PREVALENCE OF LYMPHATIC FILARIASIS, MALARIA AND SOIL TRANSMITTED HELMINTHIASIS IN A COMMUNITY OF BARDIYA DISTRICT, WESTERN NEPAL

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Abstract. Lymphatic filariasis (LF), malaria and soil transmitted helminthiasis (STH) cause major health problems in Nepal, but in spite of this very few studies have been carried out on these parasitic infections in Nepal. A cross sectional survey of all three categories of parasitic infections was carried out in Deudakala Village of Bardiya District, western Nepal. A total of 510 individuals aged 5 years and above were examined from finger prick blood for circulating filarial antigen (CFA), malaria antigen using a rapid diagnostic test (RDT), and malaria DNA using a PCR-based assay. In addition, 317 individuals were examined for soil-transmitted helminth (STH) eggs by the Kato-Katz technique. Prevalence of LF, malaria (antigen) and STH infection was 25.1%, 0.6% and 18.3%, respectively. PCR analysis did not detect any additional malaria cases. The prevalence of LF and STH infections differ significantly among different age groups and ethnic communities. The high prevalence of LF in the community studied indicates an immediate need for implementing a mass drug administration program for its control in this particular geographical area of Nepal.

Keywords: filariasis, helminth, malaria, prevalence, Nepal

INTRODUCTION

Parasitic infections cause a major public health problem in developing countries, with lymphatic filariasis (LF), malaria and soil transmitted helminthiasis (STH) comprising the vast majority of human disease burden and resulting in an estimated annual global loss of 1.2-34 million disability adjusted life-years (DALYs) (Mathers *et al*, 2008; Hotez *et al*, 2009).

LF is a vector borne parasitic disease caused by infection with *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* (Ottesen *et al*, 1990). Globally, 1,300 million individuals live in LF transmission areas, of whom 851 million are in Southeast Asia (Ottesen *et al*, 2008). Some 3.5 billion people globally are at risk of STH

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infection resulting from infection with the hookworms, *Ascaris lumbricoides* and *Trichiris trichiura* (Albonico *et al*, 2007). Malaria is a life-threatening vector borne disease caused by infection with *Plasmodium* parasites, of which *P. falciparum* and *P. vivax* are the two most prevalent species. Despite the combined efforts of 109 affected countries to control or eliminate malaria, it remains one of the most important diseases in the world's tropical regions in terms of lives lost and economic burden (WHO, 2010).

In Nepal, 25 million (90%) of the population are at risk of exposure to LF (Fig 1A) (DHS, 2012). Despite this huge problem, limited studies have been carried out on its epidemiology (DHS, 2012) and to the best of our knowledge, no major study has been carried out since 2003. As part of a national LF control program, mass drug administration (MDA) with diethylcarbamazine (DEC) and albendazole was implemented in 36 out of 75 districts in 2009/2010 treating around 12.3 million people covering 79.1% of the population living in these 36 districts (DHS, 2012).

Malaria is highly endemic in 13 districts and less endemic in the other 52 districts of Nepal (Fig 1B), and approximately 23 million people in Nepal are at risk of getting malaria (DHS, 2012). However, it has been noted that malaria incidence rate has decreased from 4.1 to 2 cases per 1,000 individuals between 2005 and 2011 as a result of malaria control program including vector control, early diagnosis and free distribution of antimalarial drugs (DHS, 2010, 2011).

The prevalence of STH is very high in Nepal ranging from 30% to 75% (Kunwar *et al*, 2006; Shakya *et al*, 2006), mainly because of limited education and lack of sanitation in households, such as inadequate disposal of human excreta and contaminated food and drinking water (Albonico *et al*, 2007).

Despite the implementations of disease prevention programs for LF, malaria and STH, the abundance of vector mosquitoes, mobile and vulnerable population, and environmental factors, such as suitable temperatures, make it very difficult to control these infections (DHS, 2010, 2012). The transmissions of LF, malaria and STH are, in some ways, related to each other as these infections are attributed to poor hygienic conditions and/or low socio-economic status (Nielsen *et al*, 2006). Earlier studies have showed a higher incidence of malaria (Nacher *et al*, 2002; Spiegel *et al*, 2003) or a lower intensity of filarial infection (De Rochars *et al*, 2004; Faulkner *et al*, 2005) in presence of helminth infection. Thus, the present study investigated the prevalence of all three parasitic infections simultaneously in the same population.

MATERIALS AND METHODS

Study design and site

The study was cross sectional and consisted of a questionnaire survey, followed by examinations of blood for circulating filarial antigen (CFA) and malaria (*P. falciparum* and *P. vivax*) antigen and DNA, and of stool samples for STH. Ethical clearance was obtained from the Nepal Health Research Council, Kathmandu (Ref. No: 134).

The study site was selected by reviewing the Annual Report of the Nepal Department of Health Services, and the study was carried out during December 2011 in Bardiya District of western Nepal, selected as it was indicated to be endemic for both LF and malaria, and MDA had not yet been introduced (DHS, 2012), but

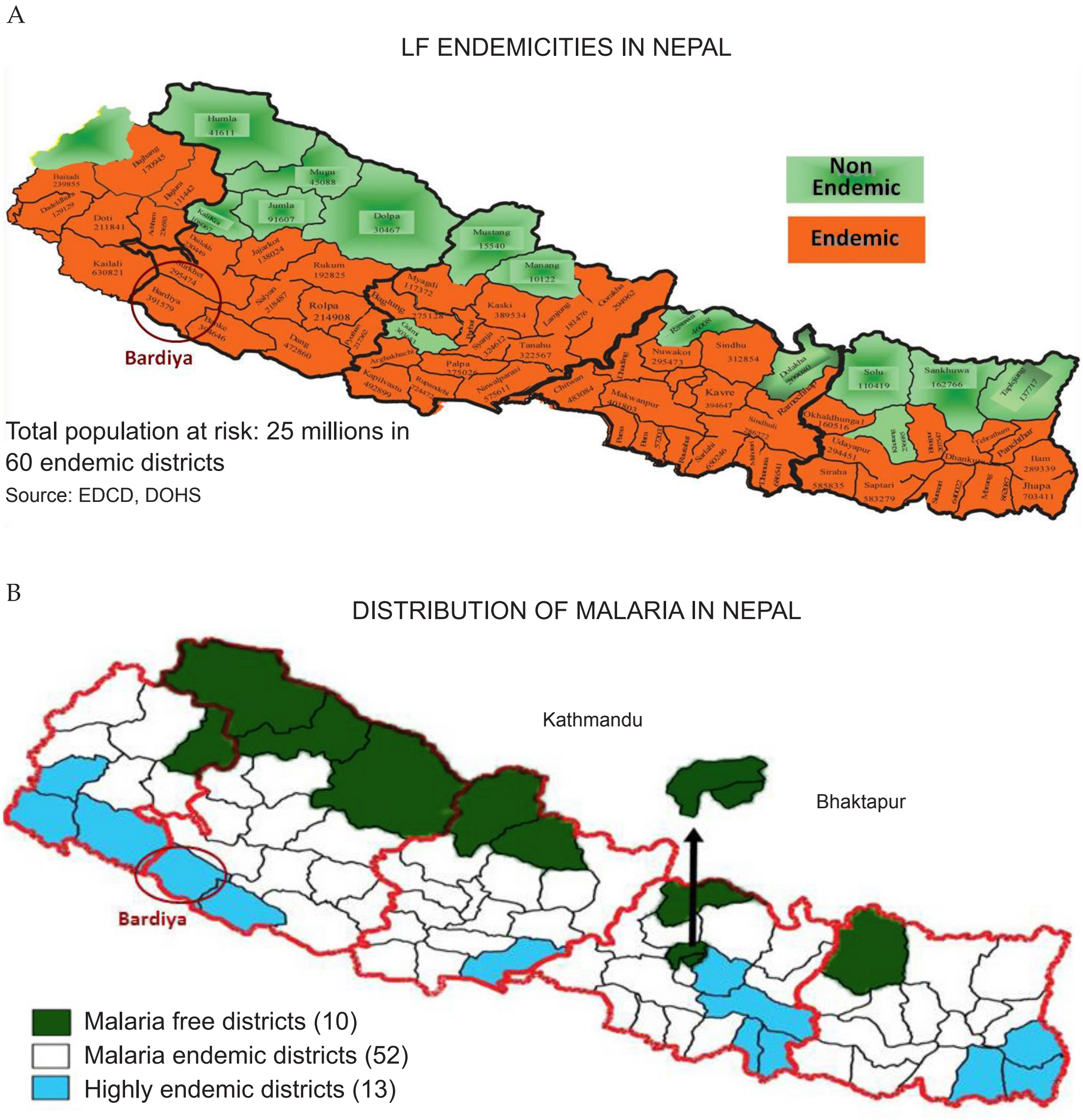

Fig 1–Endemicity of lymphatic filariasis (A) and malaria (B) in Nepal. Distribution of lymphatic filariasis was determined by using immuno-chromatographic test (DHS, 2012). Distribution of malaria was based on the abundance of two principle vectors, *Anopheles minimus* and *An. fluviatilis*, and malaria transmission rate (DHS, 2010).

no information was available on STH prevalence. The participants were of 5 years and older from Deudakala Village development committee (VDC) (smallest administrative entity in Nepal) of Bardiya District, which had a population of 19,221 in 2011 (CBS, 2012). Informed consent was obtained from every participant (or legal guardian) enrolled in the study before taking the samples.

Questionnaire

The questionnaire (in Nepali) was designed to obtain information regarding age, sex, occupation, housing conditions, educational status, medical history, self-medication behavior, household hygiene practices, birth place, migration, use of bed nets, and travel history during the past one year. The socio-economic status of the participant was determined on the basis of their housing conditions (thatched roof, corrugated roof or concrete), type of occupation (farmer, daily laborer, private or government employment), and education level (completed primary, secondary or higher education), all of which have a direct influence on the economic status of the households in Nepal.

Antigen and DNA examinations

CFA and malaria (*P. falciparum* and *P. vivax*) antigens from finger prick blood samples were determined using immuno-chromatographic test (ICT) (BinaxNOW® Filariasis; Alere, Inc, Scarborough, MA) and rapid diagnostic test (RDT) (BinaxNOW® Malaria; Alere, Inc), respectively.

In addition, finger prick blood samples were collected on filter papers (Whatman no. 3) and individually stored in plastic bags containing silica gel at room temperature until used for DNA extraction using a chelex method (Wooden *et al*, 1993) and subsequently subjected to two step PCR-based identification of malaria parasites (Rajakaruna *et al*, 2010). In brief, the first amplification was carried out using rPLU5 (5′-CCTGTTGTTGCCTTAAACTTC-3′) and rPLU6 (5′-TTAAAATTGTTGCAGTTAAAACG-3′) primers. The following thermocycling conditions (VWRi Duo Cycler, VWR/Bie&Berntsen; Bloomington, MN) were used: 94ºC for 15 minutes; 30 cycles of 94ºC for 1 minute, 58ºC for 2 minutes, and 72ºC for 2 minutes; with a final step of 58ºC for 2 minutes and 72ºC for 10 minutes. This product was used as the template in a second PCR using the conditions identical to those of the first PCR. For *P. vivax*, rVIV1 (5′-CGCTTCTAGCTTAATCCACATAACTGAAC-3′) and rVIV2 (5′-ACTTCCAAGCCGAAGCAAAGAAAGTCCTTA-3′) primers set were used, while rFAL1 (5′-TTAAACTGGTTTGGGAAAACCAAATATATT-3′) and rFAL2 (5′-ACCCAATGAACTCAATCATGACTACCCGTC-3′) primers for *P. falciparum*.

Stool examination

Participants were requested to provide stool samples the following morning. Each stool sample was examined using the Kato-Katz technique (Stool Examination Kit; Vestergaard Frandsen Group, Lausanne, Switzerland). In short, stool sample was placed in a plastic well 6 mm wide and 1.5 mm deep (containing about 40 mg of sample) and then the well was covered with cellophane strip of 30-35 mm pre-soaked overnight in 1:1 (v/v) glycerol:water solution containing 3% malachite green. Helminth eggs were counted under a light microscope (10x magnification) and the number of eggs identified was multiplied by 24 to obtain the number of eggs per gram (EPG) of feces.

Post-examination follow-up

STH-positive subjects were treated with a single dose of albendazole (400 mg) chewable tablet (Helmanil; Galpha Laboratories, Solan, Himachal Pradesh, India). Malaria- and LF-positive subjects were referred to Health Post of Deudakala VDC, where subjects were treated with chloroquine and primaquine for *P. vivax* and artemisinin-based combination therapy for *P. falciparum*, while LF-positive cases were treated with DEC.

Table 1
Number of individuals positive for lymphatic filariasis (LF), *P. falciparum* (PF) and soil-transmitted helminthiasis (STH) (and prevalence) in relation to gender and age groups.

	Gender		Age group (years)						Total
	Male	Female	5-10	11-20	21-30	31-40	41-50	>50	
For LF[a] and PF[a]									
Total examined	212	298	26	207	81	66	49	81	510
LF positive	62 (29%)	66 (22%)	5 (19%)	55 (27%)	23 (28.4%)	13 (20%)	16 (33%)	16 (20%)	128 (25.1%)
PF positive	1 (0.5%)	2 (0.7%)	0	1 (0.5%)	0	0	1 (2%)	1 (1%)	3 (0.6%)
For STH[a]									
Total examined	134	183	17	142	40	42	30	46	317
STH positive	20 (15%)	38 (21%)	10 (59%)	29 (20.4%)	10 (25%)	5 (12%)	2 (7%)	2 (4%)	58 (18.3%)

[a]Positive samples of LF, PF and STH are based on ICT, RDT and Kato-Katz test, respectively.

Table 2
Prevalence of lymphatic filariais (LF), *P. falciparum* (PF) and soil-transmitted helminthiasis (STH) in relation to ethnic groups.

	Ethnic group						Total
	Tharu	Bahun	Chettri	Dalit	Madhesi	Others	
For LF[a] and PF[a]							
Total examined	283	68	81	43	12	23	510
LF positive	86 (30%)	15 (22%)	17 (21%)	4 (9%)	4 (33%)	2 (9%)	128 (25.1%)
PF positive	3 (1%)	0	0	0	0	0	3 (0.6%)
For STH[a]							
Total examined	169	48	49	27	7	12	312
STH positive	33 (19%)	6 (12%)	9 (18%)	5 (18%)	2 (29%)	3 (25%)	58 (18.6%)

[a]Positive samples of LF, PF and STH are based on ICT, RDT and Kato-Katz test, respectively.

Data analysis

Prevalences were compared using Pearson's chi- square test by means of statistics software R, version 2.15.3 (http://www.r-project.org/) with 5% level of significance.

RESULTS

Questionnaire survey

The questionnaire survey revealed that of the 510 participants, 395 (77.5%), 73 (14.3%) and 42 (8.2%) lived in houses with thatched, corrugated and concrete roofs, respectively. Almost half the households (47.4%) had no latrines and used the open fields. Over half (54.5%) of the households were situated within the radius of 15 m from water holes. The majority of the households (96.3%) used bed nets while sleeping, with 27.1% being insecticide- impregnated bed nets. However, the impregnated bed nets had been distributed in 2005 and their conditions were not determined in this study. As regards to education, 184 (36.1%) had no formal education and only 46 (9%) were educated beyond grade 10. More than 97% of the participants had no history of travel within the past year and 80% had lived in the study area since birth or for more than 10 years. Almost all the participants (99.2%) were drinking underground water directly without any kind of water treatment. The majority of participants (68%) reported to have purchased anthelmintic drugs for self-medication 2-6 months prior to the study period, and these drugs were obtained directly from a pharmacy without any prior medical consultation and laboratory diagnosis.

CFA prevalence

Of the 128 participants, 32 (25.1%) were CFA-positive (Table 1). The prevalence of CFA is significantly different among the ethnic groups (Table 2), with the highest in the Madhesi group (33%) ($p < 0.05$). However, no significance difference was observed in the prevalence of CFA among the stratified age groups, and between males and females (Table 1).

Malaria prevalence

Three individuals (0.6%) were tested positive for *P. falciparum* (PF) by RDT (Table 1). No PCR-positive samples were detected among the remaining RDT-negative samples.

STH prevalence

Among the 317 stool samples collected, 58 (18%) individuals were positive with the Kato-Katz technique: 5 positive for *Ascaris* spp, 8 for *Trichuris* spp, and 45 positive for both *Ascaris* spp and *Trichuris* spp, but no hookworm egg were detected in any of the stool samples. However, this may have been due to the delay in microscopic examination of the samples as there was no electricity in the study site and the specimens had to be transported to Kathmandu, a journey of around 500 km. The STH- positive cases were detected in all age groups, with the highest prevalence (59%) in the 5-10 years age group ($p < 0.001$) (Table 1). Statistically, no significant difference was observed between males and females, and also among the ethnic groups ($p > 0.05$).

DISCUSSION

Parasitic infections are one of the major public health problems in developing countries, including Nepal. Among many parasitic diseases, LF, malaria and STH are highly prevalent in Nepal, where 80%-90% of the population live in endemic areas and are at risk of infection (DHS, 2012). Similar to most developing countries, poor hygienic conditions, lack of education and low socioeconomic status in Nepal favor the transmission of these parasitic infections (Rai *et al*, 2002; Banjara *et al*, 2010). This was borne out in the questionnaire survey of the participants living in Deudakala VDC, Bardiya District, western Nepal. Considering that only 3% of participants had travelled outside the area within the last year prior to the study and that more than 80% had lived in the area since birth or for more than 10 years, it is unlikely that the infections originated from other endemic areas of the country or from the neighboring country (India) where both malaria and LF are highly endemic (Sabesan *et al*, 2000; Agrawal and Sashindran, 2006).

The high prevalence (25.1%) of CFA-positive individuals, indicative of a high community prevalence of LF, could be attributed to the presence of nearby large water holes, which are potential breeding sites for the mosquito vectors of LF. *Culex quinquefasciatus*, an efficient vector of LF, has been recorded in all LF endemic areas of Nepal (DHS, 2012). Although, 95% of the participants reported sleeping under bed nets, but the conditions of the bed nets were not surveyed. In 2003, a CFA-positive prevalence of 39.8% was reported in Bardiya District, but the VDC was not identified (Sherchand *et al*, 2003). As LF is often very focal and varies considerably in prevalence from one area to another, it is difficult to compare the results from this study with the previous report. However, it is worth mentioning that the observed CFA-positive prevalence in the current study strongly indicates a relatively high prevalence of *W. bancrofti* infection in this study area. There was significant difference in the prevalence of CFA among various ethnic groups, which may reflect differences in culture and socio-economic status among these various ethnic groups. Genetic differences between ethnic groups might play a role in determining susceptibility to filarial infection (Meyrowitsch *et al*, 2010). The surprising absence in differences of CFA-positive prevalence among the stratified age groups has been also noted in the earlier study (Sherchand *et al*, 2003).

The prevalence of malaria (0.6%) was much lower than that (28%) determined in 2010 in Bardiya District (Ranjitkar *et al*, 2011). However, the samplings were conducted at two different sites within the Bardiya District. The low prevalence of malaria of this study may have been the outcome of the malaria control program, which included indoor residual spraying twice a year, improved diagnostic system using RDT, and free distribution of antimalarial drugs, such as chloroquine and primaquine for confirmed *P. vivax* cases and artemisinin-based combination therapy for confirmed *P. falciparum*-infected patients (DHS, 2012). Another explanation is that this survey was conducted in December, whereas the malaria transmission season in Nepal is from March to October with peaks in June, July and August (DHS, 2010).

The majority of STH-positive stool samples (n = 58) were positive for both *Ascaris* spp and *Trichuris* spp, with only 5 samples positive only for *Ascaris* spp and 8 samples for *Trichuris* spp. A study conducted in 2011 among school children of eastern Nepal reported 3.5% prevalence of *Ascaris* or *Trichuris* (Sah *et al*, 2013), which is much lower than that of the present study (18%). This is not surprising given the low literacy rate, and poor hygienic and socio-economic conditions of the participants. However, the relatively low prevalence of STH infection (excluding hookworms) in the studied population could be explained by their self-medicating behavior. Some 68% of the participants were taking non-prescribed anthelmintic drugs. The practice of self-medication is common throughout Nepal as it is much cheaper to buy medicines directly from pharmacies without prescription. The inability to detect hookworm eggs in stool also contributed to the low prevalence of STH infections. The observed significant differences in the prevalence of STH infection among the various age groups was in agreement with previous findings indicating that helminth infections significantly vary with subject's age and transmission level (Hotez *et al*, 2008).

In summary, to the best of our knowledge, this is the first study in Nepal, which assessed the prevalence of LF, malaria and STH infections in the same individual. There was a relatively high prevalence of LF and STH (excluding hookworms) with significant difference observed only between the ethnic groups in LF and between the age groups in STH infection, respectively. However, the prevalence of malaria was very low, most likely due to malaria prevention program and sampling being conducted during a low transmission season of the year. As MDA for LF has still not been introduced in Bardiya District, the high prevalence of LF in Deudakala VDC suggests an immediate need for such implementation program. The study has provided baseline data on the prevalence of LF, malaria and STH infections (at a single time point), which should be useful as a background information upon which to formulate larger studies in the future for monitoring and implementing national LF eradication and malaria control programs.

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