Epidemiological Analysis of Lymphatic Filariasis in an Endemic Village of Dhading District, Nepal

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With Special Paper Parasitology

By

Chandika Koju

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RECOMMENDATION

This is to certify that **Miss CHANDIKA KOJU** has completed her dissertation work entitled **"EPIDEMIOLOGICAL ANALYSIS OF LYMPHATIC FILARIASIS IN AN ENDEMIC VILLAGE OF DHADING DISTRICT, NEPAL"** as a partial fulfillment of the Master's degree of Science in Zoology with special paper Parasitology under my supervision. To my knowledge her work has not been submitted for any other degree.

.....

Dr. RANJANA GUPTA
Associate Professor
Central Department of Zoology
Tribhuvan University, Kirtipur
Kathmandu, Nepal.

Date: ……..

LETTER OF APPROVAL

On the recommendation of supervisor **Dr. RANJANA GUPTA**, this thesis of **Miss CHANDIKA KOJU** 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. TEJ KUMAR SHRESTHA
Head of Department
Central Department of Zoology
Tribhuvan University, Kirtipur
Kathmandu, Nepal.

APPROVAL

We certify that this dissertation presented by **Miss CHANDIKA KOJU** titled **"EPIDEMIOLOGICAL ANALYSIS OF LYMPHATIC FILARIASIS IN AN ENDEMIC VILLAGE OF DHADING DISTRICT, NEPAL"** is 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.

Evaluation Committee

…… **……..**

Head of Department **External Examiner**

…….

Internal Examiner

Date: …….

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Miss Chandika Koju
Exam Roll No. **: 541**
M.Sc. Zoology, Parasitology
T.U. Regd. No.: **5-1-33-378-97**
2060-2061

ABSTRACT

Lymphatic filariasis, one of the most prevalent tropical and sub-tropical diseases that lead to permanent and long term disability, is a group of infectious disorders caused by thread like nematode parasite, *Wuchereria bancrofti*. It has been identified as a major health problem globally. It has been known to be endemic in different regions of Nepal since long time. The present study was conducted from 15 January 2006 to15 December 2006 in Benighat VDC of Dhading district. A total of 521 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 in order to take information about the participating respondents before night blood sample collection. Microscopical observation showed 4/521 (0.76%) microfilaria positive cases. Regarding sex-wise distribution, it was found that there was significant difference of infection in both sexes (χ^2=7.82, P<0.005). According to age-wise prevalence, the highest microfilarial cases were found in the age group 41-50 years (2.78%) and the least was found in the age group 21-30 years (0.90%). Crude disease rate was reported to be 0.96% and the total endemicity rate was 1.73%. The main factors affecting the prevalence of the disease were lack of awareness about the disease, poor sanitation, 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 programs through mass media such as pamphlets, broacher, radio, television etc. which will help in protecting vector borne Lf.

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LIST OF ACROMYMS

ADL	Ademolymphagitic
CBos	Central Bureau of Statistics
CDR	Crude Disease Rate
CFA	Circulating Filarial Antigen
DEC	Diethylcarbamazine
DoHS	Department of Health Service
EDCD	Epidemiological and Disease Control Division
ELISA	Enzyme Linked Immunosorbent Assay
ER	Endemicity Rate
FILDAC	Filarial Diethylcarbamazine
HMG	His Majesty's Government
ICT	Immunochromatographic test
Lf	Lymphatic filariasis
MDA	Mass Drug Administration
Mf	Microfilaria
MoH	Ministry of Health
PARASED	Parasitological Research and Socio-economic Development, Nepal
PMDH	Per-man hour density
VDC	Village Development Committee
WHO	World Health Organization

I

INTRODUCTION

Lymphatic filariasis (Lf) is one of the most prevalent tropical and sub-tropical diseases that lead to permanent and long term disability. It is a group of infectious disorders and a disabling disfiguring infection caused by threadlike nematodes (Super-family filarioidea). The filarial nematodes invade the subcutaneous tissues and lymphatic vessels of mammals, producing reactions varying from acute inflammation to chronic scarring. The adult filarial nematodes live principally in the lymph nodes and lymph vessels, notably those draining the legs and genital area, where the adult worms induce allergic reactions in the sensitized tissues. The adult worm obstruct the flow of lymphatic fluid resulting in the accumulation of lymph at only one site, which cause swelling of organs; usually affecting only one or both legs, genital organs causing hydrocele, breast enlargement, swollen clitoris and vulva, enlargement of male scortum and penis. Filarial infection also causes acute fever, inflammation of lymphatic system and branchial asthmatic condition known as "Tropical eosinophilia".

In the human body the female nematode gives birth to elongated embryos, the microfilariae which are found in peripheral blood of human. These microfilariae are taken by blood sucking insects. Within the insect carrier, the microfilariae

grow into motile, infective larvae that are introduced into the human host, where they reach maturity in about a year.

Wuchereria bancrofti, Brugia malayi and *Brugia timori* are the three species of filarial nematodes that cause lymphatic filariasis (Lf) in human beings. They are also responsible for the morbidity. *W. bancrofti* is the main source of lymphatic filariasis (Lf) in Nepal (Thakur, 2000). *Loa loa, Mansonella perstans, M. ozzardi, Dipetalonema perstans* and *Onchocera volvolus* cause non lymphatic filariasis.

Lymphatic filariasis is not life threatening, but can cause permanent damage of lymphatic system and kidneys. It does not show any sign and symptom until the worms die. The dead worms block the lymph vessel resulting in the collection of fluid and inflammation. The initial inflammatory stage is characterized by granulomatous lesions, swelling in the arms, breasts, legs and genital area of males. This stage is followed by enlargement of the lymph nodes and dilation of the lymph vessels causing hardening and thickening of skin resulting the condition called elephantiasis in some of the untreated cases.

In acute cases, lymphatic filariasis involves episodic attacks of adenolymphagitic (ADL) associated with the fever and malaise causing morbidity. The Lf includes other complications like chyluria resulting the weight loss, lethargy and tropical pulmonary eosinophilia causing chronic fibrosis. The prevalence of Lf infection is increasing continuously due to rapid and unplanned urbanization which creates numerous breeding sites for the mosquitoes, the main vector for the transmission

of the disease. It is a significant cause of acute and chronic illness in both sexes, particularly in the poorer community of the society.

Globally more than 1.1 billion people i.e. 20% of the world's population in some 80 endemic countries located in tropical and sub tropical areas of the world are estimated to be at the risk of infection, from the lymphatic filarial parasites; of these 90% by *Wuchereria bancrofti* and 10% by *Brugia malayi* (WHO, 1997). 27 million people suffered from genital disease (hydrocele), more than 15 millions from lymphoedema or elephantiasis of leg, 83 million from lymphatic functional disability and 30 millions from renal pathology (WHO, 1997). The parasitic disease lymphatic filariasis is a major socio economical burden globally in tropical and subtropical areas. Approximately 1\3rd of people with infection live in India, Africa and South-East Asian countries. About 600 million people live in the endemic areas of South-East Asia constituting about 60% of the global population.

Vector control methods have been applied successfully including killing of adult mosquitoes and larva. For example, under certain condition the use of polystyrene beads can be highly effectible in *Culex* larval control and environmental management.

The chemotherapy of filariasis has been based on treatment with Diethylcarbamazine (DEC), for more than 40 years. The repeated treatment with DEC results significant reduction in the incidence of acute attacks and the risk of developing chronic disease. Control with DEC can involve mass treatment of all eligible in the community or selective treatment of those who are diagnosed as

micro-filarial positive during night blood surveys. Selective treatment is expensive, but ensures better compliance. Recent encouraging results of single dose regimens with DEC are therefore of great practical interest.

Filariasis has not been regarded as a public health problem in the world so there is no effective control of filarial parasite in most of the world's endemic areas and most control strategies are too complicated and expensive to be applied. In the past, parasite control and transmission control had eradicated filariasis in several endemic areas. It will be more appropriate to focus first on the development and implementation of simple cost effective and sustainable strategies for morbidity control (Ramchandran, 1993).

Control of lymphatic filariasis leading to its elimination is based on controlling transmission of parasites. This can be done by applying the different vector control methods.

Filariasis has been known to be endemic in different areas of Nepal since a long time. It is a public health problem in Nepal. Filariasis is much more prevalent in Nepal due to the presence of suitable environmental condition and mosquito breeding sites. In Nepal, very few researches on lymphatic filariasis have been undertaken. At present out of total population of Nepal (23.2 million approximately), 60% i.e. 13.9 millions are estimated to be at the risk of filariasis. Out of three species of lymphatic parasites; *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*, only one species i.e. *Wuchereria bancrofti* is recorded in Nepal. The previous works on filariasis were done by Jung (1973), Pradhan *et al.,* (1997) Bhusal *et al.,* (2000). More recently, Sherchand *et al.*, (2003) studied mapping of

filarial infection in 37 districts of total 75 districts of Nepal. The study showed that 33 districts of them were endemic for lymphatic filariasis. Of the 33 districts, 11 districts were having above 20% prevalence rate, 15 districts between 6-19% and 7 districts between 1-5%. The averages of 13% of the whole population in Nepal were found to be affected (Sherchand, 2003).

SIGNIFICANCE OF THE STUDY

From the above statement and different researches, it was found that the prevalence of filariasis is seen at different places of Nepal including Terai and Hilly areas. It may be due to geographical and climatic variation. 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 (2003-2015A.D.) for elimination of lymphatic filariasis in Nepal. Regarding this view, the study on lymphatic filariasis in Benighat VDC of Dhading 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 near future.

II

OBJECTIVES

General Objective

To determine the human filarial infection and its risk factors in Benighat VDC of Dhading district of Nepal

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 sex wise endemicity rate of lymphatic filariasis.
- To bring awareness about filariasis among the community.

III

LITERATURE REVIEW

Research on Filariasis in Nepal

Ghimire *et al.*, (2003) studied the prevalence of lymphatic filariasis in an endemic areas, Mahendranagar and Nagrain VDCs 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 sexeswas almost equal.

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 over all prevalence of lymphatic filariasis from 4,488 sample population was 13% and 33/37 districts 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 altitudes between 900-1500m.Of the 33 districts, 11 districts were having above 20% prevalence rate, 15 districts between 6-19% and 7 districts between 1-5%.

Sherchand (2002) conducted an epidemiological survey to determine prevalence of disease due to lymphatic filariasis in Magaragadi VDC, Bardia district of Nepal. The study population was selected above the 15 years of age group and the immumochromatographic test (ICT-filariasis test) was used to screen for circulating filarial antigens (CFA). The prevalence of lymphatic filariasis from 500 sample population was 141 which were infected with larvae of *W. bancrofti*..

Tuladhar and Sherchand, (2001) conducted an epidemiological study in three different geographical regions,viz; Terai (Sipwa VDC of Rupandehi district) and inner Terai (Dovan VDC of Palpa district) and Hill (Katunje, Golmadhi, Itachhen 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 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.

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.

Bhusal *et al.*, (1999) conducted another survey to describe the infection and intensity of *Wuchereria bancrofti* in Tokha-Chandeswori VDC of Kathmandu valley. This survey showed the overall 5.8% prevalence of microfilaraemia 13% crude disease rate in the study population. The study reported the highest microfilaraemia infection rate (11.8%) among the age group of 40-49 and main crude disease rate (36.4%) in the age group and above.

Pradhan *et al.*, (1997) carried out an epidemiological study on lymphatic filariasis in Gokarna VDC of Kathmandu valley. The study reported 12.75% microfilaria infection and 11.95% crude disease rate of *Wuchereria bancrofti* in the study population. The study showed the high crude disease rate among the females than males (crude disease rate: 8.49% in males and 16.59% in females). 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.*

Jung (1973) studied all together 9 sites which showed 4.99% to 6.15% *W. bancrofti* in all age groups and both 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% microfilaria rate was found in the urban population, 10.03% to 11.3% in the semi urban population and 0.8% to 17.69% in rural population.

The following table gives an outlook of lymphatic filariasis situation in National and Region wise based on annual reports DoHS, MoH and HMG, Nepal from 1995/96 to 2004/2005.

Table-I: National and Region wise number of filarial cases in Nepal FY 1995/96 to 2004/2005.

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	9
2001/02	1183	142	173	733	79	56
2002/03	809	63	302	334	64	46
2003/04	540	47	246	221	20	16
2004/05	549	25	274	180	50	20

Research on Filariasis in Global context

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. Seven 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% cl: 66.4-97.2) where as the specificity was 100 %(512/512). The overall prevalence of brugian filariasis as determined by dipstick is 9.4% (95% cl: 8.2-0.55) while that determined by microscopy is 0.90% (95% cl: 0.5-1.3).

Bregani *et al.*, (2005) studied the effects of thiabendazole in the treatment of symptomatic *Mansonella perstans* filariasis by treating 25 patients with thiabendazole at a single dose of 50mg/Kg for children and 3gm for adults. They found out that parasite density, eosinophilia and symptoms significantly reduced

after both one and two step therapy in most patients. This study shows that thiabendazole may be effective in *M. perstans* infection.

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 scortum. After acquired causes of lymphoedema were ruled out, the patients were treated with scrotectomy and re-construction.

Mathieu *et al.*, (2005) studied on the factors associated with participation in a campaign of mass drug treatment against lymphatic filariasis, in Leogane, Haiti. They found that absenteeism during distribution (17%), use of contraceptive drugs (12%) and pregnancy (11%) were the primary factors for failing to take drugs, while people who know that a mosquito transmits filariasis and having learnt about the MDA through posters and banners were found to be positively associated with taking drug.

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

Forid *et al.*, (2004) conducted a study to examine relationship between *W. bancrofti* microfilaria counts in human blood and parasite uptake and maturation in *Culex pipens* with observations on the effects of DEC treatment on these parameters. They found that uptake of microfilaria (Mf) and production of

infective larvae (L3) were more closely correlated with Mf counts in finger prick blood than in venous blood. Single dose DEC treatment therapy reduced Mf counts by 87.9% after 6-7 months of treatment showing that single dose DEC had a major impact on Mf ingestion and L3 production by mosquitoes.

Loymek *et al.*, (2004) determined the impact of brugian filariasis control program on intestinal helminthic infections in Narathiwat Province, Thailand. They found that 50.3% of the villagers were infected with one or more types of intestinal parasites. Double and triple infections were found in 10.9% and 1.6% of infected individuals respectively. The prevalence of intestinal parasitic infections peaked in the 1-10 years old age groups which are Pre School and young school age children. A significant reduction of intestinal helminthic infection in the post treatment stool sample was observed in 150 participants, who were examined six month after mass treatment.

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 of14 (14.2%) of E, 10 of 100 (10%) of NE and none (0%) of NNE were positive for antigenaemia.

Kyelem *et al.*, (2004) determined the impact of long term ivermectin on W. *bancrofti* and *Mansonella perstans* infection. The study was undertaken in 11 communities of S-W Burkina Faso and the drug was given under community directed treatment strategies for Onchocerciasis control. Six of the villages investigated had been treated with ivermectin at least once a year for five of six years, with a mean coverage of approximately 65% in each round. The prevalence and intensities of *W. bacrofti* and *M. perstans* microfilaraemia were significantly lower in the ivermectin treated communities.

Koyadun *et al.*, (2004) conducted a study on bancroftian antigaemia clearance and Myanmar migrants after biannual mass treatment with DEC 300mg 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 antigaemias (%) in 4 to 5 index cases (group 1) with high antigens titers (99.7-181.6 $x10^3$ 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 ($P<0.007$).

Rajendran *et al.*, (2003) studied the influence of the mass administration of DEC 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 antigenaemia 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 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 vessels 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 are described.

Hammad *et al.*, (2003) studied the impact of DEC on vector competence of *Culex pipens* L. to *W. bancrofti* Cobbold and found out that annual single dose of DEC has great potential to mediate sustained microfilaria transmission and killing the filarial parasites within the mosquito.

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.

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 normal 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.

Triteeraprapab *et al.*, (2000) conducted a study on transmission of nocturnal periodic strain of *W. bancrofti* by *Culex quinquefasiatus* 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* serves as the main vector. The Thai strains of *C. quinquefasciatus* have never been reported to transmit lymphatic filariasis. *W. bancrofti* with third stage larvae establish the potential for establishing an urban cycle of transmission in Thailand.

Bhumiratna *et al.*, (1999) assessed 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 20% antigen positive rate while other test gave lower positive rates of the same, 5.8% by thick smear and 5.3% by capillary tube technique. The ICT card test had specificity of 100% when sera from microfilaraemic subjects were positive, as when sera from *W. bancrofti* non-endemic subjects either *Brugia malayi* microfilaraemia or with other parasites and those from normal controls were all negative by the test. When it was done in *W. bancrofti* microfilaraemia sera, the ICT card test had a sensitivity of 100% using clinical and recall technique. However, the ICT card test was more positive than the other when sine in endemic normal sera (14% positive).

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

endemic population and 81% 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 infection of 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).

IV

MATERIALS AND METHODS

Study Area

Nepal is developing South Asian country that lies between 80^{o} 04' and 88^{o} 12' East longitudinal and 26^{o} 22' and 30^{o} 27' North latitude. It extends over an area of 147,181 sq. Km. It is nestled at the foothills of the Himalayas sharing its northern border with Tibetan autonomous region of China and its eastern, western and southern borders with India. Topographically, it is divided into 3 ecological regions namely mountainous, hilly and terai. Administratively, it is divided into 5 development regions, 14 zones and 75 districts. The Dhading district selected as the study area is situated in the Bagmati zone of central hilly region in the central development region.

Dhading lies about 58Km West of Kathmandu valley, at the height of 488m to 7409m from the sea level. It lies between 84^{o}20' and 84^{o}55' East longitude and 26^{o}45' and 28^{o}10' North latitude. It extends over an area of 1,962Km with population density 176/sq.Km. (CBoS, HMG, 2001). It shares its border with Nuwakot and Kathmandu in the east, Rasuwa in the North East, Makwanpur in the South East, Chitwan in the South West and Gorkha in the South. The head quarter of thes district is Dhading Besi. According to Central Bureau of Statistics, HMG (2001), the total population is 338,658 (165,864 males and 172,794 females) with

the sex ratio (M/F) 24:25. The literacy rate is 43.48% (male 53.69% and female 33.81%). 50 VDCs are included in the Dhading district. The main occupation of the people living in this district is agriculture. About 84.85% people accept agriculture as their main occupation. There are 52 health posts in this district.

Benighat VDC, located in the South West of the district was selected for the study purpose. The total population of this VDC is 8,306 (male 4204 and females 4102) with the total number of house hold 1,574. There is only one health post in this VDC. Benighat VDC lies on the Prithive Highway. Benighat extends over an area of 41sq.Km.

Preliminary Field Survey

A preliminary field survey was conducted on 15 January 2006 to know about the lymphatic filarial situation in Benighat VDC of Dhading district. A list of affected VDCs from Dhading district was prepared, consulting with several local health personals. The main aim of selecting Benighat VDC was to focus on the study of mf in unsuspected filarial patients and compare with clinically suspected patients.

Primary Data Collection

The study was conducted from 15 January 2006 to 15 December 2006. During the blood sampling in the field, sentinel sampling was chosen without separating each and every house till the target samples were achieved from the starting house. Before the blood samples collection, the respondents were gathered and were given orientation about filariasis and the aim of the study was also explained.

During the sampling, the samples from all the family members above two years age were taken. The blood samples were taken from those respondents who were at home during the collection period.

Study Design

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

Sampling Technique and Sample Size

A total of 521 blood samples were collected from the community people of Benighat VDC of Dhading district. During the survey, the blood samples were collected at night from 12am to 2:30am 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.

1. 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.
2. 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.

3. Each sampling slides were labeled with code number HH_1/A, HH_2/B etc. which were coded according to the questionnaires.
4. The blood samples were collected from the ear lobe by adding three drops of blood on slide at three different spots. Each drop of blood contained about 20ml.After blood samples were taken out from the ear lobe of the respondents, thick smears were made immediately and air dried for sometimes. The dried slides were collected in the slide box for microscopic examination.

Instrumentation

Different tools were used in this study which is as follows:

- **Questionnaire**

The questionnaire contained 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.

- **Laboratory tools for preparation of blood smear**

Materials required

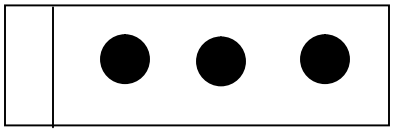
Microscopic slides, sterile lancets, tooth pick, cotton wool, gloves, measuring cylinder, slide box, compound microscope.

Reagents

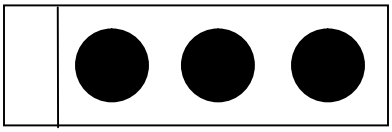
Methanol, Distilled water, Giemsa stain 5%.

- **Procedure**

After pricking the ear lobe of the respondents, three thick blood smears were prepared in each slide with the help of tooth pick or another slide. Prepared blood smears were left undisturbed for drying for about 8 to 10 minutes and kept them properly in the slide box.



Blood drops on the slide for thick smear



Thick smears of blood

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

i. 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.

ii. Dehaemoglobination of thick blood smear:

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

iii. Staining of blood smears:

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

iv. Observation:

The stained blood smears were examined in compound microscope under 5x, 10x, 40x, and 100x objective lenses. The microfilaria was identified as *W. bancrofti* on the basis of the following characters:

a) Discrete nuclei b) sheath stained c) empty space between the nuclei and body wall d) cephalic tip of tail f) tip of tail may be bent back underneath the body.

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.

Data processing and analysis

- Data editing: To detect errors and to make sure of accuracy of data either they were uniformed and well arranged, the data collected were edited as soon as possible.
- Coding: For the easy classification and tabulation of data, information was coded.

- 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 were analyzed by means of table bar diagram and pie chart.

Validity and reliability of the study:

- 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.

V

RESULTS

All together 521 blood samples were collected from the Benighat VDC. The blood samples were collected from the respondents above 2 years by using cross-sectional sampling method. The collected samples were subjected for microscopic examination to detect the human filarial infection. The study was divided into questionnaire survey and microscopic examination of blood samples. The demographical characteristics of study population were studied to know the background of respondents in the study area.

- **General Prevalence of Microfilaria**

All together 521 blood samples were collected from the Benighat VDC among the filarial suspected and unsuspected people. Among 521 samples, 4 (0.76%) were found to be infected with microfilaria (Table-2).

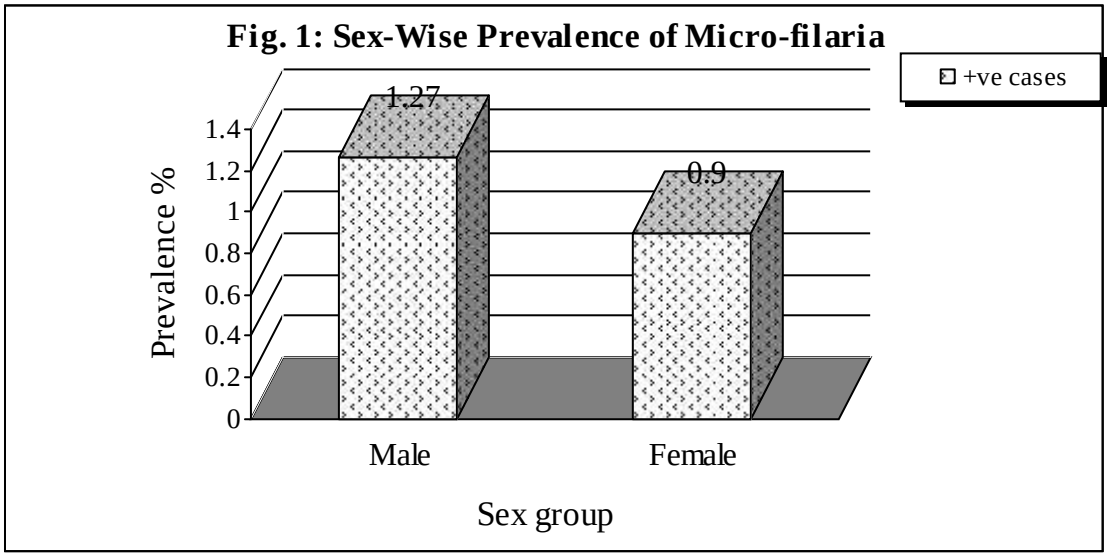
- **Sex-Wise Prevalence of Microfilaria**

Out of 521 blood samples, 237 (45.49%) were males and 284 (54.51%) were females. Among 237 male respondents, the maximum rate of infection was found to be 3 (1.27%). The minimum rate of infection was found among the female respondents which was 1 (0.90%) (Table-2, Fig-1).

Statistically there was significant difference of filarial infection between different sex groups (χ^2=7.82, P<0.05).

Table 2: Sex Wise Prevalence of Microfilaria

S.No.	Sexes	Number of Respondents (%)	Positive cases	
			No.	%
1	Males	237 (45.49)	3	1.27
2	Females	284 (54.51)	1	0.90
	Total	521 (100)	4	0.76

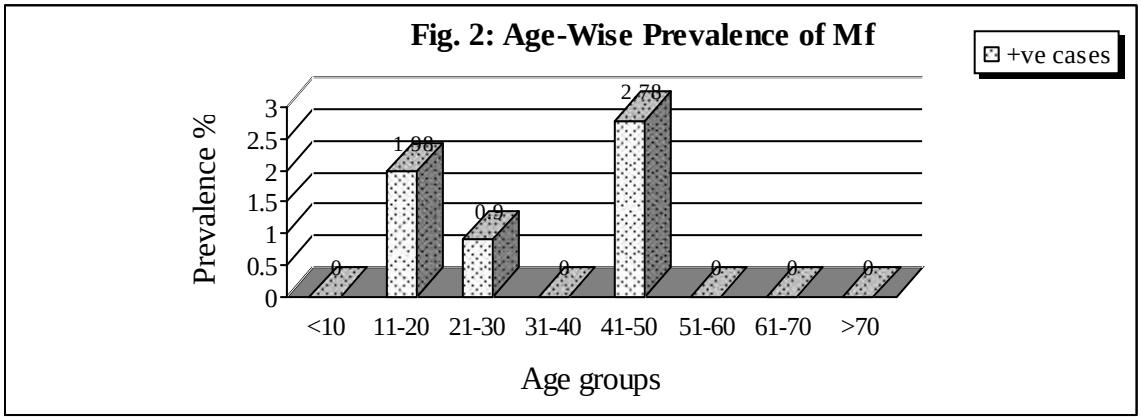


- **Age Wise Prevalence of Microfilaria**

Out of 521 total samples, the highest number 128 blood samples were examined from the age group below 10 years and less number 13 were examined from the age group above 70 years, none of which were found to be Mf positive. The highest rate of infection was found in the age group 41-50 years. Out of 36 samples from this age group, 1 (2.78%) was found to be Mf Positive. The minimum rate of infection 1 (0.90%) out of 110 samples was found to be Mf positive in the age group 21-30 years. Statistically there was significant difference of infection in different age groups (χ^2=6, P<0.05) (Table-3, Fig-2).

Table 3: Age Wise Prevalence of Microfilaria

S.No.	Age groups	Total Samples Examined	Positive cases	
			No.	%
1	<10	128	00	0.00
2	11-20	101	02	1.98
3	21-30	110	01	0.90
4	31-40	65	00	0.00
5	41-50	36	01	2.78
6	51-60	47	00	0.00
7	61-70	21	00	0.00
8	>70	13	00	0.00
	Total	521	04	0.76

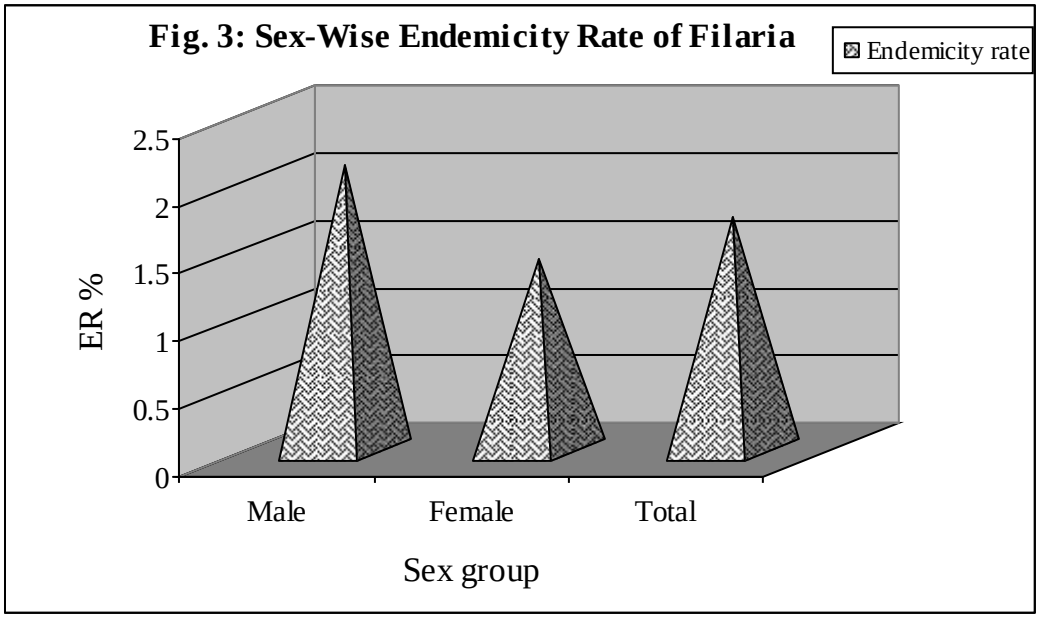


Sex wise endemicity rate of Filaria

The study showed that the total ER was 9 (1.73%). The highest ER 5 (2.11%) was found among the 237 males. The ER among the 284 females was 4 (1.41%). Statistically there was significant difference of infection in both sexes (χ^2=7.82, P<0.05) (Table-4, Fig-3).

Table 4: Sex wise endemicity rate of Filaria

S.No.	Sexes	Total number of respondents	MF	CDR	Endemicity Rate (ER)	
					No.	%
1	Male	237	3	2	5	2.11
2	Female	284	1	3	4	1.41
	Total	521	4	5	9	1.73

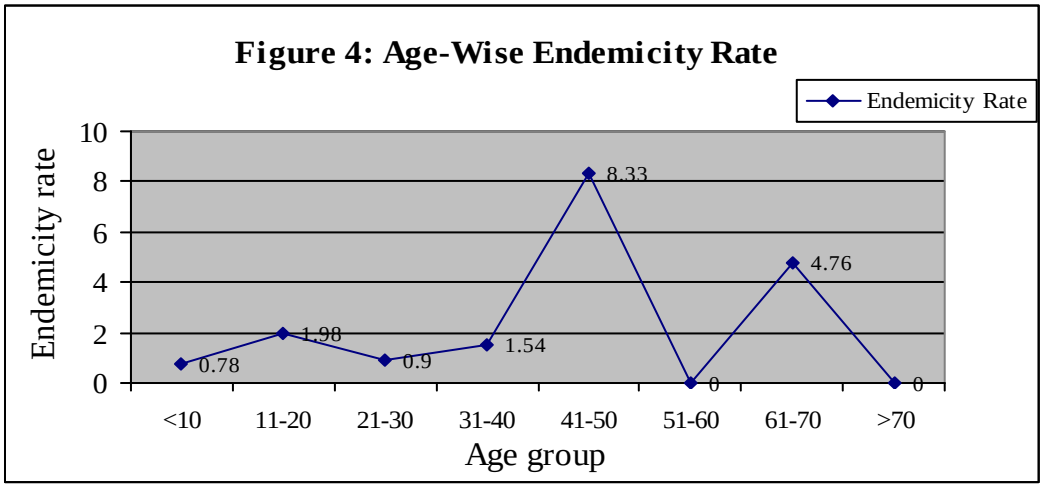


- **Age wise endemicity rate of Filaria**

Table 5 shows the age wise endemicity rate of filaria among the respondents from Benighat VDC of Dhading. The highest endemicity rate was found in the age group 41-50 years. Out of 36 respondents from the age group 41-50 years, 3 (8.33%) were recorded as filaria positive. The lowest endemicity rate was found in the age group below 10 years which was 1 (0.78%) among 128 respondents. Statistically there was significant difference of infection between the different age group (χ^2=12.66, P<0.05) (Fig-4).

Table 5: Age wise endemicity rate of filaria

S.No.	Age groups	Total samples	Mf	CDR	MF +CDR	ER	ER%
1	<10	128	0	1	00	1	0.78
2	11-20	101	2	0	00	2	1.98
3	21-30	110	1	0	00	1	0.90
4	31-40	65	0	1	00	1	1.54
5	41-50	36	1	2	00	3	8.33
6	51-60	47	0	0	00	0	0.00
7	61-70	21	0	1	00	1	4.76
8	>70	13	0	0	00	0	0.00
	Total	521	4	5	00	9	1.72

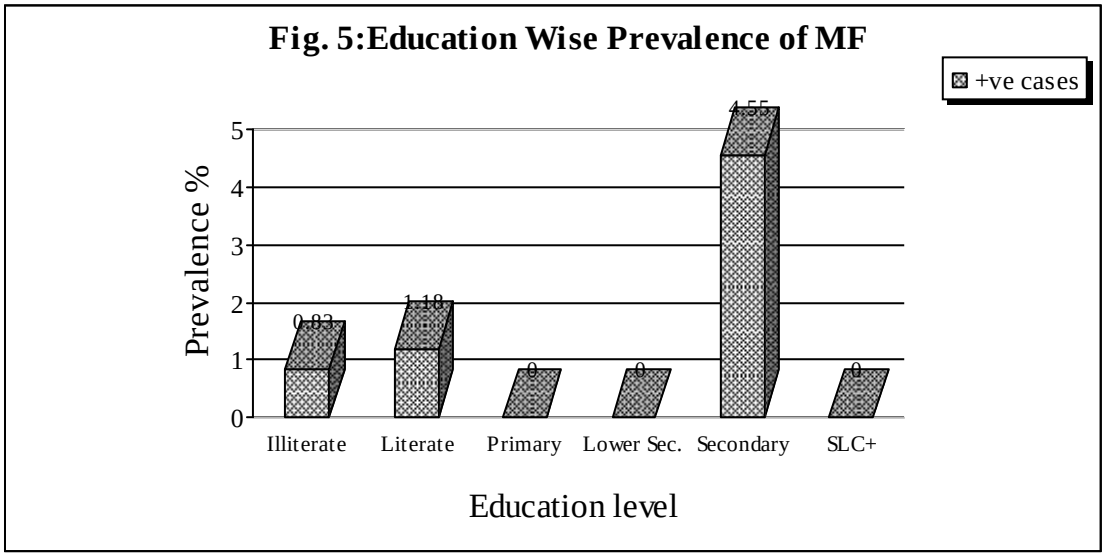


- **Education wise distribution of Microfilaria**

The study showed that total number of 4 (0.76%) were Mf positive among 521 respondents. Maximum rate 4.55% of infection was found among the respondents with secondary level education (out of 44, 1 case +ve) and minimum rate 0.83% of infection was found among the illiterate respondents (out of 121, 1 case +ve). Statistically there was significant difference of infection between different educational levels (χ^2=10.76, P<0.05) (Table-6, Fig-5).

Table 6: Education wise distribution of Microfilaria

S.NO.	Education level	Total Samples Examined	Positive cases	
			No.	%
1	Illiterate	121	1	0.83
2	Literate	85	1	1.18
3	Primary	162	0	00
4	Lower Sec.	55	0	00
5	Secondary	44	2	4.55
6	SLC+	54	0	00
	Total	521	4	0.76

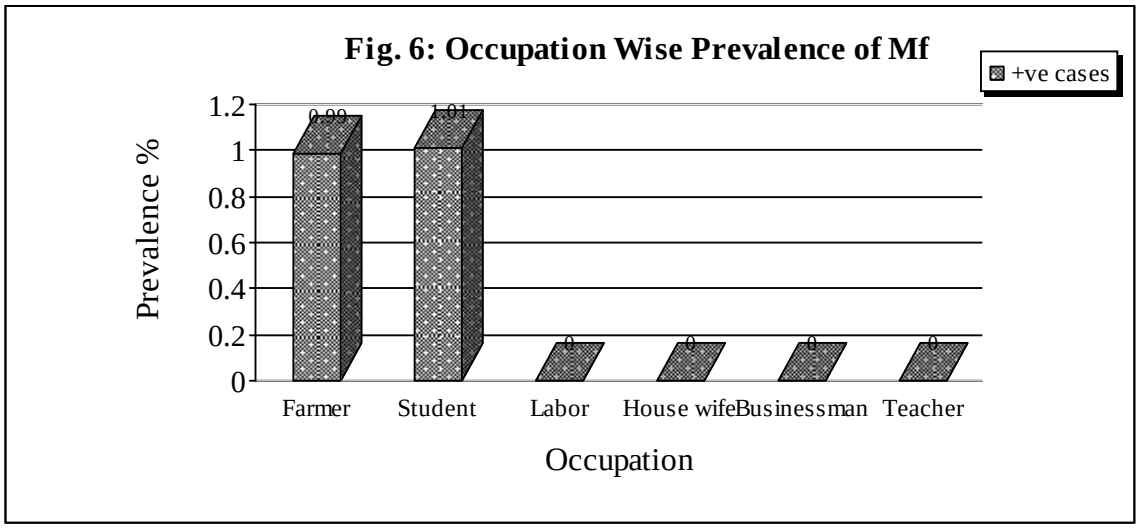


Occupation wise distribution of Mf

Out of total samples 521, the maximum number 201 of respondents were farmers and the lowest number 4 of respondents were teachers. Among the 201 farmers, 2 (0.99%) were found to be infected. Among 198 students, 2 (1.01%) were infected with Mf. None of the respondents from other occupational group were infected with Mf. Statistically there was significant difference of infection between different occupational status (χ^2=1.79, P<0.05) (Table-7, Fig-6).

Table 7: Occupation wise distribution of Mf

S.No.	Occupation	Total Samples Examined	Positive cases	
			No.	%
1	Farmer	201	2	0.99
2	Student	198	2	1.01
3	Labor	45	0	00
4	House wife	35	0	00
5	Businessman	38	0	00
6	Teacher	04	0	00
	Total	521	4	0.76



- **Prevalence of filariasis in relation to the use of mosquito net**

Out of 521 samples, 479 (91.94%) were found to use mosquito net and 42 (8.06%) did not use mosquito net. Among 479 respondents that used mosquito net, 4 (0.83%) were found to be infected with filaria. None of respondents who did not use mosquito net were found to be infected with filaria (Table-8).

Table 8: Prevalence of filariasis in relation to the use of mosquito net

S.N.	Use of mosquito net	Total No. of samples (%)	Mf +ve cases	
			No.	%
1	Yes	479 (91.94)	04	0.83
2	No	42 (8.06)	00	00
	Total	521 (100)	04	0.76

- **Prevalence of filariasis in relation to the knowledge about the Lf**

Out of 521 samples, 79 (15.16%) had knowledge about Lf and 442 (84.84%) did not have knowledge. Among 442 respondents without knowledge about Lf, 4 (0.90%) were found to be Mf positive. Out of 79 respondents having knowledge about Lf, none of them were Mf positive (Table 9).

Table 9: Prevalence of filariasis in relation to the knowledge about the Lf

S.N.	Knowledge	Total No. of samples (%)	Mf +ve cases	
			No.	%
1	Yes	79 (15.16)	00	00
2	No	442 (84.84)	04	0.90
	Total	521 (100)	04	0.76

Sex wise distribution of signs and symptoms of Lymphatic filariasis

Out of 521 samples, 5 (males 2 and females 3) were with sign and symptoms of Lf. The sign and symptoms were hydrocele, lymphoedema and swollen vessels. The total crude disease rate was recorded to be 5 (0.96%) with 2 (0.84%) males and 3 (1.07%) females. Among 237 male respondents, 1 (0.42%) was infected with hydrocele and 1 (0.42%) was infected with lymphoedema. Among 284 female respondents, 2 (0.70%) were infected with lymphoedema and 1 (0.35%) was infected with infected with swollen vessels (Table-10, Fig-7).

Table 10: Sex wise distribution of signs and symptoms of Lymphatic filariasis

S.N.	Sex	Total sample	Signs and symptoms of Lf			CDR	
			Hydrocele No. (%)	Lymphoedema No. (%)	Swollen vessel No. (%)	No.	%
1	Male	237	1 (0.42)	1 (0.42)	0	2	0.84
2	Female	284	0	2 (0.70)	1 (0.35)	3	1.07
	Total	521	1 (0.19)	3 (0.76)	1 (0.19)	5	0.96

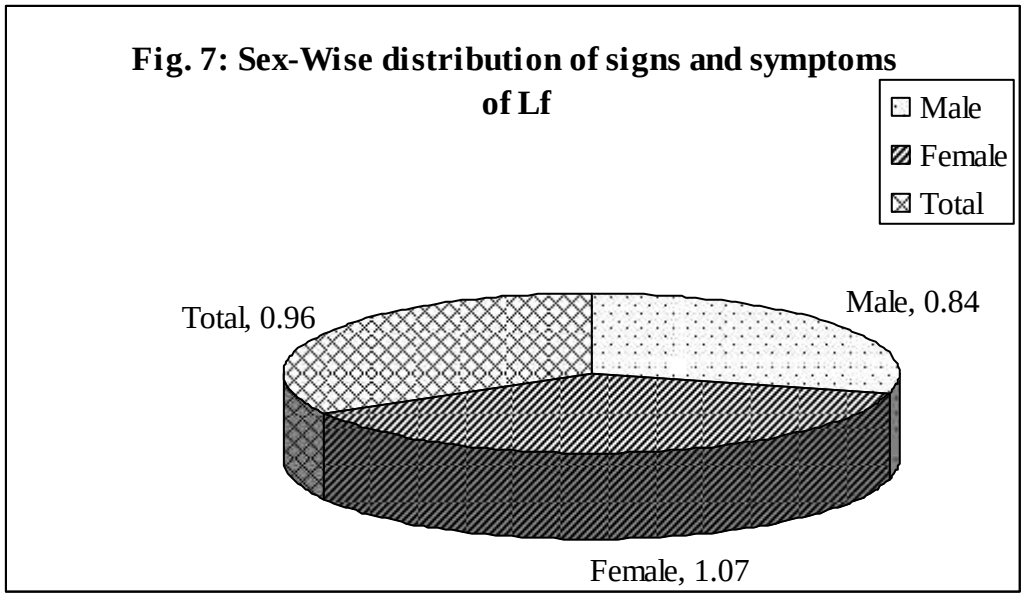


Fig. 7: Sex-Wise distribution of signs and symptoms of Lf

VI

DISCUSSION AND CONCLUSION

Lymphatic filariasis, the disabling-disfiguring parasitic disease has been identified as a major health problem with an increasing prevalence world wide. It is a major socio-economical burden globally in tropical and sub-tropical areas. At present, 1.1 billion 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 *Wuchereria bancrofti* and Brugia malayi (WHO, 2000). Filariasis has been known to be endemic in Nepal since long time (EDCD, 2005). It has been reported from different areas of Nepal. The present surveillance conducted in Benighat VDC of Dhading district shows the endemicity rate of 1.73% with overall microfilariaemia of 0.76% and crude disease of 0.96%. Jung (1973) gave first report from the 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) in the 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 population. Manandhar (2001) reported 19.9% of crude disease from Sipwa,

Dhovan 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 comparision to the endemicity rate, microfilariaemia and crude desease. Sherchand *et al*., (2000) reported 13% prevalence of microfilariaemia.

Among 4 positive microfilarial cases, 3/237 (1.27%) were males and 1/284 (0.35%) were females. Males and females were infected in the ratio of 3:1, i.e. males were thrice more susceptible to microfilaria when compared with females. This is supported by Weerasooriya *et al*., (2001) Sri Lanka. This may be due to the fact that males usually sleep outdoor without using mosquito net during summer and also due to their dressing style, only vest and pant that leads to maximum biting by mosquito in the exposed parts of the body. While females are less susceptible to mosquito biting as they along with their children sleep indoor using mosquito net.

All age groups are susceptible to filariasis. The present study showed high rate of infection in the age group 41-50 years i.e.1/36 (2.78%) while least in the age group 21-30 years i.e. (0.90%). The highest prevalence of microfilaria in the age group 41-50 years may be because of high exposure towards outer environment, lack of awareness and carelessness towards using nets, about health and hygiene. Age wise distribution of filariasis is equivalent to the length of exposure, which is supported by WHO (2001).

The maximum prevalence rate was reported in the children with the education upto secondary level i.e. 4.55% (2/44) while least in the illiterate i.e. 0.83%

(1/121). The maximum prevalence rate of filaria in the children with education upto secondary level may be due to lack of awareness about the vector borne diseases and mode of transmission of the diseases. Most of people i.e. 442/521 (84.84%) were unaware about the disease and only 79/521 (15.16%) were aware about the disease.

Occupation seems to play a major role in the infection of disease. The prevalence rate of filaria among the farmers was found to be 0.99% (2/201) and 2/198 (1.01%) positive cases of filariasis were reported among students. The infection in the farmers may be due to maximum exposure to the outer environment. They had to work in the bushy areas which are the suitable breeding sites of the mosquitoes. The farmers also reared cattle near their settlements. They use their own house as the cattle shed. They also construct the cattle sheds close to their houses which provide the favourable condition for the mosquito breeding.

RECOMMENDATIONS

After conducting the study in Benighat VDC of Dhading district, following recommendations are put forward in order to minimize the filarial infection.

- Most of the people are still unknown about lymphatic filariasis. Thus to make them familiar to Lf, awareness programs must be organized through mass media, radio, television, posters, pamphlets etc and also by conducting household awareness, group awareness programs for protecting people from vector borne lymphatic filariasis and to improve health and hygiene. Thus first priority should be given to spread awareness among the people.
- People must be made conscious to use mosquito-nets, mosquito coils, mosquito mats, fumigants and ointment for protecting from mosquito bites.
- Mass drug administration and control program must be regularized along with the monitoring of the same study population of the site throughout the elimination program in order to assess the success of the program.
- Public health education must be included from primary level education and also the teachers should be trained about the control of filariasis.
- Concerned authority should make strict rules for introducing larvicides and insecticides or filling up the ditches and pits which are the suitable sites for breeding of mosquitoes.
- Regular health check up is needed in the study area. If someone is infected with microfilaria, he/she should be immediately treated.

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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 12^{o}c, 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^{o}C or up to 2hours at 37^{o}C 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^{o}C.

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

Cutopolasm 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

Precaution 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 lead 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 Benighat VDC of Dhading District of Nepal

Date:
S.N……

1) Name of the respondent : ……..
Address : District:…….
Ward No./Tole/ Block No.……..

2) Age/sex…….

3) Education:
1. Literate☐ 2.Illiterate☐
If literate,
1. Primary. ☐ 2. Lower Sec. ☐ 3.Secondary ☐
4.S.L.C.☐ 5. Intermediate ☐ 6.Bachelor ☐
7.Master☐ 8.Others☐

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

5) Marital status:
1.Single/Married 2.Widow/Widower/Divorce

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

7) Respondent's current residence status:
1.Birth place ☐ 2.Migrate☐ 3.Temporary☐
(How long have you been staying here?)…….years/months

8) Surrounding environmental condition:
1. Clean ☐ 2.Dirty☐ 3.Bushy ☐ 4.Open Drainage☐

9) Use of the any means for the protection against mosquito bite:
1. Yes ☐ 2. No☐
If yes, which one of the following:
1. Mosquito net ☐
2. Anti mosquito cream☐
3. Smoke☐
4. Spraying insecticides☐
5. By burning mosquito coils☐

10) Do you have knowledge about the disease filariasis(elephantiasis)?
1.Yes☐ 2.No☐

If yes, how is it transmitted?
1.By mosquito biting☐ 2. Contact with disease person☐
3.Mother to foetus☐

11) Respondent current health status:
1.Healthy☐ 2.Unhealthy☐
If unhealthy since when……..yrs ……months

12) Do you have any symptoms?
1.Yes☐ 2.No☐
If yes, which of the following
1.Fever ☐ 2.Effect on genital organ or breast ☐ 3.Headache☐
4.Swollen Lymphnode☐ 5.Hydrocele☐ 6.Swollen hand or limb☐
7. Skin thick, red and swollen blood vessels☐ 8.Chyluria☐
9.Abscess Formation☐ 10.Nausea☐ 11.Epigastric Pain☐
12.Weakness ☐ 13. Lazyness☐
If yes, have you used any medicine?
1.Yes☐ 2.No☐
If yes, which of the following?
1.Ayurvedic☐ 2.Allopathic ☐
3.Herbal☐

13)According to your knowledge is the disease is more in parent's time or now?
1.Parent's Time☐ 2.Now ☐ 3.Don't know☐

14) Have you seen any person suffering from this disease?
1.Yes☐ 2.No☐
If yes, how many…….

15) Is there any person suffering from this disease in your family or relatives?
1.Yes ☐ 2.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-III

Diagrammatic representation of the life cycle of *Wuchereria bancrofti*

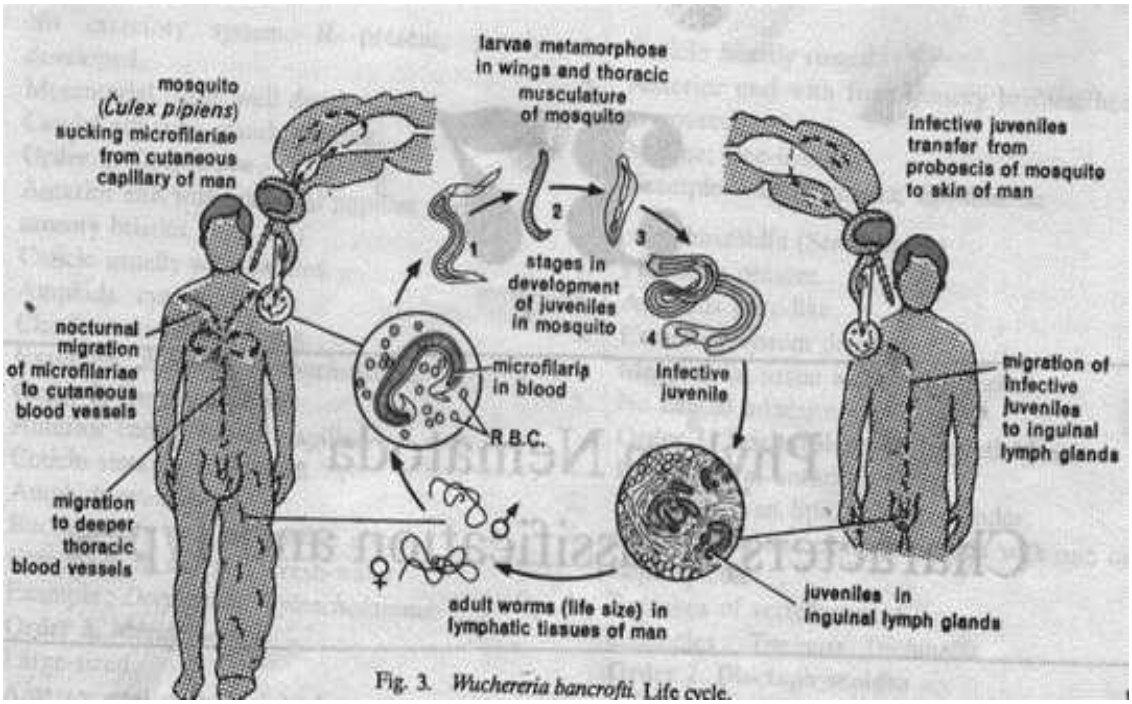

Fig. 3. *Wuchereria bancrofti*. Life cycle.