

Final Report

दर्ता नं 372
विषय NHRCC
मिति 31 Dec 2001

EPIDEMIOLOGICAL STUDY OF MICROFILARIASIS
IN THREE DIFFERENT GEOGRAPHICAL REGIONS OF NEPAL



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Nepal Health Research Council
Kathmandu
Nepal
28 December 2001



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EPIDEMIOLOGICAL STUDY OF MICROFILARIASIS IN THREE DIFFERENT GEOGRAPHICAL REGIONS OF NEPAL

Abstract:

Bancroftian filariasis is spreading as an endemic in many parts of Nepal and is a major public health problem. A survey for lymphatic filariasis was carried out in 3 places in the affected area. This paper deals with the prevalence of microfilaraemia and filarial disease studying their association with individual characteristics and variables related to exposure to the vectors. The parasitological survey was performed through a door to door conducted between Jan. 2001 – Nov. 2001 and microfilaria was examined by multiple diagnostic technique (thin smear, thick smear, buffy coat technique) using blood collected between 20.00 – 01.00. as well as ICT Card method. The individuals, male/female aged between 20 and 65 years were interviewed with questionnaire related with microfilaraemia. The risk of being microfilaraemic was greater among those who had lived in the studied area for more than 15 years. The disease prevalence was 0.31%. The chronic clinical manifestations was found in both males and female and was increased with age. The cases of hydrocele in male were noticed more in terai and innerterai but microfilaraemic cases detected in hill. The parasitological survey carried out with multiple diagnostic technique showed that smear from buffy coat of night blood was the best to find the microfilaria. ICT Card method was found very satisfactory for diagnosis of antigenemia in a *Wuchereria bancrofti* infection.

Introduction:

Bancroftian filariasis, a lymphatic filariasis resulting from infection with the mosquito borne nematode parasite *Wuchereria bancrofti* is a major public health problem, a handy capped problem in many countries especially with hot and humid climate^{1,4,7,15,16} Endemic area include Central and Northern Africa, South America, Western South Pacific and South East Asia¹.

In 1868 Otto Wuchereria found these microfilarial in the haematochylous urine of a Bahian patient in Salvador, Brazil while searching for *Schistosoma haematobium* eggs. His name marks the genus.⁵

Human infection is initiated by introduction of infective larvae *Wuchereria bancrofti* present in the saliva of a biting mosquito into the bite wound. The major vectors are various species of *Culex*, *Anopheles* and *Aedes* mosquitoes. These parasites lead to a variety of clinical manifestations such as Lymphoedema and elephantiasis of the limbs, genital disease e.g. hydrocele, chylocele, elephantiasis of the scrotum and the penis^{8,9,12} The ideal condition for the spread of filariasis is the dense population living in unsanitary environment in tropical climates where mosquito get suitable environment for breeding. The filarial worms are long, and are nematodes that inhabit in parts of the lymphatic system and subcutaneous and deep connective tissues. The parasites do not produce eggs/ ova and reproduce through microfilariae. Human, definitive host, harbour the adult worms that produce microfilariae, larvae without ova stage. The females give birth to living embryos, the sheathed microfilariae. The filarial worm, *Wuchereria bancrofti* is of nocturnal periodic type. The adults are viviparous, producing active microfilaria that rest in the day and appear in the peripheral blood at night when the *Culex* mosquito is active. A blood sucking mosquito takes the blood meal from an infected human along with the microfilariae. The infective stage develop in the mosquito and returns to a healthy human through the bite of the mosquito. The worms then mature in the circulatory system of the human body. The adults worms live in the lymph nodes resulting to elephantiasis and chyluria, escape of chyle or fat particles through urine appearing urine milky².

According to WHO Report^{33,34,35} the estimated number of infected people in Africa alone was 25 millions and was estimated at 40 as reported in the 1995 report. In North Tanzania^{10,30} 17.7% to 34.7% of the 3086 individuals were infected with microfilaria. The prevalence of this infection increased with age¹³ Similarly 13.7% of 1129 were found to be positive with microfilariasis in Kenya of also prevalence increased with age and also higher among males than in female⁹ There were some studies in Nepal²⁸ regarding the existence of filariasis in Kathmandu; similarly the Department And Disease Control Division, Ministry of Health, Nepal had also reported the occurrence of Lymphatic Filariasis in different parts of Nepal¹⁸ This is in confirmity to the contiguity of the geographical location and suitable climate in certain parts of Nepal for microfilariasis. In view of this, it is highly pertinent to enquire into the status and situation of microfilariasis so as to deal more efficiently with the problem. To establish epidemiology of the disease in different regions of Nepal and to find the prevalence of the infection on the basis of age, sex, ethnic grouping and attitude of the people regarding this study has been proposed.

Methodology:

The study area were selected according to the report published by Epidemiology and Disease Control Division, Department of Health Service, Kathmandu, Nepal (Bista et al., 2000). The study was conducted covering three different geographical regions, namely hill, inner terai and terai. Accordingly Bhaktapur, Palpa and Bhairahawa were chosen for hill, inner terai and terai region respectively. Actually, Palpa is also a hill district. But the area selected, Dovan, VDC resembles significantly with inner terai characteristics in view of its location. Survey was conducted during January and February in Bhairahawa and Palpa. In Bhaktapur, survey was conducted during February to November. In all areas, the first surveys were conducted by the researcher with the assistance of the consultants.

The houses were selected randomly for sample collection, however samples were also collected from individuals who had come to contact to our survey team. Houses from Bhaktapur and Bhairahawa were selected in three clusters. In bhaktapur, Golmadhi (ward no. 7), Byasi (ward no. 10), Itachhen and Katunje VDC (ward no. 8) were taken for sample collection. Whereas, in Bhairahawa samples were collected from ward no.1, 2 and 7 of Sipwa village. In Palpa, only one (ward no. 6) of Dovan was used for sample collection. The district health officer of Palpa told that there is no record of elephantiasis in Palpa. Again during our sample collection at Dovan (Ward no. 6), no elephantiasis cases at all were noticed. So we had stopped collecting the samples from other areas of Dovan.

During the sample collection, one questionnaire from each house was also filled for enquiring into the prevalence of the infection on the basis of age, sex, ethnic grouping and enquire into the attitude of the people regarding microfilariasis.

Each household was visited and explained the objective of the study. Firstly, they were oriented and convinced about the aim of the study. People who did not want to participate were not compelled to participate.

Night blood samples were collected (20.00 -24.00) with the help of a local health worker at Bhaktapur; the samples were processed following the methodology as described in Laboratory manual ^{2,5} In Bhairahawa samples were collected by the health post officer (Auxiliary health worker) and technician of our team with the help of District Health Officer. In Palpa samples were collected by our technician with the help of local volunteers. Besides night blood collection the urine samples were also collected from the cases of Chyluria for examination of microfilaria.

Although blood is still used for microfilaria detection and is gold standard; but this is very laborious, time consuming and not suitable for follow up. Because of this reason the individuals were clinically examined during day time for chronic manifestation of lymphatic filariasis. Soon after clinical examination, individuals were examined for circulating filarial antigens with the ICT card test for whole blood specimens (Amrad ICT, Australia) by following instructions from the manufacturers. The test detects soluble *W. bancrofti* antigens that circulate in the blood of infected human ^{3,19,29,31} This method had been reported ^{11,12,19,22} and recommended for rapid diagnosis of antigenemia in a *Wuchereria bancrofti* infection. This is an immunochromatographic test for the qualitative detection of *Wuchereria bancrofti* antigen in whole blood. The test utilizes a polyclonal antibody and a monoclonal antibody specific for *Wuchereria bancrofti*. The antibody is attached to colloidal gold and impregnated into the pink and white sample pad. The majority of blood cells will be retained in the white area of the pad and serum will flow forward into the pink area allowing any *Wuchereria bancrofti* antigen present to bind to the colloidal gold labelled polyclonal antibody. The monoclonal antibody is immobilized in a line across the membrane when card is closed, the sample and the polyclonal antibody on the pink area of the pad contact the end of the membrane. The sample and the labelled polyclonal antibody then migrate along the membrane crossing the immobilized monoclonal antibody line. In a positive sample, any microfilaria *bancrofti* antigen coupled with the gold labeled polyclonal antibody is captured by microfilaria on the membrane and pink line forms. In a negative sample, no gold labeled polyclonal antibody is captured by the monoclonal antibody on the membrane and no pink line form.

Interpretation of ICT test results:

Positive result: The test is positive if two lines (test and control) are seen in viewing window. Any visible line in the test line area indicates a positive case result. The test is positive even when; the test line appears lighter or darker than the control line.

Negative result: The test is negative if only the control line is seen. To ensure that low positive samples have had sufficient time to develop, a negative result should not be recorded until 15 minutes have elapsed from when the card is closed.

The sensitivity of microfilaria detection depends on the volume of blood sample, time of blood collection and skill and dedication of the microcopist. Later, in the same evening between 20.00 and 01.00 hours, the blood from the individuals taken for parasitological examination. The thick blood smear is the simplest for microfilaria detection and the most widely used in the field. Finger prick blood specimens, each of 40-60 microlitre, were collected. The blood smears were prepared, stained with Giemsa and examined as described in procedure manual^{2,5}

Results:

The present result of the clinical and parasitological survey from three communities of three different places: Terai, Inner terai and Hill. The details of the environment of the inhabitants, clinical manifestation of lymphatic filariasis in legs/ genitalia and the results of multiple laboratory diagnosis including socioeconomic status of the individual/ family are shown in the tables.

Table 1: Total number of samples processed from sample area:

Area	Total sample(%)	Female (%)	Male (%)
Sipwa	85 (16.4)	22 (25.88)	63 (74.1)
Dovan	17 (03.3)	6 (35.29)	11 (64.7)
Bhaktapur	410 (80.07)	210 (51.2)	200 (48.78)
Total	512 (100)	238 (46.48)	274 (53.35)

Source : Field survey

A total sample of 512 was collected from different geographical belts. Of which 85 (16.4) were from Sipwa (Bhairahawa), 17 (03.3) from Dovan (Palpa) and 410 (80.07) from Katunje, Golmadhi, Itachhen and Byasi of Bhaktapur. Of the 85 sample from Sipwa, 22 (25.88) were female and 63 (74.11) were from male. From Dovan a total of 17 samples were collected, of which 6 (35.29) were female and 11 (64.7) were male. Four hundred and ten samples were collected from Bhaktapur, of which 210 (51.2) were female and 200 (48.78) were male. The table shows male and female participated almost equally in field survey except in Sipwa, however male were found a little more in all the three places.

Table 2 : Samples in age and sex wise:

Age (years)	Total samples	Female (%)	Male (%)
Under 20	95	42 (17.36)	53 (18.96)
21 - 30	100	38 (15.7)	62 (22.96)
31 - 40	80	33 (13.63)	47 (17.40)
41 - 50	101	44 (18.18)	57 (21.11)
51 - 60	53	29 (11.98)	24 (08.88)
61 - 70	52	29 (11.98)	23 (08.51)
71 - +	31	23 (09.50)	8 (02.96)
Total	512	242 (46.48)	274 (53.35)

Source : Field survey

The total samples were divided into different age and sex group as shown in table 2. The sample was fairly divided between the age groups under 20 to 41-50 groups and the sample size declined thereafter commensurate to the trend of population. It also indicates that the age group from 20 – 50 were found actively participating from either sex in the study.

Table 3 : Survey area and cases of clinical manifestations:

Area	Total sample	Clinically suspected	Percentage (%)
Sipwa	85	25	29.4
Dovan	17	04	22.2
Bhaktapur	410	82	20.0
Total	512	111	21.67

Source: Field survey

Table 3 shows the sample size from each different survey areas. The highest number of 410 samples were collected from Bhaktapur and the least samples of 17 could be collected from Doan. Although, the cases of clinical manifestation were noticed in all three places the cases

were seen more in Sipwa. The overall percentage of cases as shown by clinical manifestation is 21.67. The families in the field survey was randomly selected.

**Table 4: Cases of clinical manifestation (Clinically suspected cases)
by age group:**

Age (years)	Total samples	Clinically suspected	Percentage (%)
Under 20	95	8	8.42
21 - 30	100	4	4.0
31 - 40	80	19	23.75
41 - 50	101	15	14.85
51 - 60	53	20	37.73
61 - 70	52	25	48.07
71 - +	31	18	58.06
Total	512	111	21.67

Source : Field survey

Table 4 shows the distribution of suspected cases in different age groups. The percentage of clinically suspected cases tended to increase with the increase in age group, except there is marked difference in the age group of 21-30. In this age group, the prevalence was the least.

Table 5: Clinical manifestation in age and sex category:

Age (years)	Total	Female	Male	
		Elephantiasis	Hydrocele	Elephantiasis
Under 20	8	1	7	0
21 - 30	4	3	0	1
31 - 40	19	8	6	5
41 - 50	15	10	5	2
51 - 60	20	11	3	6
61 - 70	25	18	2	5
71 - +	18	15	1	2
Total	111	66 (59.45 %)	24 (21.62 %)	21 (18.91 %)

Source : Field survey

Table 5 shows the number of clinically suspected cases in different age and sex groups. Altogether, 111 suspected cases were noted including 66 females and 45 males. Leg elephantiasis was more prevalent among females. The prevalence was more pronounced among females of higher age group. In male, the cases of hydrocele were more between the age of 31-50.

Table 6: Clinical manifestation cases in Sipwa

Age (years)	Total sample	Total suspected (%)	Female		Male		
			Total	Elephantiasis (%)	Total	Hydrocele (%)	Elephantiasis (%)
Under 20	21	6 (28.6)	5	0 (0)	16	6 (37.5)	0 (0)
21 - 30	9	0 (0)	2	0 (0)	7	0 (0)	0 (0)
31 - 40	18	6 (33.3)	4	0 (0)	14	5 (35.7)	1 (7.1)
41 - 50	22	8 (36.4)	8	2 (25.0)	14	5 (35.7)	1 (7.1)
51 - 60	10	4 (40.0)	3	1 (33.3)	7	3 (42.8)	0 (0)
61 - 70	4	1 (25)	0	0 (0)	4	1 (25.0)	0 (0)
71 - +	1	0 (0)	0	0 (0)	1	0 (0)	0 (0)
Total	85	25 (29.4)	22	3 (13.6)	63	20 (31.7)	2 (3.2)

Source : Field survey

In Sipwa, the prevalence of clinically suspected cases among females were noted to be very low at 3 (13.6). However, in the males, hydrocele was prevalent among 31.7% but elephantiasis was low at 3.2%.

**Table 7: Clinical manifestation cases (clinically suspected cases)
in Bhaktapur**

Age (years)	Total sample	Total suspected (%)	Female		Male	
			Total	Elephantiasis (%)	Total	Elephantiasis (%)
Under 20	73	1 (1.36)	41	1 (2.4)	32	0 (0)
21 - 30	86	4 (4.65)	33	3 (0.09)	53	1 (1.88)
31 - 40	59	12 (20.33)	29	8 (27.58)	30	4 (13.33)
41 - 50	77	9 (11.68)	34	8 (23.52)	43	1 (2.32)
51 - 60	42	16 (38.09)	25	10 (40.0)	17	6 (35.29)
61 - 70	45	23 (51.11)	29	18 (62.06)	16	5 (31.25)
71 - +	28	17 (60.71)	23	15 (65.21)	5	2 (40.0)
Total	410	82 (20.0)	214	63 (29.43)	196	19 (9.75)

Source : Field survey

In Bhaktapur, the scenario is quite different from Sipwa. The prevalence of filariasis as indicated by suspected cases was much higher among females with the suspected cases at 63 (29.43%).

Among the males prevalence was low at 19 (9.75%) but interestingly no hydrocele case was noted despite high sample size. The elder females appeared to be more vulnerable to the problem. The problem was particularly intense among the female of age group of 60 and above.

Table 8: Clinically suspected cases in Dovan:

Age (years)	Total sample	Total suspected (%)	Male Hydrocele (%)
Under 20	1	1 (100)	1 (100)
21 - 30	5	0 (0)	0 (0)
31 - 40	3	1 (33.3)	1 (33.3)
41 - 50	2	0 (0)	0 (0)
51 - 60	1	0 (0)	0 (0)
61 - 70	3	1 (33.3)	1 (33.3)
71 - +	2	1 (50.0)	1 (50.0)
Total	17	4 (23.5)	4 (23.5)

Source: Field survey

In contrast to Bhaktapur there were some cases of hydrocele but no cases of elephantiasis in Dovan in either sex. In Dovan the prevalence was found at 23.5%. Of these cases however, all hydrocele cases were noticed among males. It was distributed from low to high age group.

Table 9: Laboratory diagnosis by different methods

Area	Samples	Clinically suspected	Thin smear	Thick smear	Smear from buffy coat	ICT card
Sipwa	85	25	0	0	Not done	1
Dovan	17	4	0	0	Not done	0
Bhaktapur	410	82	15	27	39	52
Total	512	111	15	27	39	53

Among the three different methods in detection of microfilaria, smear from buffy coat was found the best. ICT card technique in antigen detection was still better for field survey in diagnosis of filariasis of all techniques.

Table 10: The socioeconomic status, educational status, occupation, knowledge, attitude and practice of an individuals participated in survey of Bancroftian filariasis in total of three different geographical regions (Terai, Inner Terai and Hill).

Table 10. 1: The socioeconomic status, educational status, occupation, knowledge, attitude and practice of the individuals participated in survey of Bancroftian filariasis from sample area: Bhaktpur

10. 1. 1. Economic status:

Status	No. of family	Percentage (%)
i. rich	0	0
ii. upper middle	2	2.46
iii. middle	26	32.09
iv. lower middle	53	65.09
v. poor	0	0
Total	81	100

The lower middle class followed by middle class were noticed more in the population of the community.

10. 1. 2. Educational status:

Status	No. of family	Percentage (%)
i. illiterate	47	58.02
ii. literate with informal education	4	4.93
iii. grade 1-5	5	6.17
iv. grade 6-8	3	3.70
v. grade 9-10	9	11.11
vi. above SLC	13	16.04
Total	81	100

Illiterate individuals were found more in study area. Only 16.04% of the cases were above SLC education.

10. 1. 3. Main occupation:

Occupation	No. of family	Percentage (%)
i. Agriculture	63	77.77
ii. Livestock	0	0
iii. Business	7	8.64
iv. teaching	1	1.23
v. Employee	1	1.23
vi. Labour	0	0
vii. House work	6	7.40
viii. Student	3	3.70
ix. Unemployed	0	0
Others	0	0
Total	81	100

The participants were predominantly agricultural worker. The term business indicates the low category of business such as selling of vegetables and groceries (corner shop).

10. 1. 4. Current residence status:

Residency	No. of family	Percentage (%)
i. Birthplace	73	90.12
ii. Migrated	8	9.87
iii. Temporary	0	0
Total	81	100

10. 1. 4. 1. Migrated :

Residency	No. of family	Percentage (%)
i. 1 - 5 years	0	0
ii. 6 - 10 years	0	0
iii. 11 - 15 years	0	0
iv. > 15 years	8	9.87

The above tables shows the participating individuals were basically the native local people. Only 8 families were migrated from other parts of Bhaktapur and all were living there for more than 15 years.

10. 1. 5. Surrounding environmental condition:

Environment	No. of family	Percentage (%)
i. clean	50	61.72
ii. Lagoon	11	13.58
iii. Dirty	18	22.22
iv. Bushy	1	1.23
v. Open drainage	1	1.23
Total	81	100

Most of the houses seems to be cleaned from outside, however many houses were found to have open sewage or dirty (damp).

10. 1. 6. Knowledge of filariasis:

Knowledge	No. of family	Percentage (%)
i. Yes	26	32.09
ii. No	55	67.90
Total	81	100

10. 1. 6. 1. If Yes:

Knowledge	No. of family	Percentage (%)
i. Mother to fetus	0	0
ii. Contact with diseased person	4	4.93
iii. Mosquito biting	4	4.93
iv. Sand flies	0	0
v. curse	0	0
vi. Any other	18	22.22

The table shows 68% of family houses do not know about the term filariasis and its causes.

10. 1. 7. Have you seen the person suffering from the disease filariasis:

Knowledge	No. of family	Percentage (%)
i. Yes	71	87.65
ii. No	10	12.34
Total	81	100

10. 1. 8. Are you suffering from the disease filariasis:

Disease	No. of family	Percentage (%)
i. Yes	62	76.54
ii. No	19	23.45
Total	81	100

The individuals of the family who claimed to have knowledge of the disease were found to give variable responses.

10. 1. 9. Knowledge of the individual:

Knowledge	No. of family	Percentage (%)
a. Preventable	22	27.16
b. Non-preventable	47	58.02
c. Do not know	12	14.81
Total	81	100

10. 1. 10. Type of treatment

Treatment		No. of family	Percentage (%)
i. Traditional	a. Dhama	0	0
	b. Puja	0	0
	c. Any other	0	0
ii. Medicinal	a. Ayurvedic	38	46.91
	b. Allopathic	41	50.61
iii. Not adopt any		2	2.46
Total		81	100

This table indicates that participants take equal interest both in ayurvedic treatment and allopathic treatment.

10. 1. 11. Places for allopathic treatment:

Place	No. of family	Percentage (%)
i. Hospital	38	46.91
ii. Health post	02	2.46
iii. Health personnel	26	32.09
iv. Drug store	14	17.28
v. Any others	01	1.23
Total	81	100

The majority of the participants take help from hospital, but health personnel and drug store personnel were also consulted.

10. 1. 12. Way of treatment:

Types of treatment	No. of family	Percentage (%)
i. Drug	71	87.02
ii. Clean the surroundings	2	2.46
iii. Both	6	7.40
iv. Any other	2	2.46
Total	81	100

According to the participants feeling the choice of treatment could be the drug.

10. 1. 13. Feeling about filariasis:

Problem	No. of family	Percentage (%)
i. Serious problem	47	58.02
ii. No problem	06	7.40
iii. Ordinary problem	26	32.09
iv. Do not know	02	2.46
Total	81	100

Majority of the individuals thought as serious problem because, it disturbs their everyday life. Other thought as an ordinary problem.

10. 1. 14. Occurrence of problem in the family:

Problem in the family	No. of family	Percentage (%)
i. Yes	31	38.27
ii. No	50	61.72
Total	81	100

The majority of the participants they do not have such problem in their family however, 38.27% of the family do have a history of such disease.

10. 1. 15. Problem common in the area:

Problem of the area	No. of family	Percentage (%)
i. Yes	59	72.83
ii. No	22	27.16
Total	81	100

Most of the participants have seen such infection in their community so they thought it could be a common, however 27.16% of the people did not think so.

Table 10. 2. The socioeconomic status, educational status, occupation, knowledge, attitude and practice of the individuals participated in survey of Bancroftian filariasis from sample area: Sipwa, Bhairahawa

10. 2. 1. Economic status:

Status	No. of family	Percentage (%)
i. rich	0	0
ii. upper middle	0	0
iii. middle	10	24.39
iv. lower middle	25	60.97
v. poor	6	14.63
Total	41	100

The residents of this Sipwa village were predominantly of lower middle class which is followed by middle class family. Some are even poor.

10. 2. 2. Educational status:

Status	No. of family	Percentage (%)
i. Illiterate	27	65.85
ii. Literate with informal education	1	2.43
iii. Grade 1-5	4	9.75
iv. Grade 6-8	5	12.19
v. Grade 9-10	1	2.43
vi. Above SLC	3	7.31
Total	41	100

Most of the participants were illiterate and about 7% of the participants were above SLC.

10. 2. 3. Main occupation:

Occupation	No. of family	Percentage (%)
i. Agriculture	34	82.92
ii. Livestock	0	0
iii. Business	1	2.43
iv. Teaching (primary school)	3	7.31
v. Employee	2	4.87
vi. Labour	0	0
vii. House work	0	0
viii. Student	1	2.43
ix. Unemployed	0	0
x. Others	0	0
Total	41	100

This table indicates that the participant's main occupation is agriculture. Hardly few others have some other profession like primary school teacher.

10. 2. 4. Current residential status:

Residency	No. of family	Percentage (%)
i. Birthplace	37	90.24
ii. Migrated	4	9.75
iii. Temporary	0	0
Total	41	100

10. 2. 4. 1. Migrated:

Residency	No. of family	Percentage (%)
i. 1 - 5 years	0	0
ii. 6 - 10 years	0	0
iii. 11 - 15 years	0	0
iv. > 15 years	4	9.75

Most of the participants were native and less than 10% were migrated and were living permanently for more than 15 years.

10. 2. 5. Surrounding environmental condition:

Environment	No. of family	Percentage (%)
i. Clean	33	80.48
ii. Lagoon	2	4.87
iii. Dirty	6	14.63
iv. Bushy	2	4.87
v. Open drainage	1	2.43
Total	41	100

The surrounding shows basically clean except in some houses that were dirty (damp).

10. 2. 6. Knowledge of filariasis:

Knowledge	No. of family	Percentage (%)
i. Yes	24	58.53
ii. No	17	41.46
Total	41	100

10. 2. 6. 1. If Yes:

Knowledge	No. of family	Percentage (%)
i. Mother to fetus	0	0
ii. Contact with diseased person	0	0
iii. Mosquito biting	18	43.90
iv. Sand flies	4	9.75
v. curse	0	0
vi. Any other	2	4.87

The table shows that 24 (58.53%) of the participants knew about the disease filariasis and 18 (43.90%) even knew about the transmission of the disease.

10. 2. 7. Have you seen the person suffering from the disease filariasis:

Knowledge	No. of family	Percentage (%)
i. Yes	28	68.29
ii. No	13	31.70
Total	41	100

The table indicates that about 68% of the participants knew about the disease.

10. 2. 8. Are you suffering from the disease filariasis:

Disease	No. of family	Percentage (%)
i. Yes	25	60.97
ii. No	16	39.02
Total	41	100

About 61% of the participants knew that they were suffering from filariasis.

10. 2. 9. Knowledge of the individual:

Knowledge	No. of family	Percentage (%)
a. Preventable	32	78.04
b. Non-preventable	6	14.63
c. Not known	3	7.31
Total	41	100

The majority of the participants think that it is preventable.

10. 2. 10. Type of treatment

Treatment	No. of family	Percentage (%)
i. Traditional		
a. Dhami	0	0
b. Puja	8	19.51
c. Any other	0	0
ii. Medicinal		
a. Ayurvedic	1	2.43
b. Allopathic	32	78.04
iii. Not adopt any	0	0
Total	41	100

About 20% of individuals believe of puja as a part of treatment.

10. 2. 11. Places for allopathic treatment:

Place	No. of family	Percentage (%)
i. Hospital	12	29.26
ii. Health post	10	24.39
iii. Health personnel	2	4.87
iv. Drug store	4	9.75
v. Others	13	31.70
Total	41	100

About 31 % of the participants seek the treatment from private clinic.

10. 2. 12. Way of treatment:

Types of treatment	No. of family	Percentage (%)
i. Drug	34	82.92
ii. clean the surrounding	0	0
iii. Both	2	4.87
iv. Any other	5	12.19
Total	41	100

This table indicates that, about 83% of the participants prefer drug is a choice of treatment.

10. 2. 13. Feeling about filariasis:

Problem	No. of family	Percentage (%)
i. Serious problem	14	34.14
ii. No problem	0	0
iii. Ordinary problem	20	48.78
iv. Do not know	7	17.07
Total	41	100

According to the table, 10. 2. 3, 48.78% of the participants think that it is an ordinary problem however, 34.14% think it as a serious problem.

10. 2. 14. Occurrence of the problem in the family:

Problem in the family	No. of family	Percentage (%)
i. Yes	19	46.34
ii. No	22	53.65
Total	41	100

This Table shows that about 46% of the participants had the problem of filariasis in their family.

10. 2. 15. Problem common in the area:

Problem in the area	No. of family	Percentage (%)
i. Yes	25	60.97
ii. No	16	39.02
Total	41	100

According to the participant's view, most think that it is a common to their area.

Table 10. 3. The socioeconomic status, educational status, occupation, knowledge, attitude and practice of the individuals participated in survey of Bancroftian filariasis from sample area: Palpa

10. 3. 1. Economic status:

Status	No. of family	Percentage (%)
i. Rich	0	0
ii. Upper middle	1	8.33
iii. Middle	2	16.66
iv. Lower middle	7	58.33
v. Poor	2	16.66
Total	12	100

10. 3. 2. Educational status:

Status	No. of family	Percentage (%)
i. Illiterate	3	25.0
ii. Literate with informal education	5	41.66
iii. Grade 1-5	0	0
iv. Grade 6-8	0	0
v. Grade 9-10	2	16.66
vi. Above SLC	1	8.33
Total	12	100

10. 3. 3 Main occupation of the participants:

Occupation	No. of family	Percentage (%)
i. Agriculture	9	75
ii. Livestock	0	0
iii. Business	0	0
iv. Teaching	0	0
v. Employee	0	0
vi. Labour	3	25
vii. House work	0	0
viii. Student	0	0
ix. Unemployed	0	0
x. Others	0	0
Total	12	100

Most participants of the area were of lower middle class, illiterate and solely dependent on agriculture work.

10. 3. 4. Current residential status:

Residency	No. of family	Percentage (%)
i. Birthplace	4	33.33
ii. Migrated	8	66.66
iii. Temporary	0	0
Total	12	100

10. 3. 4. 1. Migrated:

Residency	No. of family	Percentage (%)
i. 1 - 5 years	0	0
ii. 6 - 10 years	0	0
iii. 11 - 15 years	4	33.33
iv. > 15 years	4	33.33

10. 3. 5. Surrounding environmental condition:

Environment	No. of family	Percentage (%)
i. clean	11	91.66
ii. Lagoon	0	0
iii. Dirty	1	8.33
iv. Bushy	0	0
v. Open drainage	0	0
Total	12	100

Basically the surrounding of the participant's houses were clean and the majority of the participants were migrated from other parts of Nepal.

10. 3. 6. Knowledge of filariasis:

Knowledge	No. of family	Percentage (%)
i. Yes	6	50
ii. No	6	50
Total	12	100

10. 3. 6. 1. If Yes:

Knowledge	No. of family	Percentage (%)
i. Mother to fetus	0	0
ii. Contact with diseased person	0	0
iii. Mosquito biting	5	41.66
iv. Sand flies	0	0
v. Curse	0	0
vi. Others	7	58.33
Total	12	100

The knowledge about filariasis is variable. Half of the participants did not have the knowledge about the filariasis. Five of twelve knew the actual means of transmission of the disease.

10. 3. 7. Have you seen the person suffering from the disease filariasis:

Knowledge	No. of family	Percentage (%)
i. Yes	5	41.66
ii. No	7	58.33
Total	12	100

The knowledge about the disease was variable.

10. 3. 8. Are you suffering from the disease filariasis:

Disease	No. of family	Percentage (%)
i. Yes	7	58.33
ii. No	5	41.66
Total	12	100

10. 3. 9. Knowledge of the individual:

Knowledge	No. of family	Percentage (%)
a. Preventable	12	100
b. Non-preventable	0	0
c. Do not know	0	0
Total	12	100

Surprisingly all said that the disease is preventable.

10. 3. 10. Type of treatment

Treatment	No. of family	Percentage (%)
i. Traditional		
a. Dhami	0	0
b. Puja	0	0
c. Any other	0	0
ii. Medicinal		
a. Ayurvedic	2	16.66
b. Allopathic	10	83.33
iii. Not adopt any	0	0
Total	12	100

Allopathic is the most preferred way of the treatment by the participants.

10. 3. 11. Places for allopathic treatment:

Place	No. of family	Percentage (%)
i. Hospital	6	50
ii. Health post	4	33.33
iii. Health personnel	1	16.66
iv. Drug store	0	0
v. Others	0	0
Total	12	100

Majority of the participants recommended going to the hospital then only health personnel.

10. 3. 12. Way of treatment:

Types of treatment	No. of family	Percentage (%)
i. Drug	11	91.66
ii. clean the surrounding	0	0
iii. Both	0	0
iv. Any other	1	8.33
Total	12	100

Almost all had a feeling that they will be treated with the drug.

10. 3. 13. Feeling about filariasis:

Problem	No. of family	Percentage (%)
i. Serious problem	5	41.66
ii. No problem	0	0
iii. Ordinary problem	6	50
iv. Do not know	1	8.33
Total	12	100

The knowledge about filariasis shows a bit conflicting. About 41% of the participants think it as a serious problem and 50% say an ordinary problem.

10. 3. 14. Occurrence of the problem in the family:

problem in the family	No. of family	Percentage (%)
i. Yes	3	25
ii. No	9	75
Total	12	100

The majority of the participants did not have had a family history.

10. 3. 15. Problem common in the area:

Problem in the area	No. of family	Percentage (%)
i. Yes	5	41.66
ii. No	7	58.33
Total	12	100

Most of them do not think as common problem of the area.

Table 10. 4: The socioeconomic status, educational status, occupation, knowledge, attitude and practice of an individuals participated in survey of Bancroftian filariasis in total of three different geographical regions (Terai, Inner Terai and Hill): In Total

10. 4. 1. Economic status:

Status	No. of family	Percentage (%)
i. rich	0	0
ii. upper middle	3	2.2
iii. middle	38	28.35
iv. lower middle	85	63.43
v. poor	8	5.97
Total	134	100

The overall of the participants were basically lower middle class followed by middle.

10. 4.2. Educational status:

Status	No. of family	Percentage (%)
i. illiterate	77	57.46
ii. literate with informal education	10	7.46
iii. grade 1-5	9	6.71
iv. grade 6-8	8	5.97
v. grade 9-10	12	8.95
vi. above SLC	17	12.68
Total	134	100

Majority of the participants were illiterate. Only around 12% of the cases were above SLC education.

10. 4. 3. Main occupation:

Occupation	No. of family	Percentage (%)
i. Agriculture	106	79.10
ii. Livestock	0	0
iii. Business	8	5.97
iv. teaching	4	2.98
v. Employee	3	2.23
vi. Labour	3	2.23
vii. House work	6	4.47
viii. Student	4	2.98
ix. Unemployed	0	0
Others	0	0
Total	134	100

Occupationally around 80% of the participants were agricultural dependent.

10. 4. 4. Current residential status:

Residency	No. of family	Percentage (%)
i. Birthplace	114	85.07
ii. Migrated	20	14.92
iii. Temporary	0	0
Total	134	100

10. 4. 4. 1. Migrated:

Residency	No. of family	Percentage (%)
i. 1 - 5 years	0	0
ii. 6 - 10 years	0	0
iii. 11 - 15 years	4	2.98
iv. > 15 years	16	11.94

Majority of the participants were native and those migrated were about 15% and living there for more than 10 years.

10. 4. 5. Surrounding environmental condition:

Environment	No. of family	Percentage (%)
i. clean	94	70.14
ii. Lagoon	13	9.70
iii. Dirty	25	18.65
iv. Bushy	3	2.23
v. Open drainage	2	1.49
Total	134	100

Around 70% participants lived in clean environment; the 30 % lived in different categories of unhealthy environment.

10. 4. 6. Knowledge of filariasis:

Knowledge	No. of family	Percentage (%)
i. Yes	56	41.79
ii. No	78	58.20
Total	134	100

10. 4. 6. 1. If Yes:

Knowledge	No. of family	Percentage (%)
i. Mother to fetus	0	0
ii. Contact with diseased person	0	0
iii. Mosquito biting	27	20.14
iv. Sand flies	4	2.98
v. curse	0	0
vi. Any other	27	20.14

Majority of the individuals of the family understood of vector borne disease.

10. 4. 7. Have you seen the person suffering from the disease filariasis:

Knowledge	No. of family	Percentage (%)
i. Yes	104	77.61
ii. No	30	22.38
Total	134	100

10. 4. 8. Are you suffering from the disease filariasis:

Disease	No. of family	Percentage (%)
i. Yes	94	70.14
ii. No	40	29.85
Total	134	100

10. 4. 9. Knowledge of the individual:

Knowledge	No. of family	Percentage (%)
a. Preventable	66	49.25
b. Non-preventable	53	39.55
c. Do not know	15	11.19
Total	134	100

Majority of the participants had seen the filariasis cases and 70% of family looked suffering from filariasis. 50% of family believed that the disease filariasis is preventable.

10. 4. 10. Type of treatment

Treatment	No. of family	Percentage (%)
i. Traditional		
a. Dhami	0	0
b. Puja	8	5.97
c. Any other	0	0
ii. Medicinal		
a. Ayurvedic	41	30.59
b. Allopathic	83	61.94
iii. Not adopt any	2	1.49
Total	134	100

10. 4. 11. Places for allopathic treatment:

Place	No. of family	Percentage (%)
i. Hospital	56	41.97
ii. Health post	16	11.94
iii. Health personnel	30	22.38
iv. Drug store	18	1.43
v. Any others	14	10.44
Total	134	100

10. 4. 12. Way of treatment:

Types of treatment	No. of family	Percentage (%)
i. Drug	116	86.56
ii. Clean the surroundings	2	1.49
iii. Both	8	5.97
iv. Any other	8	5.97
Total	134	100

About 87% of the family believed that drug is the choice of treatment although, 62% preferred allopathic treatment and 50% seek the treatment from the hospital.

10. 4. 13. Feeling about filariasis:

Problem	No. of family	Percentage (%)
i. Serious problem	66	49.25
ii. No problem	6	4.47
iii. Ordinary problem	52	38.80
iv. Do not know	10	7.46
Total	134	100

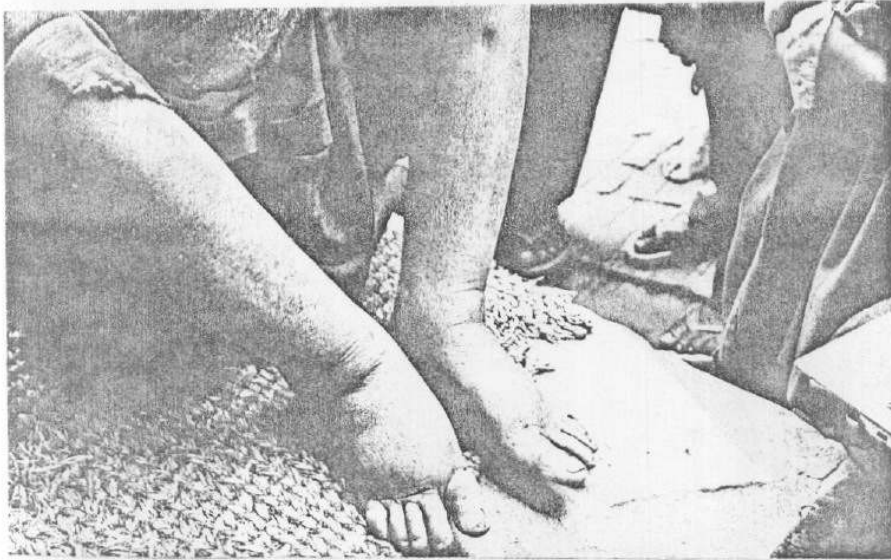
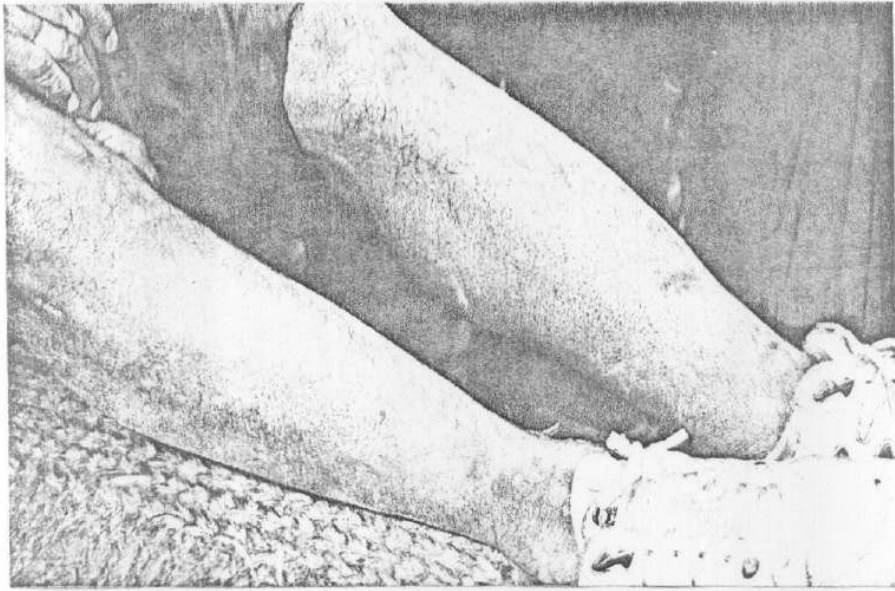
10. 4. 14. Occurrence of problem in the family:

Problem in the family	No. of family	Percentage (%)
i. Yes	53	39.55
ii. No	81	60.44
Total	134	100

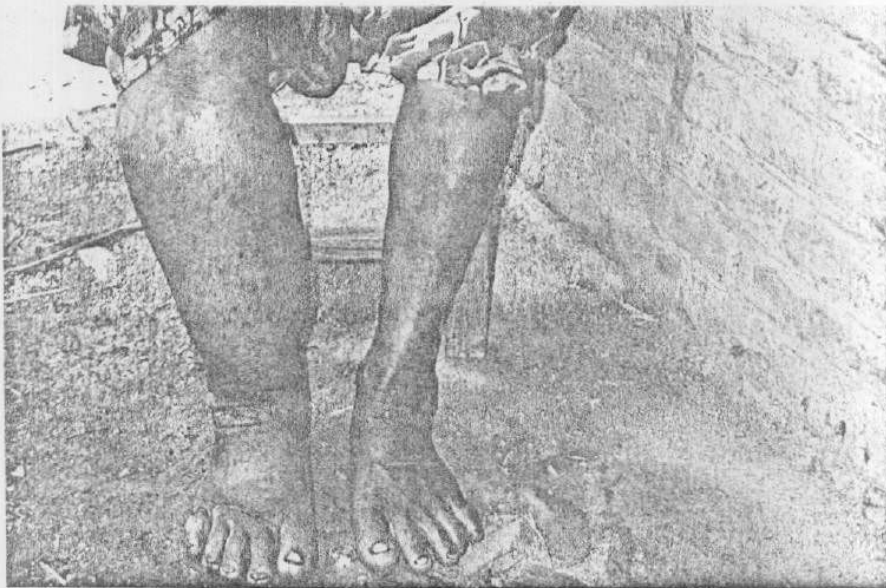
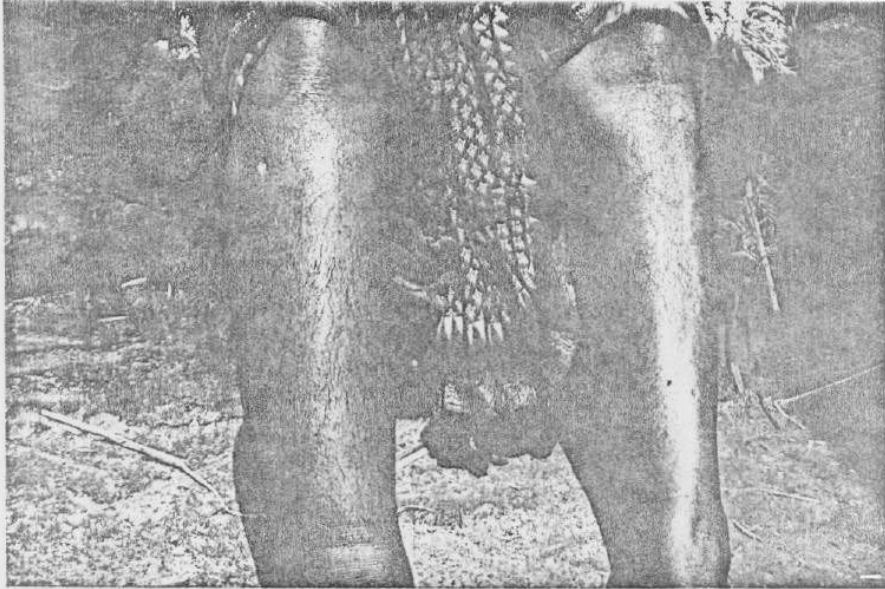
10. 4. 15. Problem common in the area:

Problem of the area	No. of family	Percentage (%)
i. Yes	89	66.41
ii. No	45	33.58
Total	134	100

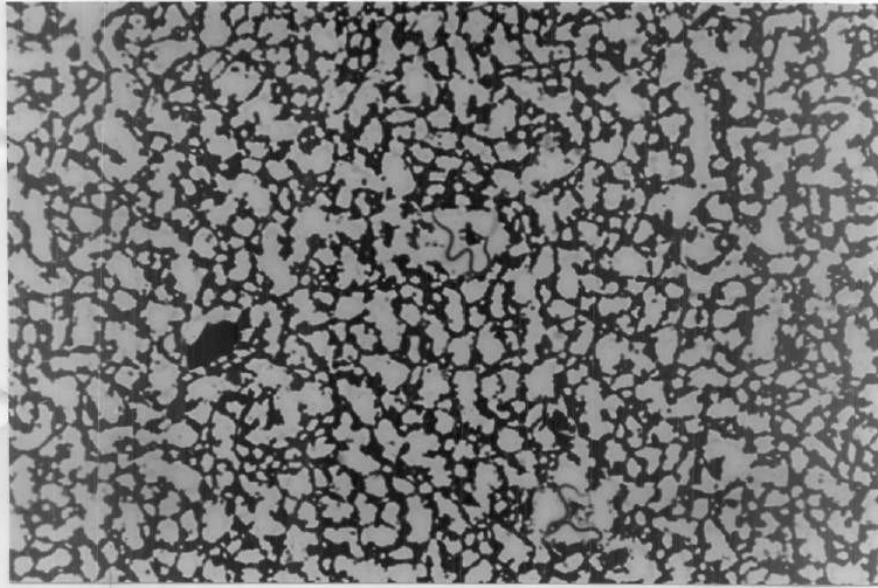
66% of the family knew about the problem within their area. 50% of the family thought as a serious problem as a handicapped feeling and 40% of the surveyed family had the filariasis in their family.



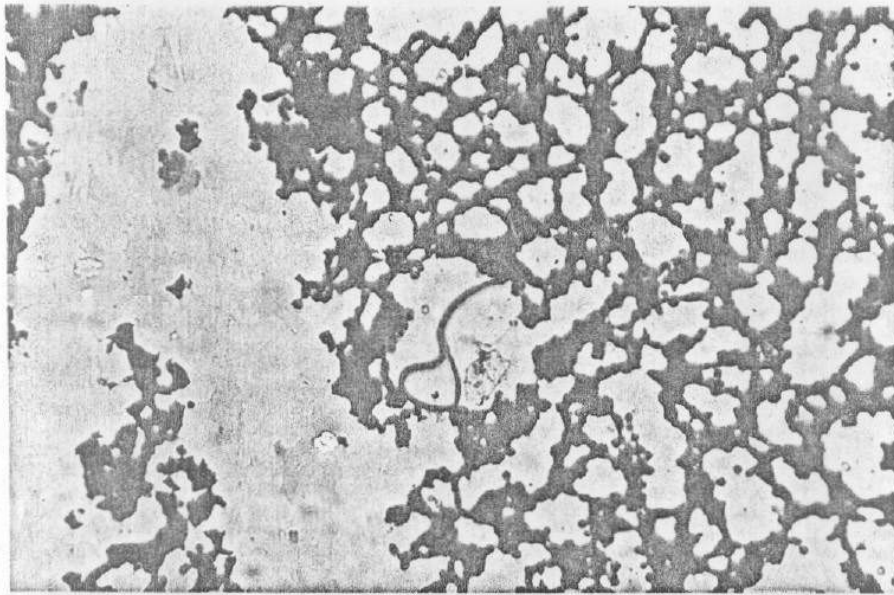
Patients showing leg elephantiasis



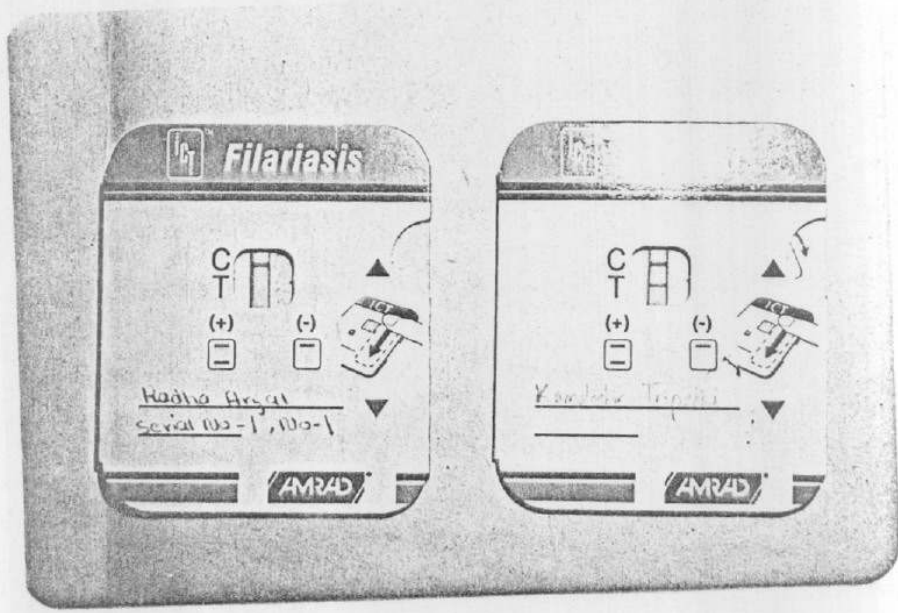
Patients showing leg elephantiasis



Microfilaria under microscope 100X



Microfilaria under microscope 200X



ICT card showing both negative (left) and positive (right) result

Discussion:

The clinical and parasitological surveys for lymphatic filariasis were carried out in three community of different geographical location Terai, Innerterai and Hill reported as epidemic of Filariasis from the Ministry of Health¹⁸. If the place is common course in lymphatic filariasis the environment factors and standard of hygiene might be important determinants of the level of disease in an affected community. Hence the environmental factors and human behavior pattern were closely studied in this survey. The densities of vector mosquito population, the climate (humidity), the dwelling and other human behavior pattern appear favorable for filariasis^{6,8,10}

At time of study, in Sipwa village of Bhairawa (terai town) and Dovan village of Palpa (Inner terai) near to Butwal town, the most houses had mud wall and thatched roof and family do not use bed nets. There are places of stagnant water, this environmental situation is different from the survey area of Bhaktapur, many houses are of 3/4 stories and outside environment is clean but inside the house open drainage known as Sagaa in local language is also noticed. In this survey the individuals of all age-group predominantly male participated in terai and inner terai; where as in Bhaktapur female participated more. In 111 (21.67%) of the cases the the clinical manifestation were noticed particularly from the age groups of 31 to 70 plus. The similar findings were reported from other parts of the globe including Indian subcontinent^{20,21,23,24,25,26} The participants in this study are classified in two groups; chronic filarial patients if there is clinical manifestation elephantiasis/ hydrocele and asymptomatic carrier if blood examination / ICT Card method showed positive but had no clinical symptom.

The survey was conducted from Jan. 2001 to Nov. 2001. Before any work started meeting was held with Public Health Officer/ village chief. Then the individual of the family were examined for clinical manifestation of lymphatic filariasis in leg/genitalia; and were then analysed. In Bhaktapur district 4 places Golmadhi, Etachhe, Byasi and

Katunge were surveyed where the number of houses recorded were 472, 456, 489, 450 respectively. The total number of houses of these 4 areas are 1867. The total population reported is 16434 (Golmadhi 4528, Etachhe 5245, Byasi 4161 and Katunje 2500). Total sample of 512 were collected. Microfilaria was detected in 39 cases showing 0.23% of the total population; and antigenemia were detected in 52 cases by ICT Card technique showing 0.31% of the population. Of the 85 samples collected from Sipwa the clinical manifestation cases were 22 (2 elephantiasis and 20 hydrocele). Similarly, 17 specimens were collected from Palpa, of which clinical manifestations were 4 and all were hydrocele. From Bhaktapur district 410 samples were collected of which there were 82 cases of clinical manifestation and all were elephantiasis. In terai and inner terai, the most common chronic clinical manifestation observed was hydrocele, whereas in hill area the most common was lymphatic filariasis in leg. The correlation between leg elephantiasis and hydrocele could not be explained. The microfilaria could not be detected from urine collected from the individuals of the surveyed area of chyluria cases. Some study²⁷ reported that leg elephantiasis had also hydrocele particularly in elderly group. This study showed that hydrocele cases were common in terai where as leg elephantiasis were more common in hilly area. There was no case of leg elephantiasis as well as hydrocele among 512 individual surveyed. The parasitological survey was carried out with multiple diagnostic technique to know the prevalence of filariasis. In spite of this, no cases microfilaraemia were detected in Sipwa and Dovan. Even ICT card technique did not show any positive except the one from case of hydrocele in primary school teacher.

In four areas of Bhaktapur district 39 cases of microfilaraemia were detected. Microfilaraemia prevalence was higher in the family members of microfilaraemia mother/ individual than in those of amicrofilaraemia mother. In this study the microfilaraemia has not been quantified. Although, some studies¹¹ had shown that many children born by microfilaraemia mother are immunologically sensitized to filarial antigens. Of the 39 microfilarimia cases from buffy coat 15 cases were detected from thin smear and 27 cases were detected from thick smear. This finding suggests that smear from buffy coat is the best to find the microfilaria from night blood. In ICT card technique found positive in 52 cases, showing that this method appeared superior for field

study. Although this method is quite expensive for the poor country like Nepal it is convenient to use. The major advantage of ICT card is that there is no need of night blood (usually taken between 21.00 - mid-night). The collection of night blood is very laborious and tedious. Blood smear negative were found ICT card positive in 52 cases among the 39 microfilarimic cases. This study shows microfilaraemia was uncommon in age group below 20 and 21-30. However, from age of 31 onward we noticed more cases of elephantiasis of which prevalence in women is higher than men. Again more than one members from single were also observed. The other studies¹⁴⁹ showed that microfilarial prevalence in the community increased with age and males generally had a higher prevalence than female of her same age group. In another study¹⁵⁰ showed the female over the age of 40 years had a higher than the male. In this study age and sex dependent microfilaraemia prevalence was not observed significantly. The adult worm was not found from any cases of the individual in the survey.

Conclusion

Bancroftian filariasis was a century old problem and still existing in many part of Nepal as an epidemic. A survey of three geographical regions conducted between Jan.2001 – Nov.2001 showed the clinical manifestations in all the places. Detection of parasite from microfilaraemia cases was found low compared with the laboratory diagnosis of antigenemia in *Wuchereria bancrofti* infection. In Bhaktapur district, many cases of leg elephantiasis were observed. The prevalence was found 0.31% in population of 16434 from a total houses 1867 of four places in Bhaktapur district. Hydrocele cases were found more in terai and inner terai area but microfilaria was not detected from the urine specimens.

Recommendation:

1. Health Education is emphasized to improve the sanitation in the community.
2. Method of detection for circulating filarial antigens for rapid diagnosis of Filariasis instead of taking night smear.
3. Microscopy of buffy coat smear of night blood is of choice if negative in thick smear.
4. Use of bed net or window net
5. Mosquito free environment or improvement in environmental sanitation.
6. Launch of immunization in the epidemic area.

Questionnaires for Filariasis Research in Nepal

1. Serial No: _____ 2. Area Code: _____ 3. Date: _____
4. Season (date of collection): 1) 2)..... 3).....
5. Ethnicity: i) Brahman, ii) Chhetri, iii) Newar iv) Tamang v) any other
6. Family Table:

No	Name	Age	Sex	Problem
1.				
2.				
3.				
4.				
5.				
7. Economic Status:
 - (i) Rich (ii) Upper middle (iii) Middle (iv) Lower middle (v) Poor
8. Educational status:
 - i) Illiterate ii) Literate with informal education iii) Grade (1 - 5)
 - iv) Grade (6 - 8) v) Grade (9 - 10) vi) Above (Above SLC)
9. Main occupation of the participant?
 - i) Agriculture ii) Livestock iii) Business iv) Teaching v) Employee
 - vi) Labour vii) Housework viii) Student ix) Unemployed x) Others
10. Participant's current residence status?
 - i) Birth place ii) Migrate iii) Temporary

If migrate or temporary, how long have you been living here?

 - i) 1 - 5 years ii) 6 - 10 years iii) 11 - 15 years iv) More than 15 years
11. Surrounding environmental condition of the house?
 - i) Clean ii) Lagoon iii) Dirty iv) Bushy v) Open drainage
12. Do you have the knowledge about the disease Filariasis (elephantiasis)?
 - i) Yes ii) No

If yes, how does the disease transmit, given below?

 - i) Mother to fetus ii) Contact with the diseased person iii) Mosquito biting
 - iv) Sand flies v) Curse vi) Any other
13. Have you seen the person suffering from the disease filariasis?
 - i) Yes ii) No

If yes, how many?Person.
14. Is it preventable?
 - i) Preventable (ii) Non-Preventable

15. Participant's current health status?

- i) Healthy ii) Unhealthy

If you are unhealthy, since when?years

16. Are you suffering from the disease filariasis?

- i) Yes ii) No

If yes, since when?years

17. Do you have any symptoms?

- i) Yes ii) No

If yes, which one given below?

- i) Fever ii) Headache iii) Effect on genital organ or breast iv) Swollen in the lymphnode
v) Hydrocele vi) Swelling in hands & legs vii) Thick skin
viii) Chyluria ix) Weakness x) Lazy feeling xi) Epigastric pain
xii) Nausia xiii) Abscess formation

18. What do you do to overcome (treat) the problem?

i. Adopt traditional cure

- a. Dhama b. Puja c. Anyother

ii. Adopt medical cure

- a. Ayurvedic treatment b. Allopathic treatment

iii. Do not adopt any particular approach.

19. Where do you go for Allopathic treatment?

- i. Hospital ii. Health post iii. Health personnel iv. Drug store
v. Any other

20. How do they treat the problem?

- i. Drug ii. Clean the surroundings iii. Both iv. Any other

21. What do you think about Filariasis?

- i. Serious problem ii. No problem iii. An ordinary problem iv. Do not know

22. Has the problem occurred in the past in your family?

- i. Yes ii. No

If yes, how frequently (whom).

23. Do you think this problem is common in your area?

- i. Yes ii. No iii. Do not know

If yes reason:

24. Types of sample taken

- i. Urine ii. Blood iii. Filter paper iv. Slide v. Other



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 A division of AMRAD Operations Pty Ltd ACN 053 997 178

ICT Filariasis for Whole Blood

25 Tests

Catalogue No. FL02

Part No. 204 Rev.2

ICT FILARIASIS WHOLE BLOOD A RAPID

IMMUNOCHROMATOGRAPHIC TEST
 for the qualitative detection of
Wuchereria bancrofti antigen
 For in vitro diagnostic use. Potents pending

Test Principle

The ICT Filariasis is an in vitro immunodiagnostic test for the detection of *Wuchereria bancrofti* antigen in whole blood. The test utilises a polyclonal antibody (PA) and a monoclonal antibody (MAb) specific for *W. bancrofti*. The PA is attached to colloidal gold and impregnated into the pink and white areas of the pad. In the first step the sample is added to the lower area of the pink & white pad. The majority of the red blood cells will be retained in the white area of the pad and serum will flow forward into the pink area allowing any *W. bancrofti* antigen present to bind to the colloidal gold-labelled PA. The MAb is immobilised in a line across the membrane. When the card is closed, the sample and the labelled PA on the pink area of the pad contact the end of the membrane. The sample and the labelled PA then migrate along the membrane crossing the immobilised MAb line. In a positive sample, any *W. bancrofti* antigen complexed with the gold-labelled PA is captured by the MAb on the membrane and a pink line forms. In a negative sample, no gold-labelled PA is captured by the MAb on the membrane and no pink line forms.

Specimen Collection

Capillary or venous blood collected in an EDTA or heparin coated container may be used.

- A) Finger prick blood collection. Collect the sample in a 100µl calibrated capillary. When sampling, care should be taken to avoid air bubbles in the capillary used to collect the blood. Should bubbles occur fill the capillary past the 100µl line to compensate.
- B) Collect venous blood by the standard venipuncture procedure into an EDTA or heparin tube.

Sample Preservation

Blood samples collected in a capillary should be used immediately. Venous blood can be used for up to three days if stored at 4° to 8°C.

Precautions and Warnings

- Optimal results will be obtained by strict adherence to this protocol. Reagents must be added carefully to maintain precision and accuracy. Do not reuse test cards.
- Biological contamination of dispensing equipment, containers or reagents can lead to false results. Observe established precautions against microbiological and serological hazards in specimen handling, disposal and throughout all procedures.
- Do not use components past expiration date.
- Keep storage boxes dry.

Limitations of Procedure

- This test is structured to indicate the presence or absence of *W. bancrofti* antigen in the sample. The absence of antigen does not exclude Filariasis caused by other nematode species.

Kit Contents

- 25 individually packaged test cards.
- 1 product insert.

RESULTS INTERPRETATION

C=Control
 T=Test



Positive Test Result

The test is positive if two lines (C and T) are seen in the viewing window. Any visible line in the T area indicates a positive test result. The test is positive even when the T line appears lighter or darker than the C line.

Negative Test Result

The test is negative if only the C line is seen. To ensure that low positive samples have had sufficient time to develop, a negative result should not be recorded until 15 minutes have elapsed from when the card is closed.

Invalid Test Result

The test is invalid if the C line does not appear. If this occurs, the test should be repeated.

Test Procedure

1. Remove the test card from the pouch just prior to use. Open the card and lay it flat on the work surface.

2. Remove and discard the adhesive liner. Ensure that the adhesive on the left hand side of the test card is exposed.

3. Fill the capillary tube to the 100µl mark using capillary action, with blood from either a finger or heel puncture.

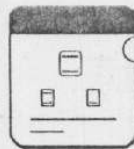
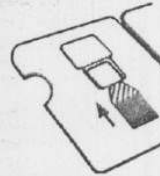
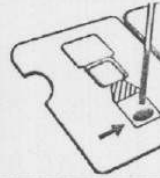
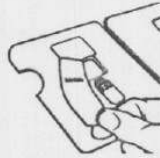
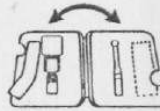
Note: When using venous blood, either a calibrated capillary or calibrated pipette may be used.

4. Add the 100µl of whole blood slowly from the capillary or the pipette onto the lower area of the pink & white pad as shown. Wait for each drop to soak in before adding the next drop. Should there be blood remaining in the capillary that will not flow out freely the tip may be gently pressed against the pad.

5. Wait until the pink area is completely wet with serum (this should take about 30 seconds to 1 minute).

6. Close the card. To ensure good test flow, press very firmly along the entire area to the right of the window. Start timing.

7. Read the result through the viewing window after two minutes. Low positive results may require up to 15 minutes to develop. Refer to the details given in this insert for test interpretation.



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