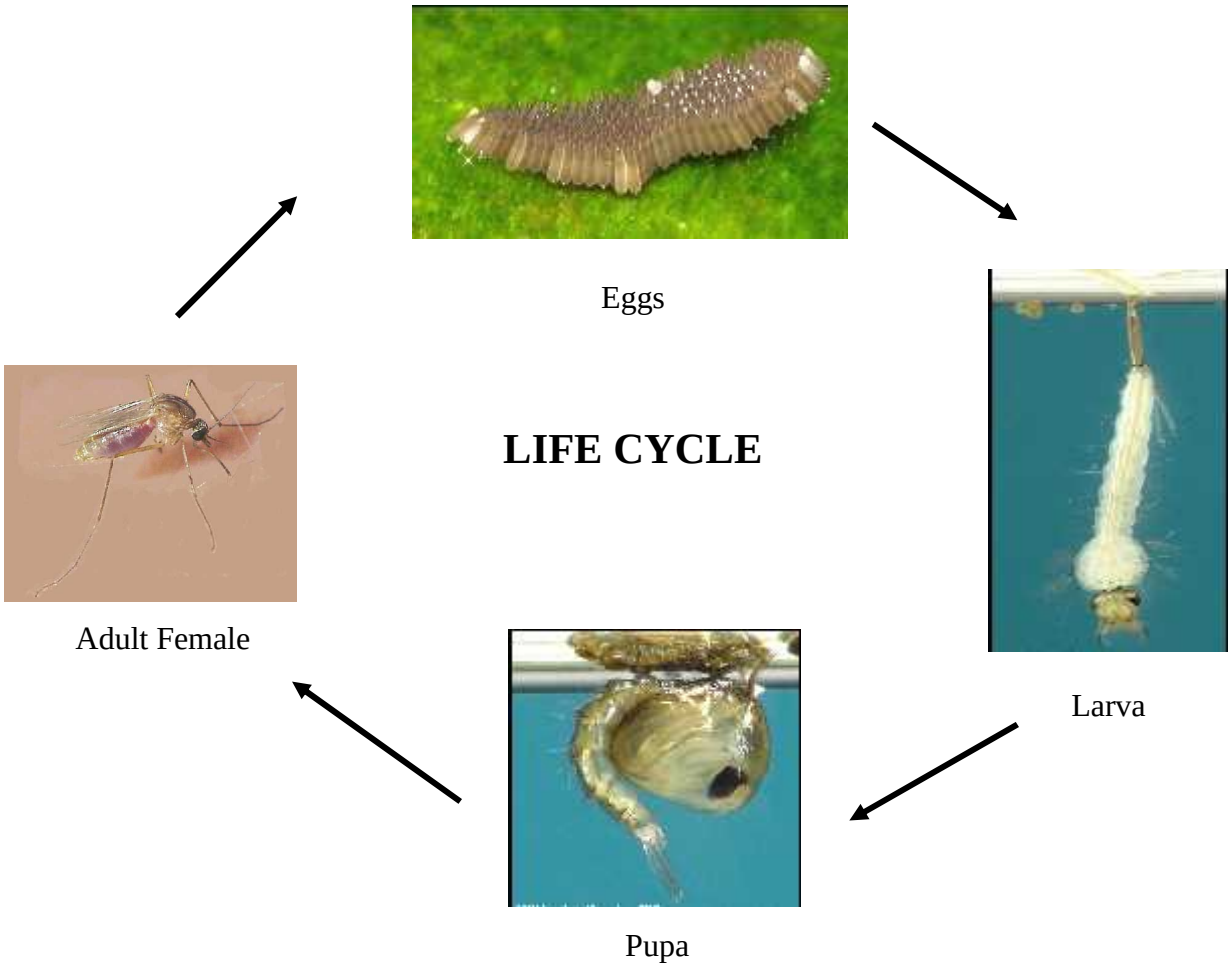
Head is globular with a pair of large compound eyes without ocelli. Two filiform antennae each with 15 jointed segments. Antennae is densely plumose in males while pilose in the females. The mouth parts is sucking type in male to suck nector of flowers, while piercing and sucking type in female to pierce the skin and suck blood of warm blooded vertebrate hosts where man is the most preferred host of *Culex quinquefasciatus*. The proboscis is without distinct pale scaled band in the vector of Lymphatic Filariasis as in *Cx. vagans* (Pradhan *et al.*, 1996). The maxillary palpi of female *Culex* vector species is very short while it is long and bent up in the male (Medical Entomology, ICPMR, 2002).

The thorax has usual three segments (viz. pro–,meso–and meta–thorax) where pleural integument is without dark and pale stripes as in *Cx. fuscocephala* and scutal integument yellowish to pale brown in *Cx. quinquefasciatus* (Sirivanakarn, 1976). In the mesepimeron one or two mesepimeral setae are present (Pradhan, *et al.*, 1990). The second pair of wings is modified into halters.

The abdomen is 10 segmented which consists of tergum and sternum on upper and lower parts respectively in each segments. The abdominal terga of *Cx. quinquefasciatus*, the vector of L.f. has basal transverse pale scaled bands (Pradhan, *et al.*, 1990b). Two pairs of spiracles i.e. mesothoracic and metathoracic spiracles are present on meso and meta thorax respectively and seven pairs of spiracles from 2nd to 8th abdominal segments are also present as respiratory organs. The 8th segment bears the terminal anus and 9th terminal gonopore. In female, the 10th bears a pair of anal cerci. The 9th bears a pair of clawed claspers in males and 10th is modified an intromittent copulatory organ called aedeagus.

ANNEX - IV

Life Cycle of Vector of Lymphatic Filariasis (*Culex quinquefasciatus*)



ANNEX - V

Process of making dilute Giemsa solution

Giemsa Stain

Giemsa stain is an alcohol -based Romanowsky's stain, it is highly flammable with flash point 12^0c, 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 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. Firstly 100ml of an empty measuring cylinder is taken.
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:

Giemsa 3.8grams

Glycerol (Glycerine) 250ml

Methanol (Methyl alcohol) 250ml

1. The Giemsa is weighted on a piece of clean paper (pre weighted) and transfered to a dry bowel 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 a water bath at 50-60^0C or up to 2hours at 37^0C 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 it 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.

Caution:

1. Giemsa stain will be spoilt if water enters the stock solution during its preparation of 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 stock stain should be transferred to a dry dispensing bottle (that can be closed tightly after use).
4. The quality of all new batches of Giemsa should be checked by using it to stain microfilarial parasite for the control purpose.
5. Thick and thin blood film should be prepared from fresh blood, dried and folded individually in paper, sealed in a plastic bag and stored in a freezer at 20^0C.

Characteristics of Giemsa stain:

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

Chromatin of parasite ..... Dark red
Cytopolasm of parasite ..... Blue
Schuffener's dots ..... Red
Maurer's dots (Clefts) ..... Red maure
Red cells ..... Grey to pale maure
Reticulocytes ..... Grey blue
Nuclei of neutrophils ..... Maure purple
Granules of eosinophils ..... Red
Cytoplasm of mononuclear cells ..... Blue grey

Photographs of the Vector i.e. *Culex quinquefasciatus* (Female)

Plate 1: A unfed female

Plate 2: The bloodfed female

Photographs of the Vector i.e. *Culex quinquefasciatus* (Male)

Plate 3: A male *Culex quinquefasciatus*

Plate 4: Anterior part of the male *Culex quinquefasciatus* showing the large palps and hairy antennae. (A typical feature of male mosquitoes)

Photographs of the Vector i.e. *Culex quinquefasciatus* (Male)

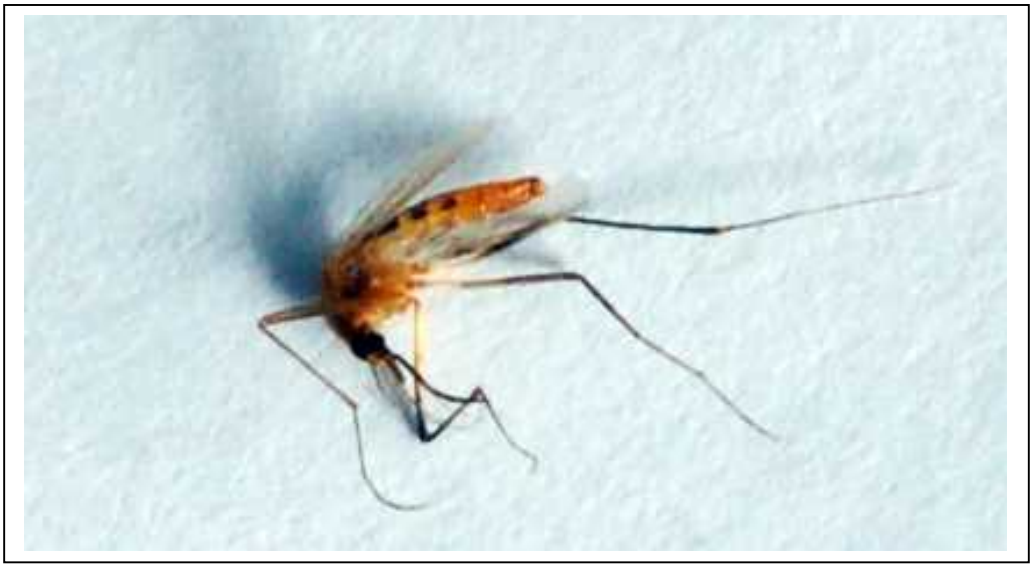
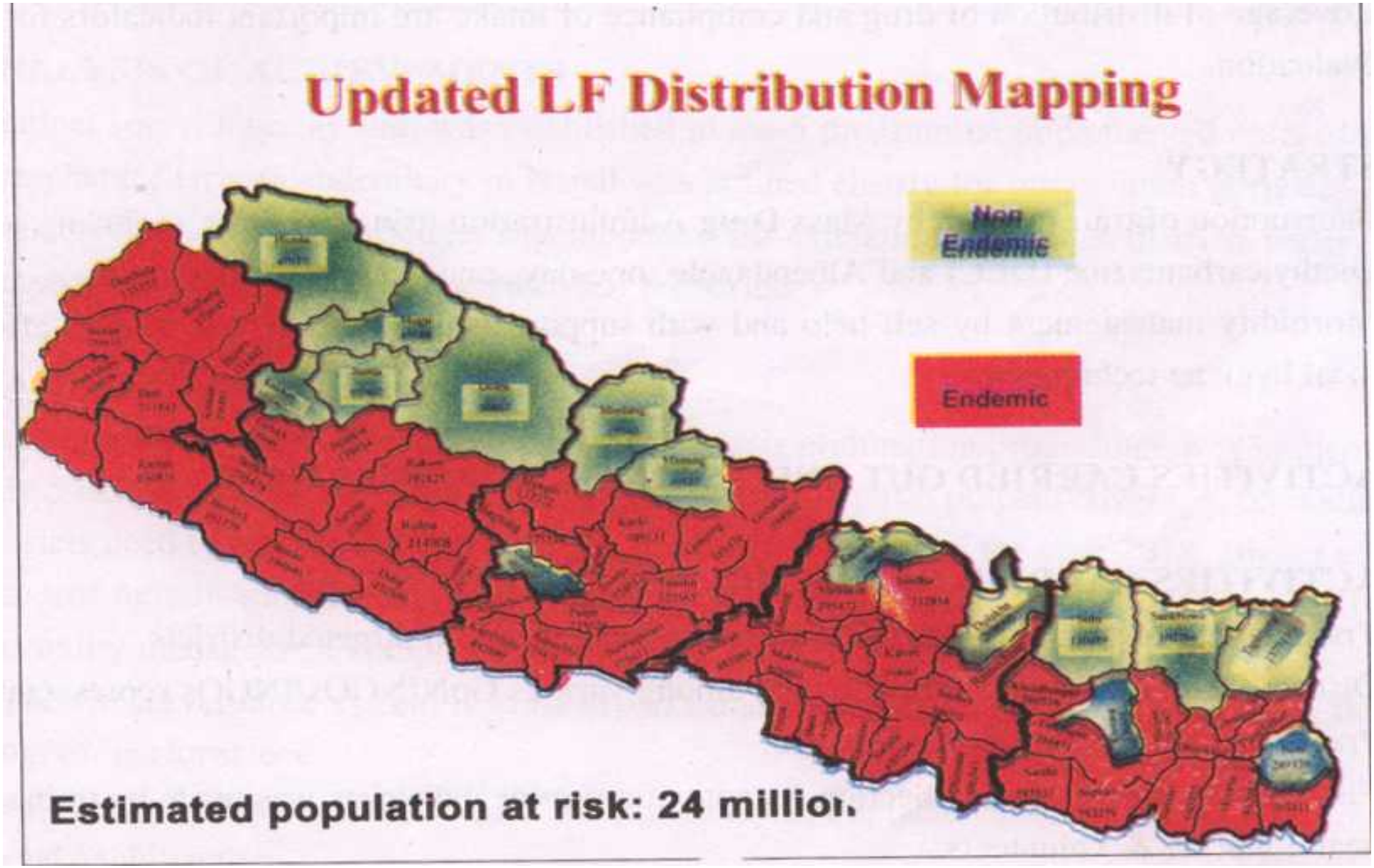


Plate 5: Lateral part of female *Culex* mosquito showing abdominal terga

Plate 6: Female *Culex* mosquito showing scutal integument and wings

Updated LF Distribution Mapping



a

b

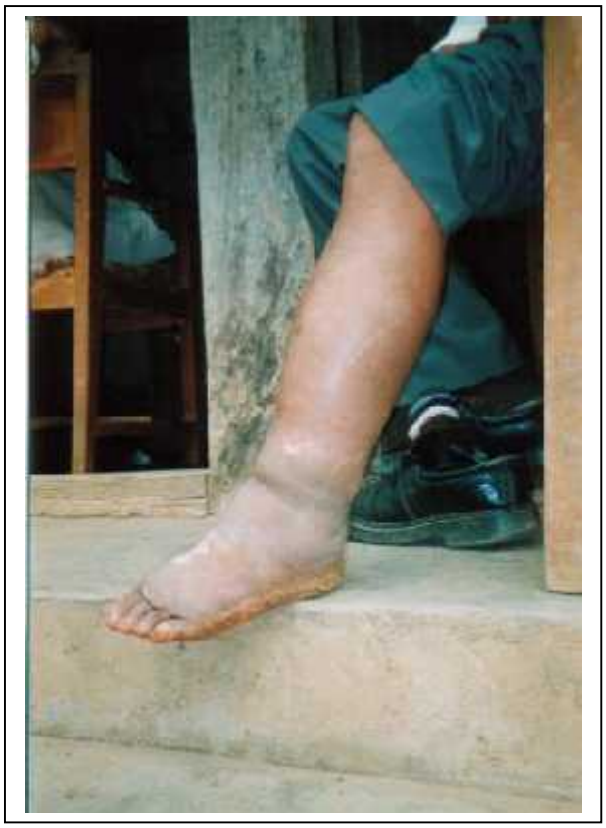
c

d

e

f

a. Daraune Pokhari in the study area from the North.
b. Bushy & insanitary environmental condition around the study area.
c. Daraune Pokhari from the West a favourable place for vector to breed.
d. Study team with researcher at the sub-health post.
e. Questionnaire survey with respondents and thesis guide at the right.
f. Mass awareness programe in the study area.



g

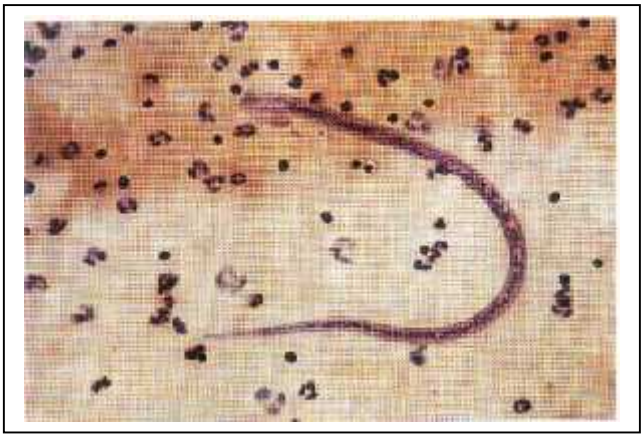
h

i

j

g. An Elephantiasis of a left leg filarial patient.
h. Highly infected legs of filarial victim with secondary infection.
i. Collection of night blood sample
j. Fixing of blood smears with methanol stain.

k



l

m

n

k. Microphotography of microfilariae
l. Microfilaria of *Wuchereria bancrofti* (100X objectives)
m. Paper cups for collecting mosquitoes
n. Observation of mosquitoes