Research

Mapping of lymphatic filariasis in Nepal

Jeevan B Sherchand[1], Valérie Obsomer[2], Garib Das Thakur[3] and Marcel Hommel*[2]

Address: [1]Infectious & Tropical Disease Research and Prevention Centre, Tripureswar, TripuraPath H.N: 416, Kathmandu P.O. Box: 10404, Nepal, [2]Liverpool School of Tropical Medicine, Disease Control Strategy Group, Pembroke Place L3 5QA, Liverpool, United Kingdom and [3]Ministry of Health, Vector Borne Disease Research and Training Centre, Hetauda, Nepal

Email: Jeevan B Sherchand - jeevan@itdrc.healthnet.org.np; Valérie Obsomer - vobsomer@iri.columbia.edu; Garib Das Thakur - thakur85@hotmail.com; Marcel Hommel* - mhommel@liv.ac.uk

* Corresponding author

Abstract

Background: Human infection with *Wuchereria bancrofti* causes a disabling parasitic disease known as lymphatic filariasis, which is a major public health and socio-economic problem in many parts of the world. At the onset of the study, little was known of the distribution of filariasis and its current importance as a public health problem in Nepal.

Methods: Epidemiological mapping was undertaken to determine the prevalence of infection by *Wuchereria bancrofti* in 37 districts of Nepal between July to December 2001. The study population above 15 years of age was selected, and the immunochromatographic test (ICT Filariasis) was used to screen for circulating filarial antigen (CFA).

Results: The overall prevalence of lymphatic filariasis from a 4,488-sample population was 13% and 33/37 districts were found to be endemic. On the basis of geographical data, the highest number of cases was found at altitudes between 500–700 m; however, a substantial number of infected individuals were found in the highly populated Kathmandu valley, at altitudes between 900–1,500 metres where transmission appears to take place. Prevalence rates above 20% were found in 11 districts (with the highest rate of 40%), 6–19% were found in 15 districts, and 0.1–5% were in 7 districts.

Information on people's knowledge, attitudes and behaviour towards filariasis was also collected by means of a structured questionnaire, which is presented and discussed in the study.

Conclusions: This is the most extensive study of lymphatic filariasis undertaken to date in Nepal. The study indicates that the prevalence of infection is far greater that was previously reported and that lymphatic filariasis should be a much higher health priority than currently given.

Background

Lymphatic filariasis (LF) is disabling parasitic disease that has been identified by the World Health Organization (WHO) as a major public health problem with an increasing prevalence worldwide [1]. At present, 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*. Over one third of the population at risk lives on the Indian sub-continent with an estimated 45 million infected individuals [2].

Approximately one third of infected individuals present with overt clinical manifestations: lymphoedema and elephantiasis of the limbs or genitals, hydrocoele, chyluria, pneumonitis, or recurrent infections associated with damaged lymphatics; the remainder are at the pre-clinical stage of infection (most often with microfilaria in their blood). To this burden of disease, must also be added the serious psychosocial consequences that these profoundly disabling lesions often have. The disease is thought to be caused primarily by adult worms living in the lymphatic vessels; the microfilariae released by the female worms circulate in the peripheral blood and are not harmful, but can be ingested by mosquitoes, which transmit the infection from person to person.

Diagnosis of bancroftian filariasis relied until recently almost exclusively on the detection and identification of microfilariae in night blood specimens [3,4]. The reason for this is that, in most geographical areas, *W. bancrofti* microfilariae have a natural periodicity, with highest intensity in the peripheral blood at night and few or none during the day [5]. The other alternative test is a DEC provocation test, where the suspected patient is given a single oral dose of 50–100 mg of diethylcarbamazine (DEC is a safe drug, normally used for the treatment for LF), followed by a blood sample 30–45 minutes later: this procedure can "flush out" microfilaria into the peripheral blood during day time and has a sensitivity that is almost comparable to that of night blood surveys [6,7]. Tests based on detection of specific *W. bancrofti* circulating filarial antigen (CFA) have been highly successful and a number of kits have recently become commercially available. The high specificity and sensitivity of the test has been confirmed in a number of studies [8–10]. More recently, ICT Diagnostics in Australia produced a simple immunochromatographic card test (AMRAD ICT) for the detection of circulating filarial antigen in serum and whole blood specimens [10,11]. In this test, antigen capture is accomplished by a monoclonal antibody with high sensitivity and specificity to adult *W. bancrofti* antigen [10]. The test is rapid and requires no equipment, which makes it suitable for use in many endemic areas in the developing world. In Nepal, no national study on the prevalence and distribution of lymphatic filariasis has been previously attempted, apart from a limited epidemiological survey in semi-urban areas of the central part of country [12–14], which established that LF was endemic. We carried out a study in different districts of Nepal to obtain baseline data on the prevalence of filariasis using the immunochromatographic test (ICT) for CFA detection together with a questionnaire and a clinical evaluation of cases, as a preliminary step for developing a strategy to eliminate lymphatic filariasis from Nepal.

Materials and Methods
Study design
Nepal is administratively subdivided into 75 districts, of which 58 districts (20 districts are in the Terai region and 38 are hill districts) are potentially endemic for filariasis; the 17 remaining districts are in mountainous regions and unlikely to be endemic since transmission does not normally occur above 2,000 meters. This preliminary study was designed to establish the approximate extent of the lymphatic filariasis problem in Nepal and provide the justification for further investigation and eventually future intervention programmes.

The study was undertaken over a period of 6 months from July to December 2001 in 37 districts, selected from the 58 potentially endemic districts. Village development committees (VDCs) are the smallest administrative entity in Nepal; we selected two or three VDCs for each district. Random selection was not feasible in these communities. The aim of the study was explained to the villagers and a request was made for volunteers to come forward. Every subject was informed of the purpose of the study and his or her consent was obtained. The study was approved by the Ethical and Research Committees of the Nepal Health Research Council.

The survey consisted of a questionnaire and a blood sample for parasitological examination and CFA testing. We aimed to select 100 volunteers over 15 years of age per VDC; however, in view of the dispersed nature of most communities tested, the study sample per VDC was usually reduced to 50–60 (hence the decision to test 2–3 VDCs per district). Apart from the target age (above 15 years of age in this study rather than school-age children), this procedure is comparable to the Lot Quality Assurance Sampling (LQAS) or the Rapid Geographical Assessment of Bancroftian Filariasis (RAGFIL) used in other studies to estimate endemicity [15–19].

Before blood sampling all individuals who participated were interviewed, using a structured questionnaire, on their knowledge, attitude and behaviour in relation to filariasis. A record of age, sex, occupation, educational status, medical history, birthplace, travel history, and use of bednet/number of bed nets used in the household, was kept. All participants were examined with the help of the local health worker for signs of clinical filariasis i.e. elephantiasis (lymphoedema), presence of non-pitting oedema, scrotal swelling (hydrocele), breast swelling and hand swelling.

Circulating filarial antigen screening
50–100 samples were tested from two or three VDCs from each district using the ICT card; the total % of positive for these samples was taken as the prevalence rate for that

district. Two technicians worked together in this survey: one technician collected finger prick blood using a sterile lancet and a capillary tube, which was filled up to the mark of 100 μl; immediately after, the other technician performed the ICT according to manufacturer's instructions. After the blood sample was taken, the participants were asked to wait for the results. The ICT card was checked after 10 minutes looking for two lines (C and T) in the viewing window: any visible line in the T area indicates a positive test result and the test was considered negative if only the C line appeared. The test was discarded as invalid, and repeated, if either no lines appeared, or only the T line appeared (i.e. without a C line).

Parasitological test
Whenever possible, night blood collections were made from 23.00 PM- 2.00 AM. Sixty microliters of finger-pricked blood were drawn for thick blood film, air dried, then stained with Giemsa solution (dilution 1 in 20) for 15–20 minutes. The parasites were examined and counted after returning to the laboratory [20] and results compared to the ICT results.

DEC provocation test
Some patients in the study were given a single oral dose of 50 mg of diethylcarbamazine (DEC), followed by a 2nd blood sample collection 30 minutes later. This procedure enabled visualisation of parasites in situations where night sampling was not acceptable and was used as quality control for the ICT. A thick blood film was prepared, air-dried, stained and examined as for the parasitological test.

Statistics
The data were analyzed by using SPSS 10.00 for entire study http://www.spss.com/.

GIS study
A GPS record of the geographical location of all study areas was taken and will be used in future to analyse the topographic distribution of lymphatic filariasis and develop prediction maps. In this preliminary study, information has been applied to a distribution of ICT positive cases in relation to altitude.

Results
District-wise prevalence of lymphatic filariasis
The overall prevalence of lymphatic filariasis from a 4,488-sample population studied from 37 districts was 582 (13%); 33/37 districts were considered endemic on the basis of this preliminary study. A distribution of antigenemia above 20% was found in 11 districts with the highest rate of 40%, 6 to 19% were found in 15 districts, 0.1 to 5% were in 7 districts. Current categorization of endemicity level is shown in Figure 1, showing confirmed

filaria-endemic districts in RED and districts contiguous to endemic districts but where endemicity was not confirmed in GREY; no study has yet been performed in the districts in WHITE. Detailed ICT results district-by-district are given on Table 1.

Age and sex-wise distribution of antigenaemia
The sample of 4,488 was made up of 2,576 males and 1,912 females. Although, the highest positivity of antigenaemia was found among males (57.4%), the difference between male and female was not statistically significant (P > 0.005). Of the total 582 ICT-positive people, the highest positive rate of 15.8% was found in the 46–50 age group whereas the lowest (10%) was amongst individuals in the 36–40 age group (Figure 2). Considering the size of the sample, these differences are not significant.

Chronic clinical filariasis
The chronic conditions of lymphatic filariasis, namely elephantiasis (lymphoedema) and hydrocele, were prevalent in the study population. Elephantiasis alone was recorded in 94 males and 127 females. 71 males were affected by more than one sequelae of lymphatic filariasis (e.g. elephantiasis and hydrocele). Breast swelling and hand swelling was also observed. While these figures are an indication of a substantial disease burden in the communities examined, they do not accurately reflect the level of burden since the study was only performed on volunteers, rather than on a random survey basis.

Parasitology and DEC provocation test
A total of 268 samples were collected by night blood sampling and DEC provocation test: 216 of these were ICT positive and 52 were ICT negative. Both ICT positive and negative samples were examined microscopically. A rate of 38% microfilaria positivity was detected from amongst the night blood of ICT positive samples and 57.4% microfilaria positivity was detected amongst the samples from the DEC provocation test. All 52 ICT negative samples of thick smears were also negative by microscopical examination. All night blood positive for microfilaria were also found positive with DEC provocation test (Table 2).

Microfilaria density
The thick smear positive samples were counted for the level of microfilaria density. The microfilarial density was classified in three levels as 1–10, 11–50 and > 50 microfilaria per 60 μl of thick blood film (Table 3)

Geographical origin (Birth place)
The respondents were asked about their birthplace, 84.4% were local people and 15.6% had migrated from elsewhere; filariasis prevalence was 12.4 % and 16.2% for local and migrants, respectively. If the respondents were not from the locality, questions were asked about their origin

Figure 1
Administrative districts of Nepal, showing the extent of the mapping exercise. Districts found positive are coloured in red, districts found negative in grey.

and travel history within the year. Nearly 44% people said that they had visited other places, mainly Terai/India where they had spent at least one week. There was no statistically significant difference in ICT positivity between the group who had a travel history and the group who did not (P > 0.005).

Geographical distribution of lymphatic filariasis
There was little difference in ICT positivity between different altitude classes: 13.8–14.0% positivity in classes below 300 m, 300–500 and 700–900; the altitude class 500–700 is the one where there is the highest proportion of positive in the ICT tested persons (16.5%) was found, while the lowest positivity was in the 900–1,500 class (9.6%). The geographical distribution of relative ICT positivity rates is illustrated on a GIS map of Nepal (Figure 3).

Treatment received for filariasis, leprosy and tuberculosis
Individuals taking part in the study were asked about taking anti-filarial drugs and 2.5% mentioned that they had taken medicine whereas 97.5% replied negatively. Questions regarding treatment history for leprosy and tubercu-

losis (TB) within the previous two years indicated that only 4% had been treated for TB or leprosy. The distribution of CFA amongst those who had been treated for TB or leprosy and those not treated was found to be highly significant (P < 0.005): ICT positivity was 13% (578/4,312) amongst the people who did not receive any treatment for TB or leprosy compared to only 2% (4/176) amongst those who had received treatment recently.

Knowledge of lymphatic filariasis
During the study period, the sample population was asked about their knowledge of lymphatic filariasis. Most of the people recognized the signs of lymphatic filariasis particularly elephantiasis by their social experience, but they had inadequate knowledge of recognition of adenolymphangitis (ADL), hydrocele, arm swelling and breast swelling as a disease of lymphatic filariasis. Of the total respondents (1,754 out of 4,488 individuals included in the study), 49.3% said that filariasis was caused by mosquito bites, 15% mentioned flies and insects, 5% blamed bad weather, 5.6% blamed poor living status, 7.5% believed that evil spirits were responsible and 9.6% did not know.
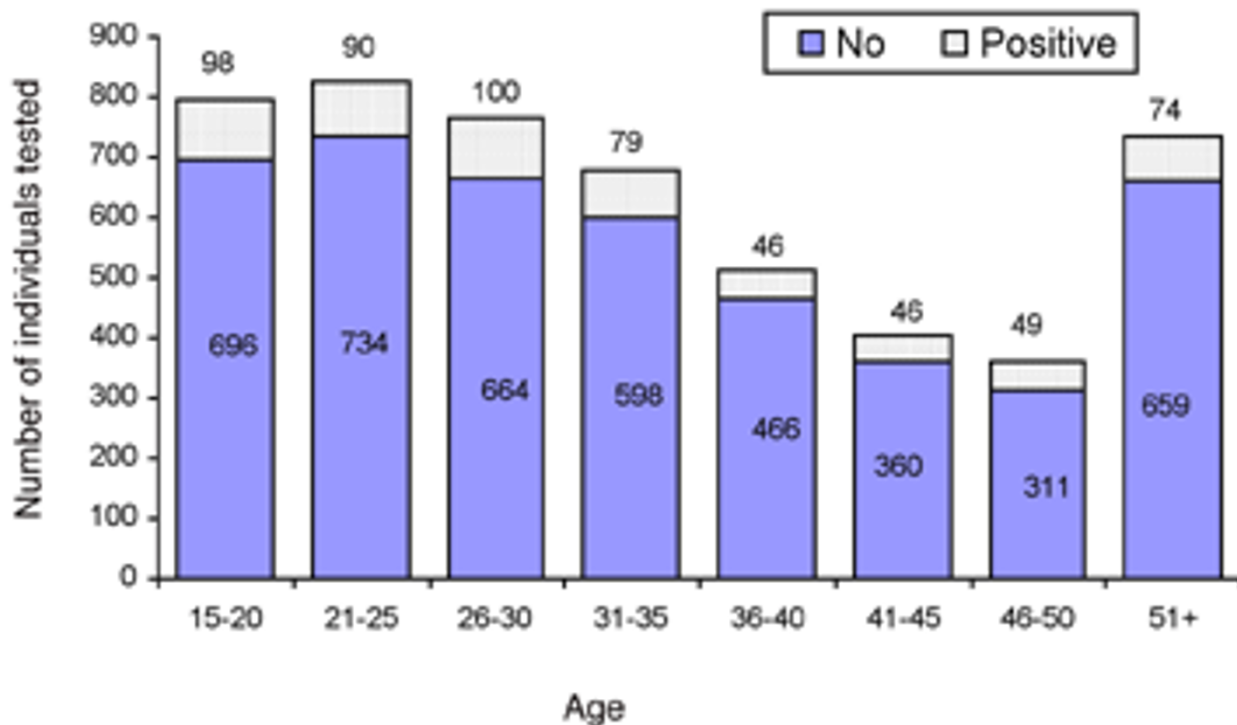
Figure 2
Age-wise distribution of antigenaemia. Negative individuals in purple, positive cases in grey

Treatment-seeking behaviour
In most rural areas, where people face many health problems, there is a general tendency to first consult the traditional healer, locally called "Dhami/Jhankri". In this study, of a total of 4,488 respondents, 30% consulted traditional healer, 38% consulted modern medicine practitioner (health assistant, doctor, nurse or private practioners), 22% used herbal medicine for self-treatment and 10% did not seek any treatment.

In answer to questions regarding other diseases, 2,625 (58.5%) replied that they were suffering from other diseases.

Use of bed-nets
Of the total number of respondents, nearly 40% people mentioned that they used bed-nets in their household. Based on the questionnaire, there was no significant difference between bed-net users and non-users with regards to the clinical signs of filarial disease, but higher ICT rates were found among non bed-net users.

Discussion and conclusions
Lymphatic filariasis has been identified as a potentially eradicable disease [18,21] and the 50th World Health Assembly 1997 passed a resolution that 'elimination of lymphatic filariasis as a public health problem' should be considered a priority by member states [18]. Delimitation of endemic localities is an essential prerequisite for planning control or elimination programmes.

The present study, in different districts of Nepal, indicates lymphatic filariasis constitutes a widespread public health problem in the country. Out of a total of 37 districts studied, 11 districts were found to have a prevalence above 20%, the highest observed rate being 40%. The finding of CFA positives (and clinical cases) in the Terai region (at an altitude between 70 and 300 metres) was to be expected in view of the rate of infection previously reported from neighbouring districts of India (e.g. 13.8% positivity in Northern Bihar) [22], but it is worth observing that the prevalence rates found in Eastern Terai districts were generally much lower (negative in Saptari, Ilam and Mahotta-
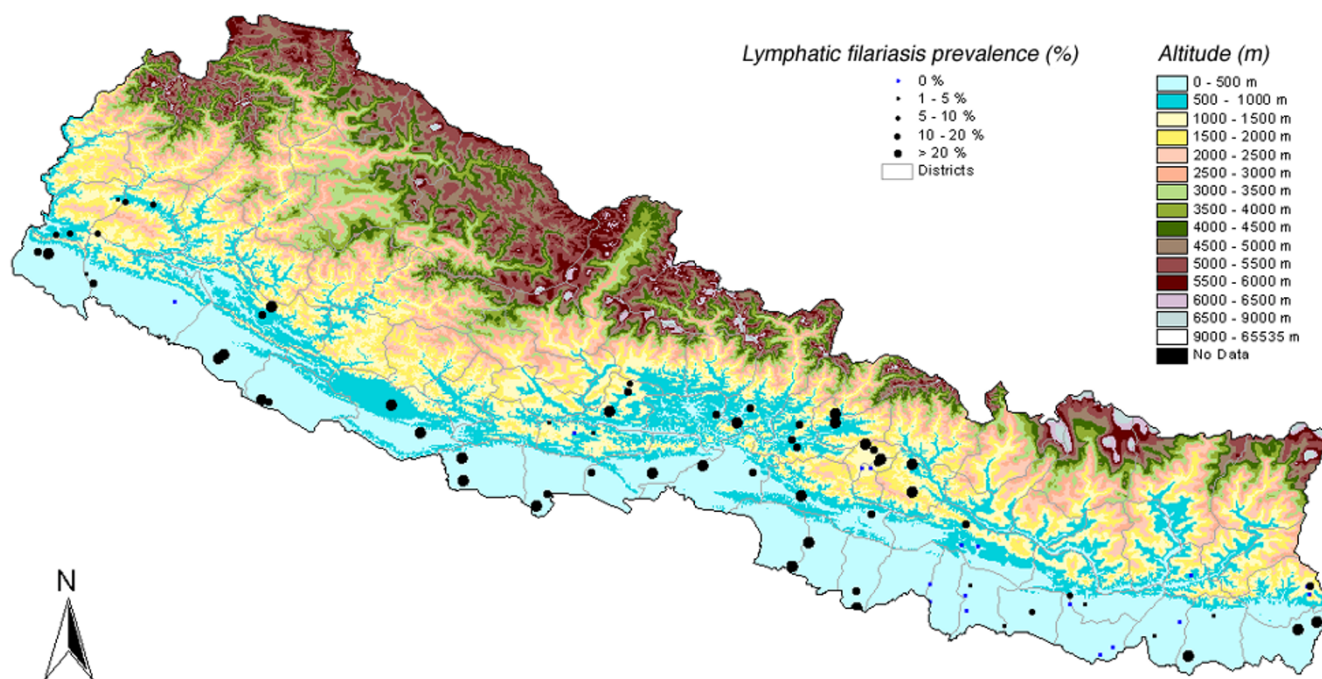
Figure 3
Geographical distribution of filariasis. Black dots indicate the site of testing and size of the dot is proportional to the percentage of prevalence. The GIS map is colour-graded with respect to altitude.

ri districts). Perhaps the most important finding in this preliminary study was the high prevalence observed in hill districts (the highest altitude tested was 1,400 metres), which was not explained by migration of population from Terai districts: this finding is important since Kathmandu valley is the most populated area of Nepal and CFA prevalence was particularly high in the VDCs tested in that area (e.g. 20% in Kathmandu and Bhaktapur, 26% in Kavre). These findings are in keeping with Jung's survey [12], which had indicated a prevalence of 25% in a small sample of the population and had identified *Culex* (*Cx.*) *quinquefasciatus* as the main vector in all the surveyed areas. A small study conducted by Pradhan et al., [13] in Gokarna VDC of Kathmandu valley reported more than 12% of microfilarial infection in the community and 12 species of mosquitoes have been identified (*i.e. Anopheles (A.) nigerrimus, A, vagus, A. willmori, A. kessele, Culex (Cx.) fuscocephala, Cx. gelidus, Cx. psedovishnui, Cx. quinquefasciatus, Cx. sinensis, Cx. vishuni, Cx. whitmori* and *Cx. tritaeniorhynchus*); in this study *Cx. quinquefasciatus* was also found to be the predominant species. In another VDC of Kathmandu valley (Tokha-Chandeshwori VDC), a night blood survey of 978 villagers showed a prevalence of 5.8% microfilaria by thick blood films and 9.6% of villagers were found to present clinical signs and symptoms of

filariasis [14]. It will be necessary, in further studies, to define the precise geographical limits of the endemic zone (particularly the maximal altitude, since many of Nepal districts are mountainous) and whether transmission occurs in urban areas as well as rural areas. Others have suggested that an increased prevalence of the disease may be a consequence of changes in the demographic characteristics of at-risk countries. Crowded living conditions, housing quality, and inadequate waste disposal and sanitation facilities combined with seasonal migration between endemic rural areas and non-endemic urban areas have all been shown to contribute to the growing "urbanization" of the disease [23,24].

Lymphatic filariasis occurs in individuals of all ages and both sexes but prevails in those of low socioeconomic status [17]. As the chronic manifestations of lymphatic filariasis appear most frequently later in life, clinical and pathological investigations have focused on the adult population. Our present study indicates that the prevalence of chronic forms of lymphatic filariasis is age-dependent in both sexes, but generally higher in males than in females. This observation is in keeping with similar studies of chronic filarial conditions from South India

Table 1: District-wise prevalence of lymphatic filariasis in Nepal

Districts	ICT-Positive (%)	Total
Ilam	0 (0.0)	102
Jhapa	24 (24.0)	100
Morang	14 (13.6)	103
Sunsari	1 (1.0)	101
Udayapur	6 (4.0)	150
Siraha	5 (3.3)	152
Dhanusha	1 (0.7)	152
Mahottari	0 (0.0)	102
Sindhuli	7 (4.7)	150
Saptari	0 (0.0)	100
Rautahat	24 (19.0)	126
Kavre	26 (26.0)	100
Kathmandu	22 (20.0)	110
Lalitpur	0 (0.0)	100
Banke	21 (20.8)	101
Kailali	9 (6.0)	151
Kanchanpur	20 (20.0)	100
Bhaktapur	20 (19.8)	101
Kapilbastu	24 (24.0)	100
Makawanpur	17 (16.8)	101
Chitwan	19 (18.4)	103
Rupandehi	18 (17.6)	102
Dang	31 (29.8)	104
Nawalparasi	23 (22.8)	101
Dhading	22 (14.7)	150
Nuwakot	30 (29.4)	102
Surkhet	18 (17.3)	104
Doti	10 (6.7)	150
Dadeldhura	10 (6.5)	153
Bardiya	41 (39.8)	103
Palpa	3 (2.0)	152
Kaski	11 (7.3)	150
Syanja	22 (14.7)	150
Gorkha	20 (19.6)	102
Tanahu	24 (16.0)	150
Parsa	32 (20.3)	158
Dhankuta	7 (4.6)	152
Total	582 (13.0)	4488

Note: Figures in parenthesis indicate percentages

[25,26] and Varanasi, North India [27,28]. Hydrocele was the most frequent clinical sign, while elephantiasis of the leg was comparatively rare.

In this study, the use of the ICT filariasis test for the detection of antigenaemia proved of high sensitivity and efficiency. When ICT was compared to night blood samples and day samples after DEC provocation, the results were predictably twice as high as parasitology, in keeping with published validation studies of ICT, which had shown thick film to be of low sensitivity. The prevalence rates observed need to be considered with caution because of the non-random nature of the survey technique: while such information is sufficient for defining endemicity of inter-vention units, more accurate prevalence rates will need to be determine in selected sentinel sites to be used for monitoring future elimination programmes. It is of interest to note that this survey has provided sufficient information for advocacy purposes which has resulted in the publication of a National Programme for the Elimination of Filariasis in Nepal in April 2002.

In the context of this preliminary survey, the finding of significantly lower ICT rates in individuals receiving TB or leprosy treatment (2% compared to 13% in non-treated individuals) merits discussion in view of the recently described relationship between *Wolbachia* infection and filarial survival [29]: long-term antibiotherapy, such as is

Table 2: Distribution of microfilaria among different samples

	Parasitological Examination	
Description of samples	Thick smear- DEC provocation test	Thick smear-night blood examination: (at 23:00 to 2.00 AM)
ICT positive samples (n = 216)	124 (57.4%)	82 (38%)
ICT negative samples (n = 52)	0	0
Total samples (n = 268)	124	82

Table 3: The number of positives in ICT according to density of microfilariae as determined by thick blood film.

	Density of microfilariae				
MF density	0	1–10	11–50	>50	Total
ICT positive	92	81	32	11	216

given for the treatment of tuberculosis, would be the sort of intervention needed to eliminate adult worms by destroying their *Wolbachia* symbionts. The observation of significantly higher ICT rates in households not using bed-nets is also noteworthy. Both observations emphasize the need for further studies looking at possible interactions and cross-benefits between different national intervention programmes.

Authors' Contributions
M. Hommel and J.B. Sherchand designed the study, analyzed the data and wrote the first draft of the manuscript. G.D. Thakur provided advise and logistical support to the fieldwork. J. B. Sherchand organized and conducted the work in the field. V. Obsomer advised on geographical issues, transferred the field data to GIS and produced the map. All authors contributed to the writing and editing of the manuscript.

Competing interests
None declared

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