I

INTRODUCTION

Lymphatic filariasis is a parasitic disease which causes obstruction to flow of lymphatic fluid and swelling of the infected area. It is one of the most prevalent tropical disease caused by nematodes, inhabiting the lymphatic. Lymphatic filariasis, also known as elephantiasis is a dramatically disabling, disfiguring disease usually affecting one or both legs or causing hydrocele and equally grotesque enlargement of the male scrotum. Infection also causes acute fever, inflammation of the lymphatic system and the bronchial – asthmatic condition known as “tropical pulmonary eosinophilia” (WHO Geneva, 1995).

The nematodes of the superfamily “Filarioidea” are generally referred to as the filarial worms or simply filariae. These include some of the most important nematode parasites of human and domestic animals from the medical and veterinary view points. An infection caused by any of parasitic filariae is known as “filariasis”. There are eight main species of filarial nematode infecting human beings. They are, *Wuchereria bancrofti, Brugia malayi, Brugia timori, Onchocerca volvulus, Loa loa, Mansonella perstans, Mansonella streptocerca* and *Mansonella ozzardi*. Diptera vectors transmit all these (Cheng, 1986).

Lymphatic filariasis first of all do not show any sign and symptom, until the adult worm die. The disease can permanently damage

lymph system and kidneys, but is usually not life threatening. Because lymph system does not work properly as nematodes block the lymph vessel, fluid is collected and swelling is caused in the arms, legs, breasts, and in man the genital area. The swelling is known as lymphoedema. The entire leg, arm or genital area may swell to several times its normal size, hardening and thickening of the skin takes place, which is now called elephantiasis.

Acute manifestation of lymphatic filariasis involves episodic attacks of adenolymphangitic (inflammed lymph nodes) associated with the fever and malaise. Each attack may incapacitate the patient for several days. Such attacks are significant cause of morbidity. Other complications may include chyluria (milky urine), which is painless but results in weight loss and lethargy, and tropical pulmonary eosinophilia (asthma and cough), which result in chronic pulmonary fibrosis.

The disease is global in its distribution, lymphatic filariasis is a major global and socio economical burden in the tropics and subtropics. About 1.1 billion people (20% of the world's population) in some 80 endemic countries located in tropical areas of the world are at risk of infection by *Wuchereria bancrofti* and *Brugia malayi.* About one – third of population with this infection live in India, Africa and South – East Asian countries (WHO, 1997).

The problem of lymphatic filariasis is found to be grave in India, Indonesia and China. These three countries together account for more

than two third of the total infected population. India alone accounts for around 50 percent of the total cases occur world wide. In India most of the cases are due to bancroftian filariasis (Ghai and Gupta, 1999).

Although bancroftian filariasis is rarely life threatening it causes much suffering and disability. It represents a significant impediment to economic development, as a result of lost working hours, and the costs of treating the sick and controlling the vectors of disease (Rozendaal, 1999).

In most of the world's endemic areas there is no effective filariasis control. This is mainly because filariasis has not been regarded as a public health priority, and because most control strategies are too complicated and expensive to be sustained. In the past, parasite control and transmission control have eradicated filariasis in several endemic areas. However eradication will be very difficult to achieve in most places. 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).

Outward manifestation of Bancroftian filariasis, such as lymphoedema occur in a relatively small proportion of infected people in filariasis - endemic areas, and usually several years after infection with the parasite. Thus infection status is largely "hidden". Because of the hidden nature of the disease (Dreyer, 1997), its magnitude and public

health impact are often not recognized by government officials, even in areas of intense transmission.

Nepal is a small country mostly covered with hills. The distribution of lymphatic filariasis is varied according to topographic areas ranging in altitude from 70m. in terai ecological zone to 1400m. in the high hill areas. The disease is more prevalent in the terai areas than in the hills. The distribution and magnitude of the disease is gradually increasing in rural areas as well (DoHS, MoH, HMG, Nepal, 2004).

Lymphatic filariasis is a major public health problem in Nepal. The disease is a major cause of morbidity, primarily lymphoedema of legs and hydrocele. It impedes socioeconomic development in many endemic areas of Nepal. It is a disease of poverty, affecting the poorest of the poor and usually contracted in childhood often before the age of five and can lead to lifetime disability.

Out of total population of Nepal (23.2 million), approx. 60% (13.9 million) are estimated at risk of filariasis. Out of three species (*Wuchereria bancrofti, Brugia malayi, Brugia timori*) of lymphatic filarial parasites, only one species i.e. *Wuchereria bancrofti* has been reported in Nepal (Thakur, 2000). Sherchand *et al.,* (2002) studied mapping of lymphatic filariasis in Nepal, the average of 13% people were found to be infected on the basis of 37 districts. The analysis of HMIS (Health Management Information System) data of last five years (2000/01 to

2004/05) of lymphatic filariasis cases recorded and reported from various levels of government health institutions and/or epidemiological sample survey carried out in 37 districts in 2001 by ICT (Immuno chromatography) card test method revealed that 50 out of total 75 districts of the country have been identified as endemic to LF (Red areas).

In 1993 the "International Task Force for Disease Eradication" identified lymphatic filariasis as one of only six diseases meeting the criteria for being eradicable or potentially eradicable. Since that time, efforts towards this goal have moved ahead rapidly. Recognizing that this disease of disability was a major health drain on the economics, wellbeing and development of the 80 mostly poor nations where lymphatic filariasis remained endemic and that tools were available that could eliminate it, the World Health Assembly passed a resolution in 1997 proposing as a public health goal the global elimination of lymphatic filariasis. The principal strategy for achieving this goal was to prevent transmission of this mosquito – borne parasite by eliminating the infectious microfilariae from the blood of affected humans through one – yearly, single – dose treatment with a combination of two drugs (chosen from among DEC, albendazole and ivermectin) of entire population at risk for lymphatic filariasis. Such treatment should continue for four to six years, the estimated reproductive life span of the adult stage parasite (Ottesen, 2002).

National programme were active in 38 of the 80 countries where LF is endemic, reaching almost 90 million people, in 2002. The expansion required to meet the targets of reaching 350 million people by 2005 and all 1.1 billion at risk globally by 2020 is now the major challenge of what has become the global program to eliminate lymphatic filariasis, coordinated by the WHO and supported by a global alliance of partners (Ottesen, 2002). For any country to be successful in eliminating lymphatic filariasis from its territory, the first epidemiologic approach to be adopted is to assess the current magnitude and geographic distribution of the disease.

With WHO's global strategy to eliminate lymphatic filariasis as a public health problem and governments political commitment, Epidemiology and Disease Control Division, Department of Health Services, Ministry of Health has formulated a National Plan of Action (2003 – 2015 AD) for elimination of lymphatic filariasis in Nepal. To meet this target national project has been conducted in districts of Nepal. Present study was also a part of that project.

II

OBJECTIVES

General objectives

- To determine the prevalence of Bancroftian filariasis and situation of mf by means of cross-sectional survey in Maharajganj VDC, Kapilbastu District of Nepal.

Specific objectives

- To determine the prevalence of microfilariae in the people of survey area.
- To study the knowledge, attitude and practices among different people towards filariasis.
- To study the filarial situation in relation to education and occupation of the people.
- To determine age – wise and sex – wise filarial infection.
- To study the Endemicity Rate of lymphatic filariasis.

III

LITERATURE REVIEW

Ancient Filariasis:

The symptoms of Bancroftian filariasis has been mentioned as "elephantiasis arabicum" in the ancient Hindu literature, viz., Sushatha (600BC). The term "Malabar leg" was applied to the condition by Clark in Cochin in 1709AD. Our present knowledge of filariasis owes much to the investigations carried out towards the end of the nineteenth and the beginning of the twentieth centuries. Microfilariae (the 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 a patient with haematochyluria. Lewis (1872) in India demonstrated the same in the peripheral blood. Adult worms were found by Bancroft in Brisbane in 1876 and named *Filaria bancrofti* by Cobbold in 1877. This species was included in the genus *Wuchereria* in 1921. (Arora and Arora, 2001).

Sir Patric Manson, working in China in 1878, observed the development of *W. bancrofti* in the mosquito *Culex quinquefasciatus* and established the essential role of the vector. This was the first demonstration that female *Culex* mosquito could harbour an infective agent of a parasite. A year earlier he confirmed the hypothesis that this nematode was the

cause of elephantiasis (Cheng, 1986). In 1881 he described nocturnal periodicity of *W. bancrofti,* the microfilariae present in greatest numbers in the peripheral blood during night hours.

Recent Data on Filariasis in Global Context:

Dixit *et al.,* (2005) studied the temporal dynamics of microfilariae (mf) in human blood, and the biting activity and mf density in the vector. It was found that the biting periodicity appeared to be much more stable in all subjects; the peak appeared at 02:37 with a range between 00:39 and 03:22. The peak in mf frequency was earlier by 3 hours and 25 minutes, whereas the peak of mf frequency in the vector was later by about 2 hours 4 minutes. The observed synchronization between the rhythms of vector and host appears to be essential for effective transmission of diseases.

Hornberger *et al.,* (2005) studied on idiopathic scrotal elephantiasis. Scrotal lymphoedema is a condition that has historically been described in areas endemic to filariasis. An unique case of a 22 year – old man with idiopathic lymphoedema isolated to the scrotum was presented. After acquired causes of lymphoedema were ruled out, the patient was treated with scrotectomy and scrotal reconstruction.

Simonsen *et al.,* (2005) observed on false positive reactions in the rapid Now ® Filariasis card test. Survey was carried out in an endemic Tanzanian village. It was observed that individuals who were positive in

Now ® Filariasis test at 10 min after specimen application were also positive in the Trop Bio ELISA for CFA (circulating filarial antigen), and thus appeared to be truly positive. Many of the test cards that were negative at 10 min developed a positive line later, but these lines appeared to be falsely positive when the Trop Bio test was used as a gold standard. Close examination revealed that true and false positivity lines could be distinguished on their shape and colour.

Mathieu *et al.,* (2005) conducted an intense health – education campaign followed by a mass drug administration (MDA) with diethylcarbamazine and albendazole in Leogane, Haiti. Questionnaire based interviews were used to explore the knowledge – attitude - practice (KAP) of 304 subjects. The primary reasons given for failing to take the drugs were absenteeism during the distribution (17%), use of contraceptive drugs (12%) and pregnancy (11%). In a multivariate analysis, being male, knowing that a mosquito transmits the disease, and having learned about the MDA through posters and banners were found to be positively associated with taking the drugs.

Khan *et al*., (2005) showed a significant correlation between DNA damage/suppressed cell – proliferative response and the chronicity of *Brugia malayi* infection, and this may reflect on the development of hypo responsiveness.

Dixit *et al.,* (2004) reported the population dynamics of *Culex quinquefasciatus* filaria vector in Raipur city of Chattisgarh state. The

vector was recorded throughout the year with higher densities during the months of March (44.29 pmh), February (41.29 pmh), August (38.58 pmh) and April (37.17 pmh). The lower densities were recorded during July (17.05 pmh), September (16.82 pmh) and November (16.64 pmh). The Gudhyari and Amanaka localities recorded the highest and lowest densities of *Culex quinquefasciatus* respectively.

Sizwitch Wongkamchai and Loymek *et al*., (2004) studied on impact of filariasis control programme on intestinal helminthes infections in Narathiwat Thiland. Study was conducted in 539 villagers of 9 villages endemic for Brugian filariasis. It was 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. A significant reduction of intestinal helminthes infection in the post treatment stool sample was observed in 150 patients who were examined six month after mass treatment.

Gyapong *et al.,* (2003) conducted study on the geographical distribution of human infection with *W. bancrofti* was investigated in four West American countries (Benin, Burkina Faso, Ghana and Togo) using a commercial immuno chromatographic test for filarial antigen. Study was carried out in 401 randomly selected communities. The results revealed that prevalence in the adult population of some communities exceeded 70% and that, over large areas of Burkina Faso, community prevalence were between 30% and 50%. Most of Togo, Southern Benin and much

of Southern Ghana appeared completely free of the infection. Although there were foci on the Ghanian coast with prevalence of 10% - 30%, such high prevalence did not extend into coastal Togo or coastal Benin.

Pacella *et al.,* (2003) conducted a study on a 23 – year old man immigrated from Sri Lanka, for an acute painful volume increase of the right scrotum without fever. Clinical diagnosis reveals an inflammatory spermatic cord and epididymis with a purple nodule of the middle portion. The nodule was excised and sent to pathologist that diagnosed a filarial infection.

Weerasooriya *et al.,* (2003) carried out an enzyme – linked immuno sorbent assay (ELISA) to detect filaria – specific urinary IgG4 was tested in samples from 203 children less than five years old and their parents (165 mothers and 127 fathers) in Sri Lanka. There were four IgG4 positive children within 58 days after birth, suggesting the transfer of the antibody from mothers. No positive children were found between days 65 and 417. After day 1000, the number of the positive individuals and the level of IgG4 increased quickly. The children of urinary IgG4 – positive parents showed a higher IgG4 positive rate that those of negative parents. The children of positive mothers had a higher prevalence than those of negative mothers, whereas the positivity of fathers was not associated with that of their children.

Tobian *et al.,* (2003) studied on risk factors for hydrocele as a consequence of *Wuchereria bancrofti* infection, 342 men more than 15

years of age in an endemic area in Papua New Guinea were evaluated. These observations suggest that filarial pathology of the male genitalia is under-reported when evaluated by physical examination alone and that duration and intensity of infection are risk factors for hydrocele..

Schuetz *et al*., (2001) studied on evaluation of the whole blood filariasis ICT test for short term monitoring after antifilarial treatment. It was filariasis test, a rapid screening tools that will be useful for defining the prevalence and distribution of *W. bancrofti* as a part of the global programme to eliminate lymphatic filariasis. The results demonstrated that antigens level persists in microfilarial negative persons for up to three years after treatment. Different strategies for monitoring control programme may have to be considered.

Weerasoriya *et al.,* (2001) reported 4.4% of prevalence of microfilariaemia 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). The prevalence of elephantiasis was generally higher among females (4.2%) than among males (1.4%). This manifestation showed a linear increase in prevalence after the age of 40 years.

Witt *et al.,* (2001) reported that Lf is acquired in childhood often with as many as one third of children were infected before 5 years of age.

Initial damage to lymphatic system by the parasites generally remains sub clinical for many years or gives rise only to non specific presentations of lymphadenopathy, however especially after puberty the characteristic clinical features of adult disease syndrome (lymphoedema, hydrocele) manifest themselves.

Massaga *et al.,* (2000) reported the prevalence of *W. bancrofti* in 31.8% of 1025 inhabitants (32.1% of female and 31.5% of male). In Hale area in Northeast Tanzania, clinically 6.9% of examined individuals had elephantiasis and 28.5% males aged 15 years and above had hydrocele. Both clinical manifestations and microfilaria prevalence increased with age.

Meyrowitsch *et al.,* (1998) carried out a survey on 13500 individuals in 24 provinces of Vietnam. The highest prevalence of microfilariaemia were observed in low land i.e. 0.9 – 5.5%. The most common type of chronic clinical manifestation was shown to be leg elephantiasis.

WHO (1997) in India: Filariasis is major public health problem next only to malaria. As percent estimates, about 428 million people with 28 million mf carriers and 21 million clinical cases were spread in 13 states 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 *B. malayi* was confined to the western coast of Kerala and a few pockets in six other states. Both the infections were

nocturnally periodic. In the Nicobar group of Island, diurnally sub – periodic infection transmitted by *Aedes (Finlaya) niveus* group was detected about three decades back.

WHO (1997) carried out a study in Northern Ghana in a rural community where filariasis disease is highly endemic (41% of the population age over 10 years is microfilarimic with *W. bancrofti* and 3% has chronic disease), showed that LF can be a major social and economic burden on poor communities and that the disability and indirect economic loss associated with adenolymphangitis (ADL). Another study carried out in a 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 control.

Filariasis in Nepal:

Ghimire *et al.*, (2003) conducted a study on prevalence of lymphatic filariasis in Mahendranagar and Nagrain VDCs of Dhanusha district in the Terai plain region of Nepal. The prevalence of microfilariaemia in Mahendranagar was higher than Nagrain VDC. A total of 1085 finger-prick thick blood smear samples were collected, 468 from Mahendranagar and 617 from Nagrain VDC, from 22:00 – 2:00 hr. 25/468 (5.3%) of Mahendranagar and 14/617 (2.3%) from Nagrain VDC were found positive for *W. bancrofti*. Although the participation of both sexes are almost equal, the prevalence was found to be higher in females.

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 above 15 years of age group was selected and the immuno chromatographic test (ICT – filariasis) was used to screen for circulating filarial antigens (CFA). The prevalence of lymphatic filariasis from 500 sample population was 141 infected with larvae of *Wuchereria bancrofti* were found from the genus *Culex quinquefasciatus*.

Manandhar (2001) conducted an epidemiological study of microfilaria in three different 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*., (2000) studied the prevalence of *W. bancrofti* infections in Tokha – Chandeshwori VDC of Kathmandu in 1998. A survey of 978 nocturnal blood samples collected in the VDC indicated an overall prevalence of 5.8% for microfilariaemia and the crude disease rate of *W. bancrofti* was recorded to be 13% in the study area. The highest microfilariaemia infection rate was recorded 36.4% in the age group of 70 and above.

Pradhan *et al*., (1997) reported 24.6% endemicity rate with the overall, 12.75% microfilarial infection (15.09% in male and 8.9% in female) and 11.95% crude disease rate (8.49% in male and 16.59% in female) of *Wuchereria bancrofti* in Gokarna VDC of Kathmandu valley and

identified 12 species of mosquitoes *(Anopheles nigerrimus, Anopheles vagus, Anopheles willmori, Anopheles kessele, Culex fescocephella, Culex gelidus, Culex pseudovishnui, Culex whitmori, and Culex tritaeniorhynchus)* from the study area. Among these species *Culex quinquefasciatus* was found to be more prominent.

Jung, (1973) studied all together 9 sites, which showed 4.99 to 6.15% crude disease rate in all the age groups and both the sexes in the urban population, 6.6 to 10.3% in the semi – urban population and 1.2 to 17.8% in the rural population. Similarly 7.1 to 9.16% microfilariae rate was found in the urban population, 10.03 to 11.3% in the semi – urban population and 0.8 to 17.69% in the rural population.

The following table gives an outlook of national and region wise lymphatic filariasis situation based on Annual Reports, DoHS, MoH, and HMG, Nepal from 1995/96 to 2004/05.

Table I: National and Region - wise Number of Filarial cases. Nepal FY 1995/96 to 2004/05.

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

According to Annual report, DoHS, MOH, and HMG, Nepal, 2004/05, the number of lymphatic filariasis cases reported in Mountain Region was 4, Hilly Region was 192 and in Terai Region was 350. This data reveals that highest prevalence of LF was in Terai Region.

OPD cases of Filariasis patients in Kapilbastu district of Nepal was reported 487 in 1995/96, it was 386 in 2000/01 and 142 in 2004/05 (Annual Report, DoHS, MOH, HMG, Nepal).

IV

MATERIALS AND METHOD

STUDY AREA:

Nepal is a small country. It is located from 80°4’ to 88°12’ East longitude and from 26°22’ to 30°27’ North latitude. It is administratively divided into 5 development regions, 14 zones and 75 districts. Twenty districts are in the Terai region, 38 are hilly districts and the remaining 17 districts are in the mountainous region. The Kapilbastu district where the present study was carried out is terai district situated in Lumbini zone and included in Western development region.

Kapilbastu is situated over 200 km west of Kathmandu. Its latitude is 27°4’ North of the equator, while the longitude is 83°0’ East of the prime meridian. Its altitude reaches a maximum of not above 1000m from MSL. It is surrounded in the east by Rupandehi district (Nepal), in the north by Arghakhanchi district (Nepal), in the west by Dang – Deukhuri district (Nepal) and in the south by Uttar Pradesh state (India). The average annual rainfall in this district ranges from 100 to above 250cm, and the temperature range is 10 – 45°c. According to Central Bureau of Statistics, HMG (2001), the population of this district is 4,81,976 (2,47,875 male and 2,34,101 female). This population is 2.08% of total population of Nepal. The total area covered by this district is 1738 sq. km, while the population density per sq. km is 277 (CBoS, HMG, 2001).

The total no of household in the district is 72,932 and the average household size is 6.61. Literacy of the district is 41.8% out of which 53.3% are males and 29.5% are females (CBoS, HMG, 2001). The ethnic groups include Muslims, Tharu, Maithili, Bhojpuri, Brahmin, Chhetri, Magar, Kurmi, Kanu, Raj Bansi, Yadav, Baniya and others. The whole district encompasses 77 VDCs and one municipality. The health facilities of this district include 2 Primary health centers (PHCs), 6 Health posts (HPs), 67 Sub – health posts (SHPs), Bir Hospital and Shiva Raj Hospital among others. The headquarter of Kapilbastu district is Taulihawa.

The VDC of this district selected for the present study is Maharajganj situated in Southern part of the district. The total population of this VDC is 13,709 (7,175 male and 6,534 female). The total number of household is 2,103 and the average household size is 6.52 (CBoS, HMG, 2001). Out of total population 71.52% people depend on agriculture. Human habitation is surrounded with poor sanitation and people are mostly farmers.

MATERIALS: Tools used in this study were as follows:

Laboratory Tools:

Materials required:

Microscopic slides, sterile lancets, cotton wool, globes, mask, measuring cylinder, dropper, slide box, compound microscope, a slide stand.

Reagents:

Methanol, Giemsa stain 10%, Distilled water, Oil immersion.

Study population: On consulting with the several local health personals and a list of district profile of the filarial infected VDCs, Maharajganj VDC ward no.1 was choosen as a study site for cross-sectional sampling.

Population size: A total of 505 blood samples were collected from the people of Maharajganj VDC ward no.1. The questionnaire of the same population was taken.

Study design: Epidemiological cross-sectional survey design was applied as a research tool in this study.

Orientation program:

Before the collection of blood samples from the selected study area, all the respondents were gathered and were orientated about the disease filariasis, its effects on public health and prevention and control methods.

Questionnaire survey:

The format of questionnaires included name, age, sex, occupation, education, marital status, relation with the household and family size, surrounding environment, respondents current health status, filariasis symptom in family member. The structural questionnaire was prepared, pre-tested and piloted before administration in community.

Human blood sampling: Human blood samples were drawn out by ear-lobe prick method.

Sampling technique:

In the study field the blood samples were collected during night time at 10:00 PM to 2:30 AM, when the people were in relaxed condition in their beds.

To ensure the better condition during the study, the following precautions were taken:

- Questionnaire were distributed to the respondents who could fill themselves and for those who could not, were filled by interviewing their elders to record their family background, knowledge about the disease and the clinical history of patients.
- The sample slide, lancet was properly cleaned and dried.
- Each sample slides were labeled with code number 1A/1HH, 2A/1HH etc. Which were coded similar to questionnaires.
- The blood samples were collected from the ear lobe about 60ml by adding three different films, each film containing about 20ml. Thick blood films were prepared immediately after the blood sample taken out from the person and air dried for some time. The slides were collected in the slide box for microscopic examination.

Procedure:

Three thick blood films were prepared on a slide by earlobe prick method. The blood samples were collected during night time at 10:00PM to 2:30AM. Prepared blood smear was allowed to dry for 8 – 10min.

Each smear of the blood samples contains approx. 20ml. Hence 60 ml of blood was used to determine the density of microfilaria.

After the collection of blood samples, they were stained using Giemsa stain. The staining process was done by following method:

- Dehaemoglobination of thick blood smear: The dry film was kept in tap water for 5 to 10 minutes. Lysis of RBCs takes place and the film becomes clear. The film was dried at room temperature.
- Fixing of blood smear: The dried film was now dipped once in methanol for about 5 sec. It was done for fixing of nematodes in blood smear. The film was let to dry again at room temperature.
- Staining: The film was then put in 10% Giemsa stain for 30 minutes for staining purpose. After washing the film was dried again and examined under compound microscope.
- Observation: Microfilariae were observed and identified as *Wuchereria bancrofti* on the basis of following observation:

i. Sheath stained ii. Discrete nuclei iii. Empty space between the nuclei and the body wall iv. Cephalic space as long as it is broad v. No nuclei in tip of tail vi. Tip of tail may be bent back underneath the body.

DATA PROCESSING AND ANALYSIS:

Data were edited, information were coded and according to the need of objectives all the data were classified, tabulated and interpretated. Data were analyzed by means of tables and illustrations and also by statistical tools.

V

RESULTS

The present study was conducted in ward no.1 of Maharajganj VDC. A total of 505 human population were surveyed by cross-sectional technique for lymphatic filariasis. The study was conducted by questionnaire survey analysis and collection and examination of blood samples.

⟩ General prevalence of microfilaria (mf):

Table 2 revealed that out of 505 blood samples examined for detection of the human microfilarial infection, 50 blood samples showed the presence of microfilaria. Hence general prevalence of mf was found to be 9.90%.

⟩ Sex-wise prevalence of microfilaria:

Among 269 male blood samples, 30 (11.15%) were mf positive and out of 236 female blood samples, 20 (8.47%) were mf positive. Statistically, the difference in prevalence of microfilaria in both the sexes was found to be significant ($\chi 2 = 1.01$, $P>0.05$, $df=3$) (Table 2).

Table 2: Prevalence of microfilaria in Maharajganj VDC of Kapilbastu district.

Sex	Total sample examined	Mf positive	
		No.	%
Male	269	30	11.15
Female	236	20	8.47
Total	505	50	9.90

⟩ Age and Sex - wise prevalence of microfilaria:

⟩

⟩ Age and Sex-wise prevalence of microfilaria:

Out of total 505 blood samples, the highest number (136) of blood samples were examined from 11 – 20 years age–group. Out of this, 12 samples were found to be mf positive. The highest rate of infection was found in 61 – 70 years age – group. Out of 15 total samples from this group, 3 (20%) were found to be mf positive. Two mf positive samples (22.22%) were of males and 1 (16.67%) was of female. Whereas the least rate of infection was found in ≤ 10 years age group. Out of total 120 samples, 8 (6.66%) were found to be mf positive in which among the 68 male blood samples, 5 (7.35%) were found to be mf positive and out of 52 female blood samples, 3 (5.77%) were found mf positive. Statistically, the difference in the prevalence of microfilaria in different age groups were found to be significant ($\chi 2 = 5.40$, $P>0.05$, df=15) (Table 3).

Table 3: Age and sex - wise prevalence of microfilaria.

Age group (years)	Total			Male			Female		
	No. of samples	Mf positive		No. of samples	Mf positive		No. of samples	Mf positive	
		No.	%		No.	%		No.	%
≤ 10	120	08	06.66	68	5	07.35	52	3	05.77
11 – 20	136	12	08.82	70	6	08.57	66	6	09.09
21 – 30	089	11	12.35	43	8	18.60	46	3	06.52
31 – 40	068	09	13.23	34	6	17.65	34	3	08.82
41 – 50	042	04	09.52	25	3	12.00	17	1	05.88
51 – 60	028	02	07.14	16	0	00.00	12	2	16.67
61 – 70	015	03	20.00	09	2	22.22	06	1	16.67
>70	007	01	14.28	04	0	00.00	03	1	33.33
Total	505	50	9.90	269	30	11.15	236	20	8.47

) Education-wise distribution of microfilaria:

The maximum numbers of respondents were illiterate, i.e. 226, whereas minimum number of respondents were with primary education i.e.84. Out of 226 illiterate population, 92 were males in which 11 (11.96%) showed mf positive case, and 134 were females in which 10 (7.46%) showed mf positive case. The highest rate of infection was found in literate (having informal education) population. In this group out of 95 total samples, 14 (14.74%) mf positive cases were reported. The lowest rate of infection was found in population with secondary + higher educational status. Here out of 100 total samples, 8 (8%) showed mf positive cases. Statistically, the difference in the distribution of microfilaria in different educational status was found to be significant ($\chi 2 = 3.21$, $P>0.05$, $df=7$) (Table 4).

Table 4: Education-wise distribution of microfilaria.

Education status	Total			Male			Female		
	No. of sample	Mf positive		No. of sample	Mf positive		No. of sample	Mf positive	
		No.	%		No.	%		No.	%
Illiterate	226	21	09.29	92	11	11.96	134	10	07.46
Literate	095	14	14.74	64	11	17.19	031	03	09.68
Primary	084	07	08.33	51	04	07.84	033	03	09.09
≥ Secondary	100	08	08.00	62	04	06.45	038	04	10.53
Total	505	50	9.90	269	30	11.15	236	20	8.47

) Occupation-wise distribution of microfilaria:

Out of 505 studied population, 145 (95 males and 50 females) people were farmers. Among them 17 (17.89%) males were found to be mf positive and 6 (12%) females were found to be mf positive. The highest percentage (16.67%) of filariasis positive case was recorded among labor whose total studied population was 6. Out of 151 students, 11 (7.28%) mf positive cases were recorded. Teachers and others were found to be not infected by microfilaria. This may be because they have a better living style and are literate, possess a good home and have knowledge of disease. Statistically, the difference in the distribution of microfilaria in different occupation was found to be significant ($\chi 2 = 11.88$, $P>0.05$, $df=17$) (Table 5).

Table 5: Occupation-wise distribution of microfilaria.

Occupation	Total			Male			Female		
	No. of sample	Mf positive		No. of sample	Mf positive		No. of sample	Mf positive	
		No.	%		No.	%		No.	%
Farmer	145	23	15.86	95	17	17.89	50	6	12.00
Student	151	11	07.28	84	05	05.95	67	6	08.96
Labor	006	01	16.67	06	01	16.67	00	0	00.00
House wife	076	05	06.58	00	00	00.00	76	5	06.58
Business man	062	05	08.06	50	04	08.00	12	1	08.33
Teacher	005	00	00.00	05	00	00.00	00	0	00.00
Other	002	00	00.00	02	00	00.00	00	0	00.00
Child	058	05	8.62	27	03	11.11	31	2	06.45
Total	505	50	9.90	269	30	11.15	236	20	8.47

) Knowledge of filariasis among mf positive and negative people:

Only a few people have the knowledge of lymphatic filariasis. 81.39% people were unaware of filariasis. They do not have knowledge about the disease, its vector and transmission. This was due to lack of education, illiteracy or awareness about the disease. Out of 411 people who had no knowledge of lymphatic filariasis, 39 (9.49%) were mf positive, whereas out of 94 people who had knowledge of lymphatic filariasis, 11 (11.70%) were mf positive (Table 6).

Table 6: Distribution of microfilaria in relation to knowledge of LF.

Knowledge about filariasis	People		Mf positive	
	No.	%	No.	%
Yes	094	18.61	11	11.70
No	411	81.39	39	09.49
Total	505	100	50	9.90

) Distribution of filariasis in relation to the use of mosquito-nets:

Out of total 505 studied population, 285 (56.44%) were found to use bed - nets while sleeping. Among them 35 (12.28%) were mf positive. Whereas, out of 220 (43.56%) people who were not using bed - nets, 15 (6.82%) were mf positive. Mf positive was found more in net using people. This may be because of very hot sunny day people work in the fields till late night and early in the morning, which are peak biting hours of mosquitoes so the chances of mosquito biting is more (Table 7).

Table 7: Distribution of Lf in relation to the use of mosquito nets.

Use of mosquito nets	People		Mf positive	
	No.	%	No.	%
Yes	285	56.44	35	12.28
No	220	43.56	15	06.82
Total	505	100	50	9.90

J Status of Clinical Manifestation: Age and Sex – wise.

The total of 505 persons were examined. Ten persons had signs and symptoms of lymphatic filariasis. Among them 4 were males and 6 were females. Hence overall crude disease rate was recorded to be 10 (1.98%), male 0.79% and female 1.19%. The highest crude disease rate was recorded in 41-50 years age-group. Out of 42 people from this group, 4 (9.52%) had signs and symptoms (as shown in the Table 8) of lymphatic filariasis. Whereas the least crude disease rate was found in 21-30 years age-group. Out of total 89 people, 1 (1.12%) had signs and symptoms of lymphatic filariasis. No signs and symptoms of lymphatic filariasis was recorded in the people of the age-groups ≤ 10 years, 11-20 years and >70 (Table 8).

Table 8: Clinically manifested persons in the study population.

Age group (year)	Total							Male		Female	
	Total sample	Total cases		Swollen limbs	Swollen nerves	Elephantiasis	Chyluria	Total cases		Total cases	
		No.	%					No.	%	No.	%
≤ 10	120	00	0.00	0	0	0	0	0	000	0	000
11 - 20	136	00	0.00	0	0	0	0	0	000	0	000
21 -30	089	01	1.12	0	1	0	0	1	100	0	000
31 – 40	068	03	4.41	2	0	0	1	0	000	3	100
41 – 50	042	04	9.52	3	0	1	0	1	025	3	075
51 - 60	028	01	3.57	1	0	0	0	1	100	0	000
61 - 70	015	01	6.67	1	0	0	0	1	100	0	000
>70	007	00	0.00	0	0	0	0	0	000	0	000
Total	55	10	1.98	7	1	1	1	4	0.79	6	1.19

⟩ Endemicity rate (ER) of LF in Maharajganj VDC of Kapilbastu district:

The overall endemicity rate of lymphatic filariasis in Maharajganj VDC of Kapilbastu district in 505 total studied population was found to be 11.68% of 59 samples. In this microfilariaemia (mf) was 49 (9.70%), CDR was 9 (1.78%) and MF+CDR was 1 (0.20%) (Table 9).

Table 9: Endemicity rate of LF in Maharajganj VDC of Kapilbastu district.

Site	Total sample	MF		CDR		MF + CDR		ER	
		No.	%	No.	%	No.	%	No.	%
Maharajganj	505	49	9.70	9	1.78	1	0.20	59	11.68

⟩ Sex - wise endemicity rate of lymphatic filariasis:

Out of 505 total studied population, 269 were males in which the overall endemicity rate was found to be 12.64% of 34 samples and among 236 females, the overall endemicity rate was found 10.59% of 25 samples. Statistically, the difference in the sex-wise ER was found to be significant ($\chi 2 = 0.51$, $P>0.05$, $df=3$) (Table 10).

Table 10: Sex wise endemicity rate of lymphatic filariasis.

Sex	Total sample	MF	CDR	MF+CDR	ER	(%)
Male	269	30	4	0	34	12.64
Female	236	19	5	1	25	10.59
Total	505	49	9	1	59	11.68

⟩ Age – wise endemicity rate of lymphatic filariasis:

The overall study showed that the highest endemicity rate was found in the age group 61 – 70 years (26.67% of 4 samples) and the least endemicity rate was found in the age group ≤ 10 years (6.67% of 8 samples). Statistically, the difference in the age-wise ER was found to be significant ($\chi 2 = 10.97$, $P>0.05$, $df=15$) (Table 11).

Table 11: Age – wise endemicity rate of lymphatic filariasis.

Age group (years)	Total sample	MF	CDR	MF + CDR	Endemicity rate	
					No.	(%)
≤ 10	120	08	0	0	08	06.67
11 – 20	136	12	0	0	12	08.82
21 – 30	089	11	1	0	12	13.48
31 – 40	068	09	3	0	12	17.65
41 – 50	042	03	3	1	07	16.67
51 – 60	028	02	1	0	03	10.71
61 – 70	015	03	1	0	04	26.67
> 70	007	01	0	0	01	14.29
Total	505	49	9	1	59	11.68

⟩ Age and Sex - wise endemicity rate:

The overall study showed that the highest endemicity rate was found in the age group 61 – 70 years (26.67% of 4 samples), among which endemicity rate of males was found to be 33.33% of 3 samples and endemicity rate of female was found to be 16.67% of 1 sample. The least endemicity rate was found in the age group ≤ 10 years (6.67% of 8 samples), among which endemicity rate of males was found to be 7.35% of 5 samples and endemicity rate of females was found to be 5.77% of 3 samples (Table 12).

Table 12: Age and sex - wise endemicity rate.

Age group (years)	Total			Male			Female		
	No. of sample	Endemicity rate		No. of sample	Endemicity rate		No. of sample	Endemicity rate	
		No.	%		No.	%		No.	%
≤ 10	120	08	06.67	68	5	07.35	52	3	05.77
11 – 20	136	12	08.82	70	6	08.57	66	6	09.09
21 – 30	089	12	13.48	43	9	20.93	46	3	06.52
31 – 40	068	12	17.65	34	6	17.65	34	6	17.65
41 – 50	042	07	16.67	25	4	16.00	17	3	17.65
51 – 60	028	03	10.71	16	1	06.25	12	2	16.67
61 – 70	015	04	26.67	09	3	33.33	06	1	16.67
> 70	007	01	14.29	04	0	00.00	03	1	33.33
Total	505	59	11.68	269	34	12.64	236	25	10.59

VI

DISCUSSION AND CONCLUSION

According to fifth report of the WHO expert committee on filariasis, issued in 1991 almost 751 million people were living in endemic areas and were at risk. Of these 72.8 millions were infected with *W. bancrofti* and 5.8 million with *B. malayi* and *B. timori* lymphatic filariasis. Lymphatic filariasis has been estimated as endemic in some 80 countries including 120 millions of people (WHO Geneva 2000).

Filariasis has been known to be endemic in Nepal since long time (Epidemiology and Disease control Division, March 2000). Microfilaria has been reported from different areas of Nepal. The first report was given by Jung (1973) from central Nepal. In the urban population, he reported 4.99% to 6.15% crude disease rate in all the age groups, and both the sexes, 6.6% to 10.3% in the semi urban population and 1.2% to 17.8% in the rural population. Similarly the study showed 7.1% to 9.16% microfilariamia in the urban population, 10.03% to 11.3% in semi urban population and 0.8% to 17.69% in the rural population. The present surveillance study was carried out in ward no.-1 of Maharajganj VDC of Kapilbastu district. The total population of the VDC was 13,709, out of which 505 people were choosen as samples. Out of 505 samples examined for microfilaria, 50 cases (9.90%) were recorded positive for microfilaria, out of which 30 were males and 20 were females. Similar finding was also given by Jung, (1973). In the present study the

endemicity rate of lymphatic filariasis was recorded 11.68% which is less than Pradhan *et al.*, (1997) who had reported 24.6% endemicity rate with the overall, 12.75% microfilarial infection and 11.95% crude disease rate in Gokarna VDC of Kathmandu valley. Serchand *et al.* (2002) surveyed in 37 districts of Nepal and reported 13% prevalence of microfilaria. In the present study highest prevalence rate of microfilaria 20% was recorded in the age group 61-70 years. The lowest prevalence rate 6.66% was recorded in the age group ≤ 10 years. The highest prevalence rate of microfilariae may be due to maximum exposure towards outer environment, lack of awareness and knowledge of filariasis, carelessness towards using mosquito-nets etc. Mf positive was found more in mosquito-net using people. This may be because of hot day people prefer to work in the fields till late night and early in the morning, which are peak biting hours of mosquitoes, so the chances of mosquito biting is more.

The children and students of age group ≤ 10 years are at higher risk of infection with Lf. This may be due to low socioeconomic status of their parents. They go to the field and sleep outdoor without bed-nets, hence are exposed to mosquito biting. They do not have knowledge about the disease and its protective measures. The highest crude disease rate was recorded in the age group 41-50 years and the lowest crude disease rate was recorded in the age group 21-30 years. The signs and symptoms of filarial disease increase with increase in age. Age is equivalent to the length of exposure has also been supported by WHO Geneva (2001).

Present study showed that male to female ratio for the disease was 3 : 2. Prevalence of filariasis is higher in males than in females. Similar finding was also given by Weerasoriya *et al.*, (2001) Sri Lanka who had reported prevalence was significantly lower in female than in male. This may be because males are mostly involved in field work, where chances of mosquito biting are more. The favourable breeding season of the mosquito is the summer season. During this period males usually sleep outdoor and hence are more exposed to the mosquito biting. While females mostly sleep indoor with their children. They use mosquito nets to prevent the children from mosquito biting and hence are less exposed to mosquitoes.

The present study showed 1.98% crude disease rate. Similar finding was also given by Jung, (1973) who had reported 1.2 to 17.8% CDR in the rural population. Whereas this finding was less than the Manandhar (2001) who had reported 19.9% crude disease rate in three different geographical regions of Nepal. Crude diseases were found to be higher among the females than the males. This is supported by Pradhan *et al.*, (1997) who had reported 8.49% CDR in male and 16.59% in female in Gokarna VDC of Kathmandu valley.

Illiteracy also seems to play a major role in the infection of disease. Least positive cases 8% were recorded from people with secondary and higher educational status, while the maximum positive cases were recorded from illiterate and literate with informal educational status. This may be due to lack of awareness and knowledge of people about the disease filariasis and its vectors as well as preventive measures. Lymphatic filariasis is a disease of poor

environmental condition with low socioeconomic status, low literacy rate and high percentage of illiterate health education status (WHO 1997).

Occupation was also found to be a major factor for prevalence of the disease. Among farmers, 15.86% positive cases of filariasis were recorded, 7.28% were recorded among students, and 6.58% were recorded among housewives. No positive case was recorded among teachers. The farmers keep cattle in the same house where they live. Cattle sheds are one of the most favourable breeding sites for the vector mosquito and hence have more chances of being bitten by mosquitoes. They have habit of working in the field during morning and evening time which is also a suitable time for mosquito biting. Most of the surrounding environment of the house is dirty with dead and decaying organic matters, stagnant water, bushy places, open drainage which are suitable sites for mosquito breeding. Hence people living in that environment have maximum chances of mosquito biting, which increases the disease prevalence. They are illiterate, poor and unaware of the diseases. These are risk factors which increases the disease prevalence. Comparatively teachers and others have a better life and possess healthy life style and have knowledge of the disease, hence no positive case was recorded from these groups.

VII

RECOMMENDATIONS

- Awareness programme should be launched and expanded through mass media, radio and television to give the knowledge of lymphatic filariasis to local people. These programmes must include the methods for protecting vector borne lymphatic filarial disease and to improve sanitation, health and hygiene.
- The cause, early symptoms, detection and preventive measures of control of the disease should be made familiar to the people.
- The control or elimination of breeding sites of mosquitoes in stagnant water in ditches is possible by improving sanitation systems and hygiene in general. Where such improvements are impossible or economically unfeasible, larvicides can be applied to breeding sites.
- For protection from mosquito bite, people should be made familiar to use mosquito net, mosquito coil, mosquito mat, bagon spray, fumigation and ointment.
- Mass treatment programme should be conducted after the identification of the communities where the infection exists. The control programme must be regularized along with the monitoring of the same study population.
- The insecticides should be sprayed to control local vectors, which are known to be effective against them.
- Health education should be emphasized to improve the health status of the community.

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Annex-1

Questionnaires for Filariasis cross-sectional survey in Maharajganj VDC, Kapilbastu district of Nepal.

S.N…… Date :……
1. Name of respondent……

2.Address. District……..
VDC/Municipality……
Ward No./Tole/Block No……

3. Age/Sex……..

4. Education. I) Illiterate ☐ II) Literate☐
If literate. I) Primary ☐ II) Lower secondary☐ III) Secondary☐
IV) SLC ☐ V) Inter☐ VI) Bachelor
VII) Master☐ VIII) Others☐

5. Occupation. I) Farming☐ II) Business☐ III) Teaching☐
IV) Labor ☐ V) House wife☐ VI) Student☐
VII) Unemployed ☐ VIII) Others☐ IX) Child ☐

6. Marital status. I) Single/Married II) Widow/Widower/Divorce

7.Relationship with the head of household /family size…….....

8. Respondent's current residence status.
I) Birthplace☐ II) Migrate☐ III) Temporary☐
(How long have you been here……)

9. Surrounding environmental condition.
I) Clean☐ II) Dirty☐ III) Bushy☐ IV) Open drainage☐
V) Stagnant water ☐ VI) Open night disposal☐

10. Do you use any means to protect mosquito biting? Yes ☐ No.☐
If yes, which one
I) By spraying medicine ☐ II) Burning perfume mosquito coil☐
III) Smoking ☐ IV) Mosquito net☐
V) Domestic herbs☐

11. Do you have the knowledge about the disease filariasis (elephantiasis)?
Yes ☐ No.☐
If yes, how does this disease transmit?
I) By mosquito biting ☐ II) Contact with the disease patient☐
III) Mother to fetus☐
12. How can we prevent this disease? May you give any suggestion?
Yes☐ No. ☐
I)…….
II)……

13. According to your knowledge, is the disease filariasis more in parent's time or now?
I) Parent's time ☐ II) Now ☐ III) Don't know ☐

14. Respondent's current health status.
I) Healthy☐ II) Unhealthy☐
If you are unhealthy, since when? ……Years.
Do you have any symptoms? Yes☐ No. ☐
If yes, which one given below?
I) Fever ☐ II) Headache☐ III) Effect on genital organ or breast☐
IV) Swollen lymphnode☐ V) Hydrocele ☐ VI) Swollen hands and legs☐
VII) Thick skin☐ VIII) Chyluria ☐ IX) Swollen nerves☐
X) Weakness☐ XI) Lazy feeling☐ XII) Epigastric pain☐
XIII) Nausea ☐ XIV) Abscess formation☐
If yes, have you used any medicine? Yes☐ No.☐
If yes, which medicine…….

15. Have you seen the person suffering from the disease filariasis?
Yes ☐ No ☐
If yes, how many? ……person.

16. Is there person suffering from the disease filariasis in your family or in relatives?
Yes☐ No☐
If yes, who is he/she? …… (Your relation).

Signature of the patient/attendant with consent for participation.

.....
Signature

Thank you, very much for your valuable time.
Result of blood examination. Positive ☐ Negative ☐
If positive, Number of microfilaria per 20ml ……..

Annex-2

METHOD OF MAKING DILUTE GIEMSA SOLUTION

Giemsa stain:

Giemsa stock stain is an alcohol based Romanow sky stain, it is highly flammable with flash point 12°c, which 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. The concentration of stain is low and the staining time is long. Care must be taken to prevent water from entering the stock stain.

Preparation of reagents of Giemsa stains (stock solution):

Purchase ready made or prepare using the following formula.

To make about 500 ml stain, 3.8 grams of Giemsa power, 250 ml of glycerol (Glycerin) and 250 ml of Methanol (Methyl alcohols) are required.

Weight the Giemsa on a piece of clean paper (pre weighted) and transfer to a dry brown bottle of 500 ml capacity that contains a few glass beads. Using a dry cylinder measure the methanol and add to the stain, mix well. Using the same cylinder, measures the glycerol, and add to the stain, mix well. Place the bottle of stain in a water bath at 50-60°c, if not available at 37°c, for up to 2 hours to help the stain to dissolve, mix well at intervals. Label the bottle, and mark it flammable and toxic. Store at room temperature in the dark place. If kept well Stoppard, the stain is stable for several months.

For use: Filter a small amount of the stain into a dry stain-dispensing container.

Caution:

1. Giemsa stain will be spoilt if water enters the stock solution during its preparation or storage.

2. Methanol is toxic and highly flammable, therefore handle it with care and use well away from an open flame.

Making a Giemsa 10% working solution:

In a measuring cylinder of 100 ml put 90 ml distilled water and 10 ml of Giemsa stock solution and mix gently. And used as a Giemsa stain working solution.

Character of Giemsa stain:

Staining with Giemsa stain gives different colors to different cell parts:

Chromatin of parasite …….Dark red.

Cytoplasm of parasite……..Blue.

Schuffner's dot ……...Red.

Maurer's dots (Clefts) ……Red mauve.

Red cells…….Grey to pale mauve.

Reticulocytes……...Grey blue.

Nuclei of neutrophils……Mauve purple.

Granules of eosinophils……Red.

Cytoplasm of mononuclear cells……Blue grey.

Annex-3

Structure of *Wuchereria bancrofti*

Adult worms: The adult male measures 2.5 to 4 cm in length by 0.1 mm in thickness, with a ventrally curved tail-end and contains two spicules of unequal length. The adult female measures 8 to 10 cm in length by 0.2 to 0.3 mm in thickness, with a narrow and abruptly pointed tail-end. They are long-hair like transparent nematodes (often creamy-white in colour). They are filiform in shape and both ends are tapering, the head-end terminating in a slightly rounded swelling. Females are ovo-viviparous. Males and females remain coiled together and can only be separated with difficulty (females are usually more numerous than males and the later are difficult to find). The life span of the adult worm is several years (5 to 10 years).

Embryos (Microfilariae): Microfilaria after passing through the lymph nodes, find their way by the main lymphatic trunks into the circulating blood. They are very active and can move both with and against the blood stream. The embryo measures about 290 *M*m in length by 6-7 *M*m in breadth. When dead and stained with Romanowsky's stains or Giemsa stains the embryos shows the following morphological peculiarities:

i) A hyaline sheath is a structure less sac which is much longer than the larval body so that the larva can move forwards and backwards within it.

ii) Cuticula is lined by sub cuticular cells and is seen only with vital stains.

iii) Nuclei appear as granules, which do not extend up to the tip of the tail (terminal 5 percent) and is a distinguishing feature of mf (*W. bancrofti*). At the anterior end, there is a space, called the cephalic space.

iv) The granules are broken at definite places serving as the landmarks for identification of the species. They include the following: (a) Nerve ring, an oblique space, (b) Anterior v-spot, represents the rudimentary excretory system and posterior v-spot or tail-spot, represents the terminal part of the alimentary canal (anus or cloaca).

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

vi) Innen Korper of Fiileborn or Central (Internal) Body of Manson extends from the anterior v-spot to the G-cell 1. It represents the rudimentary alimentary canal.

In the human body the larval forms do not undergo any further development unless they are taken up by their appropriate intermediate host (mosquito). If these microfilariae are not sucked up by the mosquito, they die in course of time. The life span of microfilariae in the human body has been found to be as long as 70 days (Chatterjee, 2002).

Annex-4

Life cycle of *Wuchereria bancrofti*

The life cycle of *Wuchereria bancrofti* is digenetic i.e. it completes its life cycle in two hosts: man and mosquito.

The definite host is man, in whose lymphatic system the adult worms are harboured.

The intermediate host is a mosquito, in which the microfilariae undergo further development, after which they become infective to man. A large number of species of mosquito belonging to the genus *Culex,* sometimes *Aedes, Anopheles*, *Mansonia*, *Psorophora* and *Coquillettidia* act as intermediate host.

Development of Microfilaria in the mosquito:

Microfilaria in the peripheral blood circulation is ingested by the mosquito when the appropriate female mosquito vector bites an infected human host during its blood meal within the appropriate time. On reaching the mid-gut of the mosquito, they lose their sheaths quickly, and penetrate the gut-wall within an hour or two and migrate to the thoracic muscles.

In the next 2 days they changes to a thick, short, sausage-shaped form with a short spiky tail, measuring 124 to 250 *M*m in length by 10 to 17 *M*m in breadth. This is the first-stage larva, with a rudimentary digestive tract.

The larva moults first time in 3 to7 days time. The second-stage larva measures 225 to330 *M*m in length by 15 to 30 *M*m in breadth.

On the 10^{th} or 11^{th} day, the larva moults second time, after which the tail atrophies to a mere stump and the digestive system, body cavity and genital organs are fully developed. This is the third stage larvae (L3) which measures 1500 to 2000 *M*m in length by 18 to 23 *M*m in breadth. It is the infective stage to man and enters the proboscis sheath of the mosquito. When the infected mosquito bites the human host, the larva gets injected during blood-meal (Chatterjee, 2002).

Infection to the man and development into adult worms:

When the infected mosquito bites a human being, the third stage larvae (L3) are not directly injected into the blood stream like malarial parasites but are deposited on the skin near the site of puncture. Then they either enter through the puncture wound or penetrate through the skin on their own. There after, they enter into lymphatics and settle down in lymph nodes where they develop into adult worms after moulting twice. Sexes are separate with distinct sexual dimorphism. They become sexually mature in about 5-18 months.

The mature worms can live for many years in their host depending in part on the extent and the host immune response. Their mean life span is 4-6 years but they can survive up to 15 years or more (Cheesbrough, 1998).

After copulation, the male fertilizes the female and the gravid females give birth to larvae. The new generation of the microfilariae or first-stage larvae is emitted which passes either through the thoracic duct or the right lymphatic duct, to the venous system and pulmonary capillaries and then to the peripheral circulation. Thus the life cycle is completed.

The appearance of microfilaria in the peripheral blood showed marked periodicity. Nocturnal periodicity that is occurrence of mf in the peripheral circulations at night mostly after two hours at rest.

Several hypothesis have been contributed to explain nocturnal periodicity:

1. There is a chemo tactile force that attracts the microfilariae to the saliva of the mosquito hosts (vectors); which are more plentiful at night.
2. During sleep the relaxation of the host induces the microfilariae to migrate into the peripheral circulation.
3. The migration results from a response to oxygen and carbon dioxide supply.
4. The microfilariae survive for only a short period and it in during nocturnal period that they are most abundant and readily found in the peripheral circulation.

None of these hypothesis is completely satisfactory. The microfilaria is most plentiful in the peripheral circulation between 10:00 PM to 2:00 AM (Cheng, 1999).

In case of diurnal sub-periodic and nocturnal sub-periodic strain of *Wuchereria bancrofti*, microfilariae can be found in the peripheral circulation throughout the 24 hours, with only a slight increase in number during day and night hours respectively. The mf is chiefly in the capillaries and in the lungs when not circulating in the peripheral blood (Haslett *et al*., 1999).

The different periodicities of mf correspond with the biting habits of their principal vectors. For e.g., the nocturnal periodic mf are transmitted by night

biting habits of mosquitoes. This adaptation enhances their chance of onward transmission.