

Effect of untreated bed nets on blood-fed *Phlebotomus argentipes* in kala-azar endemic foci in Nepal and India

Albert Picado^{1/+}, Vijay Kumar⁴, Murari Das², Ian Burniston¹, Lalita Roy², Rijal Suman², Diwakar Dinesh⁴, Marc Coosemans³, Shyam Sundar⁵, Kesari Shreekanth⁴, Marleen Boelaert³, Clive Davies¹, Mary Cameron¹

¹London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK ²BP Koirala Institute of Health Sciences, Dharan, Nepal ³Institute of Tropical Medicine, Antwerp, Belgium ⁴Rajendra Memorial Research Institute of Medical Sciences, Patna, India ⁵Banaras Hindu University, Varanasi, India

Observational studies in the Indian subcontinent have shown that untreated nets may be protective against visceral leishmaniasis (VL). In this study, we evaluated the effect of untreated nets on the blood feeding rates of Phlebotomus argentipes as well as the human blood index (HBI) in VL endemic villages in India and Nepal. The study had a "before and after intervention" design in 58 households in six clusters. The use of untreated nets reduced the blood feeding rate by 85% (95% CI 76.5-91.1%) and the HBI by 42.2% (95% CI 11.1-62.5%). These results provide circumstantial evidence that untreated nets may provide some degree of personal protection against sand fly bites.

Key words: untreated nets - *Phlebotomus argentipes* - visceral leishmaniasis - HBI

Visceral leishmaniasis (VL), also known as kala-azar, is an important vector-borne disease in the Indian subcontinent where 150 million people are at risk and more than 40,000 cases are reported annually (WHO 2005). In India, Nepal and Bangladesh, VL is caused by *Leishmania donovani* (Laveran & Mesnil) (Kinetoplastida: Trypanosomatidae), which is exclusively transmitted by *Phlebotomus argentipes* (Annandale & Brunetti) (Diptera: Psychodidae) (Dinesh et al. 2000).

Control of VL in the India subcontinent is based on detection and treatment of cases and vector control. Indoor residual spraying (IRS) is currently the major form of reducing *P. argentipes* indoor density in the region. IRS was chosen based on the successful control of VL following the malaria eradication programmes in the 1970s (Ostyn et al. 2008). Despite these efforts, the current strategy of IRS is failing to control VL in the Indian subcontinent. The use of insecticide treated nets (ITN) and, specifically, long-lasting insecticidal nets (LN) have been postulated as alternatives or complements to IRS (Ostyn et al. 2008). The KALANET community trial (ClinicalTrials.gov CT-2005-015374) demonstrated that comprehensive distribution of LN reduces the indoor density of *P. argentipes* by 25% (Picado et al. 2009).

Other measures including blood feeding rates (percentage of blood fed females) and human blood index (HBI) the proportion of blood meals taken on man

(Boreham 1975)] are applied to monitor vector control interventions and can be used to assess personal protection (Githeko et al. 1996, WHO 2006). The use of ITN may enhance the barrier effect of the bed net and/or reduce the blood feeding rate of those sand flies that cross through the netting, as shown in laboratory bioassays with *Phlebotomus perniciosus* (Newstead), *Phlebotomus papatasi* (Scopoli) (Maroli & Majori 1991) and *Lutzomyia longipalpis* (Lutz & Neiva) (Oliveira Filho & Melo 1994). However, untreated nets may also provide some personal protection against *P. argentipes* based on observational studies on VL in Nepal and Bangladesh (Bern et al. 2000, 2005). Unfortunately, personal protection estimates in VL endemic areas are difficult to generate. This is because human-landing catches, or experimental hut studies, are difficult to justify ethically where the disease is potentially fatal and when, unlike malaria, there is no effective prophylactic to provide to field workers put at risk by such experiments.

The objective of this study was to use the distribution of untreated nets in the households included in the KALANET trial for the night of capture to investigate the impact of untreated nets on the blood feeding rates and anthropophagic behaviour of *P. argentipes*.

Twelve highly endemic clusters for VL included in the KALANET trial were selected in India and Nepal (6 in each country) based on past VL incidence. In September 2006, 10-min aspirations were conducted in 25 randomly selected households per cluster. The 10 households with the highest number of *P. argentipes* in each cluster and their 10 nearest cattle sheds per cluster were monitored from September 2006 for 15 and 16 months in Nepal and India, respectively. LNs were distributed in all the houses in six of the clusters (3 intervention clusters per country) in November and December 2006 in Nepal and India, respectively. The other six clusters were used as controls and did not receive LNs. Monthly

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+ Corresponding author: albert.picado@lshtm.ac.uk

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captures were performed with CDC light traps (LT) set up in the 10 selected houses one night per month from 6 pm–6 am. The following morning between 6–9 am sand flies were collected in the same houses and cattle sheds by aspiration for 15 min each.

From December 2006 in Nepal and from January 2007 in India (1st month that LNs were provided to trial clusters), untreated bed nets were lent to the 10 selected households during the sand fly collection nights in intervention and control clusters. In intervention households LNs were returned on the following morning. The use of untreated nets for the night of capture was required to attribute any observed reduction on *P. argentipes* indoor density in intervention clusters to the comprehensive distribution of LNs rather than to a local household effect induced by the presence of the LN during the night of capture (Picado et al. 2009).

Informed consent was obtained from the head of the households where sand flies were collected. *P. argentipes* collected by LT and aspiration were examined under a binocular dissecting microscope for identification of gender and blood meal status. Blood-fed *P. argentipes* were squashed individually onto Whatman's #1 filter paper to determine blood meal origin.

An ELISA was used to determine the origin of the blood in fed *P. argentipes* collected in the 6 KALANET clusters in Nepal. The sand flies were tested for human, bovine, goat, dog, rat and chicken blood. The ELISA protocol used was based on the technique described by Svobodova et al. (2003).

As untreated nets were only provided to households during sand fly collection nights after the first three or four months in Nepal and India, respectively, it was hypothesised that any observed change between pre and post-intervention blood feeding rates and human index

in female *P. argentipes* would be best explained by the personal protection provided by the untreated nets.

Since reduction in blood feeding rates in the LN clusters could be due to the personal protection provided by the untreated nets on the night of sand fly capture as well as the presence of LNs throughout the cluster, the effect of untreated nets on blood feeding rates was tested only on control clusters in India and Nepal. However, the effect of untreated nets on human index was assessed using the results from households and cattle sheds from both the intervention and control clusters in Nepal.

The effect of untreated nets was assessed as a “before and after intervention” design using two sets of data: (i) pre and post-intervention LT collections were compared in control households in India and Nepal to determine the effect of untreated nets on the blood feeding rates and (ii) pre and post-intervention collections were compared from intervention and control households and cattle sheds in Nepal to assess the effect of untreated nets on HBI. A negative binomial mixed model (adjusted by country when required) using household or cattle shed as a random effect to control for repeated measures was used. A dichotomous variable representing the time (i.e. pre and post-intervention) was the explanatory variable. In the blood feeding rate and HBI models the number of blood-fed *P. argentipes* and *P. argentipes* with human blood per household (and shed) were the response variables. The interaction term between intervention and time variables was tested to determine whether the effect of the use of untreated nets varied between the two groups.

Ethical clearance to conduct this study was obtained from the Indian Council of Medical Research, the Ethical Committee of the BP Koirala Institute of Health Sciences (Dharan, Nepal) and the corresponding bodies at the In-

TABLE I

Number of female *Phlebotomus argentipes* collected and percentage of blood fed females by cluster before and after use of untreated nets the night of capture in control clusters in India and Nepal

Country	Pre-intervention			Post-intervention		
	LT nights	Females	Blood fed (%)	LT nights	Females	Blood fed (%)
India						
C09	32	113	22 (19.5)	96	106	5 (4.7)
C06	40	85	41 (48.2)	118	112	1 (0.9)
C05	40	83	22 (26.5)	110	121	2 (1.6)
Total	112	281	85 (30.2)	324	339	8 (2.4)
Nepal						
C60	30	83	7 (8.4)	120	106	1 (0.9)
C56	30	117	16 (13.7)	120	554	19 (3.4)
C55	30	49	6 (12.2)	120	75	1 (1.3)
Total	90	249	29 (34.3)	360	735	21 (2.9)
India and Nepal	202	530	114 (21.5)	684	1074	29 (2.7)

the number of nights of capture using CDC light traps (LT nights) per cluster in the three pre- and the 12 post-intervention months is also presented.

TABLE II

Number of blood fed females and human blood index (HBI) for *Phlebotomus argentipes* collected by cluster in households and cattle sheds in Nepal using CDC light traps and aspiration according to the sampling in the three pre and the 12 post-intervention months

Cluster	Group	Pre-intervention blood fed <i>P. argentipes</i>			Post-intervention blood fed <i>P. argentipes</i>		
		Household n (HBI)	Cattle shed n (HBI)	Total n (HBI)	Household n (HBI)	Cattle shed n (HBI)	Total n (HBI)
C51	intervention	28 (75)	3 (67)	31 (74)	4 (0)	25 (4)	29 (3)
C52	intervention	12 (92)	0 (0)	12 (92)	4 (50)	0 (0)	4 (50)
C53	intervention	4 (100)	1 (100)	5 (100)	4 (100)	0 (0)	4 (100)
Total intervention		44 (82)	4 (75)	48 (81)	12 (50)	25 (4)	37 (19)
C55	control	4 (50)	0 (0)	4 (50)	1 (0)	1 (0)	2 (0)
C56	control	12 (83)	0 (0)	12 (83)	38 (87)	15 (27)	53 (70)
C60	control	8 (88)	0 (0)	8 (88)	4 (50)	0 (0)	4 (50)
Total control		24 (79)	0 (0)	24 (79)	43 (81)	16 (25)	59 (66)
Total intervention and control		68 (81)	4 (75)	72 (81)	55 (75)	41 (12)	96 (48)

stitute of Tropical Medicine (Antwerp, Belgium) and the London School of Hygiene and Tropical Medicine (UK).

A total of 1,064 female *P. argentipes* were collected using LTs from 58 households in the six KALANET control clusters. Of these, 143 (11%) were blood-fed. As shown in Table I, a large post-intervention reduction in blood-fed rates was observed in all six control clusters (from 21.5 to 2.7%). The negative binomial mixed model demonstrates that the percentage of female *P. argentipes* with blood meals dropped significantly by 85.5% (95% CI 76.5-91.1%, $p < 0.001$) after the untreated nets were introduced.

In order to determine the human blood index, the 168 blood-fed *P. argentipes* collected in Nepal using LT and aspiration in households and cattle sheds over 15 months were analysed for blood origin. Of these samples, 61.9% fed on human blood, 22.6% on bovine, 4.2% on dog, 3% on goat and 0.6% on chicken. We were unable to identify the blood origin for 10.1% of the samples. A total of four samples had mixed blood meals: two human/bovine, one human/goat and one human/chicken mixtures were found. These results show that *P. argentipes* in VL endemic foci in Terai are mainly anthropophilic but will feed on bovine blood meal when available.

To evaluate the effect of untreated nets on HBI in Nepal, the ELISA results summarised in Table II were dichotomised (i.e. human blood vs. non-human blood). Samples not identified were considered as non-human blood in the analyses to avoid overestimations of the effect of untreated nets. The negative binomial mixed model found that HBI was reduced significantly by 42.2% (95% CI 11.1-62.5%, $p = 0.014$) when untreated nets were used. The overall reduction in the control and intervention clusters was significantly different (interaction term: $p = 0.014$). This result suggests that the massive distribution of LNs in intervention clusters had an effect on the blood feeding behaviour of *P. argentipes*, as HBI was reduced to a greater extent

in intervention compared to control clusters (Table II). This phenomena has been described in laboratory reared sand flies (Maroli & Majori 1991, Oliveira Filho & Melo 1994) and field trials with mosquitoes in India (Sampath et al. 1998, Dinesh et al. 2000), but it could not be tested (by comparing post-intervention HBI between intervention and control clusters) in this trial due to the massive reduction of blood-fed *P. argentipes* in households post-intervention.

These results provide circumstantial evidence that even untreated nets provide personal protection against sand fly bites. Blood-fed rates in LT catches decreased by 85.5% in houses using untreated nets. Further, HBI was reduced by 42.2% suggesting that female *P. argentipes* are diverted from humans when untreated nets are used (the later effect seems to be enhanced by the use of LNs). This result is consistent with observational studies that have detected a significant negative association between the ownership of untreated bed nets and the incidence of VL in endemic areas in Nepal and Bangladesh (Bern et al. 2000, 2005); however, other studies in Nepal have failed to detect such an effect (Schenkel et al. 2006). Since there were no concurrent controls, the reduction in blood feeding rate and HBI could have been due to another confounding time-associated factor in our study. For example: (i) meteorological factors that may alter the frequency of vector-host contact, e.g., changes in temperature or humidity and (ii) changes in host availability, e.g., more domestic animals may have been available as alternative hosts during the course of the trial. Specific entomological trials should be conducted to corroborate the results observed in this study.

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