

# Reducing Visceral Leishmaniasis by Insecticide Impregnation of Bed-Nets, Bangladesh

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The effect of insecticide-treated materials on reducing visceral leishmaniasis (VL) is disputable. In Bangladesh, we evaluated the effect of a community-based intervention with insecticide impregnation of existing bed-nets in reducing VL incidence. This intervention reduced VL by 66.5%. Widespread bed-net impregnation with slow-release insecticide may control VL in Bangladesh.

The governments of Bangladesh, India, and Nepal have committed to eliminate visceral leishmaniasis (VL) by 2015 (1). Reducing VL incidence by controlling sandflies, the vector of *Leishmania* spp. parasites, through integrated vector management is a key strategy of elimination programs (2). Community-based intervention with insecticide-treated materials, such as distribution of long-lasting insecticide-treated bed-nets or mass bed-net impregnation programs with slow-release insecticide tablets, could be possible vector-control components of integrated vector management if they are found effective in reducing VL incidence (3). We evaluated the effect of a community-based intervention with impregnation of existing bed-nets in reducing VL incidence in VL-endemic villages of subdistrict (upazila) Godagari, district Rajshahi, Bangladesh.

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## The Study

The study comprised all 72 VL-endemic villages in Godagrai, distributed in 5 unions (Deopara, 36; Rishikul, 15; Gogram, 12; Pakuria, 6; and Mohanpur, 3). The intervention area was 36 villages in Deopara union comprising 2,512 households (11,426 inhabitants), and the control area was the 36 villages from other 4 unions comprising 3,143 households (14,021 inhabitants) (Figure 1). The bed-net impregnation intervention program with KO Tab 1-2-3 (Bayer Environmental Science, Bayer [Ply] Ltd., reg. no. 1968/011192/07, 21 Isando, South Africa, CODE 05682036 C) was conducted during February–March 2008. All households from all 79 villages in Deopara union, including households in 36 VL-endemic villages, were invited to participate in bed-net dipping (Figure 1). Details about the surveys and intervention are given in the online Technical Appendix ([wwwnc.cdc.gov/EID/article/19/7/12-0932-Techapp1.pdf](http://wwwnc.cdc.gov/EID/article/19/7/12-0932-Techapp1.pdf)). We measured VL incidence in the intervention and control areas before and after intervention during September 2006–March 2007 and December 2009–January 2010, respectively. Household screening for VL cases in the previous 12 months was performed by trained field research assistants. Past VL cases were confirmed through document analysis and checking of hospital registers. A new VL case was defined by using the definition for new VL case of the National Kala-azar Elimination Guideline (4). VL incidence was expressed by number of VL cases (newly found plus past VL cases) per 10,000 persons. The field research assistants also conducted an in-depth interview with each household head by using a structured questionnaire in every 11th household and in households where they found new and past VL cases to collect sociodemographic characteristics of the surveyed community and VL-related knowledge and practice. A total of 556 household heads (254 and 302, respectively, in the intervention and control areas) were interviewed. Sociodemographic and knowledge, attitude, and practice variables between 2 areas with  $p$  values  $<0.2$  were extended to 5,655 households by using statistical tools, and the validity was checked by comparing the distribution of each variable before and after random extension (online Technical Appendix Table). This helped us to investigate the eventual confounding effect of socioeconomic and knowledge, attitude, and practice variables on VL incidence reduction.

We evaluated the effect of the intervention on VL incidence in different ways. First, we compared reduction of VL incidence at the population level. Second, we compared reduction of VL-affected households in the 2 areas by a difference-in-difference method. Then, we examined the consistency of the effect of the intervention by measuring protection of the population from VL in the intervention area and protection of households from

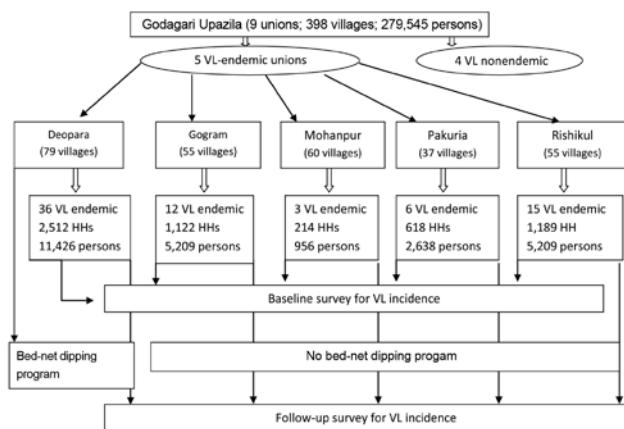


Figure 1. Design of study of reducing visceral leishmaniasis by insecticide impregnation of existing bed-nets, Bangladesh, 2006–2010. VL, visceral leishmaniasis; HH, households.

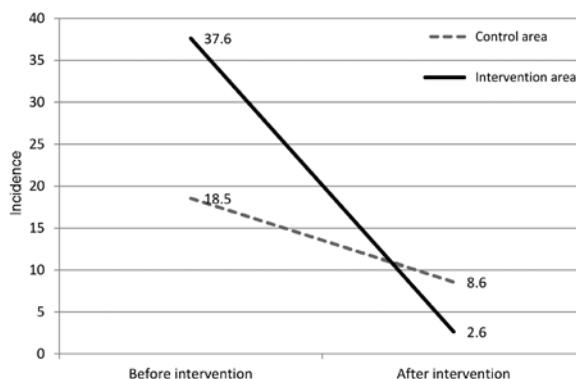


Figure 2. Visceral leishmaniasis incidence (cases per 10,000 persons) in intervention and control areas before and after intervention, Bangladesh, 2006–2010.

VL by the intervention through unadjusted and adjusted longitudinal logistic regression models. Data management and statistical analysis were conducted by using Epi Info version 3.2.2 (Centers for Disease Control and Prevention, Atlanta, GA, USA) and Stata 10.1 (Stata Corp, College Station, TX, USA), respectively. The International Centre for Diarrhoeal Disease Research, Bangladesh, and the Ethical Review Committees of the Special Program for Research and Training in Tropical Diseases/World Health Organization (WHO) approved the study. Informed written consent was obtained from each household head and from the persons with suspected VL for any study-related interventions.

The 2 areas differed regarding knowledge of the household head about VL symptoms, VL transmission, and household education (online Technical Appendix). A total of 2,239 (89.1%) of the 2,512 household heads from the study area of Deopara participated in the bed-net dipping. The use of impregnated bed-nets was also very

high (99.8%), as found by random nightly observation in a subsample of households in the intervention area.

Before intervention, 69 VL cases were found, resulting in a VL incidence of 27 per 10,000 persons in the study area. VL incidence in the intervention area, 37.6 cases per 10,000 persons (43/11,426), was significantly higher than in the control area (18.5/10,000) (26/14,021;  $p = 0.0036$ ). In intervention and control areas, 3 and 4 households, respectively, had multiple persons with VL. After intervention, VL incidence in intervention and control areas was 2.6 (3/11,426) and 8.6 (12/14,021) cases per 10,000 persons, respectively. During follow up, annual VL incidence declined in both areas, but the reduction was significantly greater in the intervention area (decrease of 35 cases/10,000 persons) than in the control area (decrease of 9.99/10,000;  $p = 0.001$ ) (Table 1; Figure 2). The effect of community-level intervention, measured by difference-in-difference method, was 66.5% (Table 1). Using odds ratios in the longitudinal logistic regression model, we

Table 1. VL incidence and affected households before and after bed-net impregnation program, Bangladesh, 2006–2010\*

Group	Bed-net impregnation		Rate changes (p value)	% Reduction† compared with control (p value)‡
	Before, no. (%) affected	After, no. (%) affected		
<b>HH§</b>				
Intervention, n = 2,512	40 (15.92)	3 (1.19)	-14.73 (<0.0001)	-70.52% (0.0007)
Control, n = 3,143	21 (6.68)	10 (3.18)	-3.50 (0.0476)	
Total, n = 5,655	61 (10.79)	13 (2.30)	-8.49 (<0.0001)	
<b>Population¶</b>				
Intervention, n = 11,426	43 (37.63)	3 (2.63)	-35.01 (<0.0001)	-66.49% (0.001)
Control, n = 14,021	26 (18.54)	12 (8.56)	-9.99 (0.023)	
Total, n = 25,447	69 (27.12)	15 (5.89)	-21.22 (<0.0001)	

\*VL, visceral leishmaniasis; HH, households.

†Effect of intervention:  $(B/A) - (D/C)$  Where A = baseline value for VL-affected HH per 1,000 HH/VL incidence per 10,000 persons in the intervention area; B = post-intervention value for VL-affected HH per 1,000 HH/VL incidence per 10,000 persons in the intervention area; C = baseline value for VL-affected HH per 1,000 HH/VL incidence per 10,000 persons in the control area; D = post-intervention value for VL-affected HH per 1,000 HH/VL incidence per 10,000 persons in the control area. The effect is negative or positive if the VL-affected HH per 1,000 HHs/VL incidence per 10,000 persons is decreased or increased after intervention. Then the percentage reduction by intervention is calculated as  $(E/[A]) \times 100$ .

‡p values were calculated by Z statistic for pre- or post-rate differences between intervention and control areas.

§Incidence per 1,000 HH.

¶Incidence per 10,000 persons.

Table 2. Estimation of protection of households by the VL intervention using longitudinal logistic regression model with and without adjustment for confounders, Bangladesh, 2006–2010\*

Model; parameter	Odds ratio (95% CI)	Estimated protection by intervention at household level, % (95% CI)	p value
Simple, without adjustment for confounders; intervention	0.13 (0.030–0.557)	87 (44.3–97.0)	0.006
Full model, with adjustments for confounders			
Intervention	0.13 (0.03–0.56)	87 (44.3–97.0)	0.006
Family size >5 persons	1.75 (0.99–3.11)		0.054
HH head occupation, labor	2.38 (1.37–4.12)		0.002
Precarious (mud/thatched) house	4.64 (0.56–38.69)		0.156
HH head without any knowledge of VL symptom	0.25 (0.13–0.46)		<0.001
HH head without any knowledge of VL transmission	0.57 (0.33–0.98)		0.042
Having bed-net at home	0.49 (0.12–1.98)		0.319
Use of bed-net for protection against mosquito bites	2.57 (0.81–8.21)		0.109

\*The intervention effect and covariates are tested in 2 different panel logistic regression models; simple not controlling for any covariates, full model controlling for confounders. VL, visceral leishmaniasis; HH, household.

found that 85.8% (95% CI 44.0%–96.5%;  $p = 0.005$ ) of the population in the intervention area was protected from VL by the intervention.

The total number of household heads was 5,655, with 2,512 and 3,143 in the intervention and control areas, respectively. Before intervention, VL-affected households were 15.9 and 6.7 per 1,000 households in the intervention and control areas, respectively. After intervention, VL-affected households declined 13 times and 2 times, respectively, in the intervention and control areas compared with VL-affected households before intervention. The effect of the intervention in reducing VL-affected households in the intervention area compared with the control area was 70.5% by difference-in-difference analysis (Table 1). Again, using odds ratios in the longitudinal logistic regression model, we estimated the crude protection of households in the intervention area from VL by the intervention as 87% compared with those in the control areas. The protective effect of the intervention remained independent when adjusted for possible confounders (Table 2).

## Conclusions

The community-based bed-net impregnation with slow-release insecticide significantly reduced VL incidence in VL-endemic areas. We used the difference-in-difference method for impact calculations because it is recommended by impact evaluation experts when effects of disease significantly differ between intervention and control, such as in our study (5–10). The protective effect was consistent and independent, as shown by the longitudinal logistic regression model. The differences in calculated effect and estimated protection at the household and community levels were due to households with multiple VL cases. Our findings agree with those of Ritmeijer et al. (11), who found a 59% reduction in VL by bed-net impregnation in Sudan. Our findings, however, were not consistent with those of Picardo et al. (12), who found no additional protection by random villagewise distribution of commercial insecticide-treated bed-nets compared with

existing vector-control practice in India and Nepal. This discrepancy might be explained by the different delivery (commercial bed-net vs. existing bed-net impregnation) and coverage achieved (patchy villagewise vs. all villages in the area) by the intervention. We recommend mass coverage of VL-endemic villages with bed-net impregnation with slow-release insecticide for controlling VL in Bangladesh.

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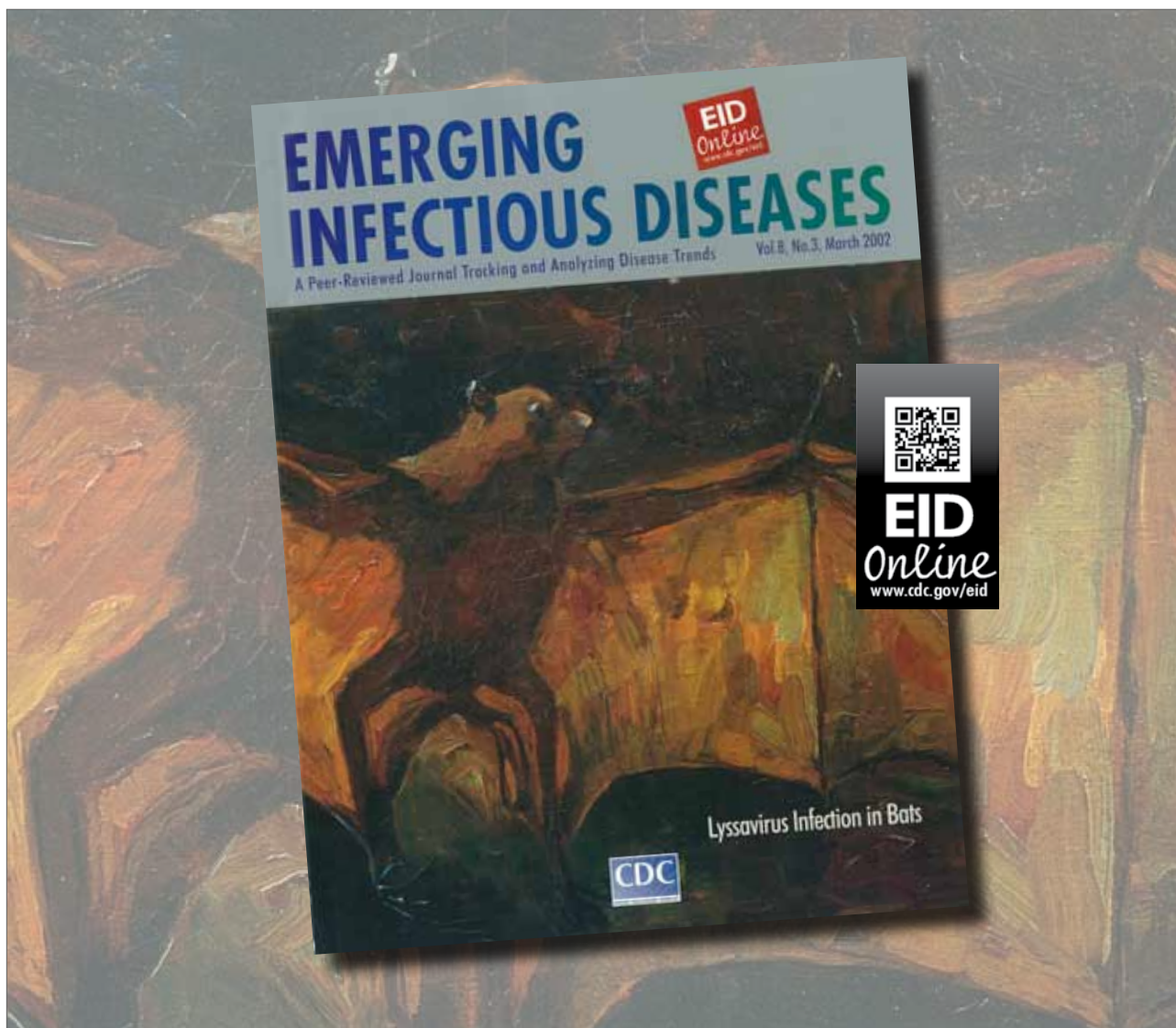
Dr Mondal is a member of the WHO Advisory Panel for *Leishmania* control and a member of the Regional Technical Advisory Group on Kala-azar Elimination in the Indian subcontinent, South-East Asia Regional Office, WHO, in New Delhi, India. His primary research interests include control of infectious diseases, such as visceral leishmaniasis, tuberculosis, and neglected tropical diseases.

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# Reducing Visceral Leishmaniasis by Insecticide Impregnation of Bed-Nets, Bangladesh

## Technical Appendix

### Methods Used to Evaluate the Impact of a Community-based Intervention with Impregnation of Existing Bed-Nets in Reducing Visceral Leishmaniasis Incidence in Visceral Leishmaniasis–Endemic Village of Subdistrict Godagari, District Rajshahi, Bangladesh

#### Study Design, Study Area, and Population

The study design was a quasi-experiment with an intervention community and a control community. Visceral leishmaniasis (VL) incidence was measured before and after intervention from both intervention and control areas by repeated cross-section surveys.

The study was carried out in the Godagari upazila (subdistrict) of Rajshahi district, Bangladesh. According to the 2001 Census of Bangladesh, Godagarai has a total population of 279,545 living in 9 unions and 398 villages. A union is the smallest administrative unit in Bangladesh, and all public health–related activities are centered on the union basis. VL has been reported from only 5 (Deopara, Rishikul, Gogram, Pakri and Mohonpur) of 9 unions of the subdistrict. The total number of villages affected by VL in these 5 unions was 72 (out of 286): 36 in Deopara, 15 in Rishikul, 12 in Gogram, 6 in Pakuria, and 3 in Mohanpur. In Bangladesh, there have been no vector control activities since 1985 except in the malaria-endemic southeastern hilly part of the country, and there is no overlap of VL- and malaria-endemic areas. Since September 2011, the national program has introduced indoor residual spraying with Deltamethrin in VL-endemic villages.

#### Baseline Survey

From September 2006 through March 2007, trained field research assistants (FRAs) conducted a screening questionnaire by house-to-house visit in the 72 villages of the

abovementioned unions to detect patients in whom VL had been diagnosed over the preceding 12 months and those who had chronic fever (>2 weeks). In those fever cases, the trained FRAs examined for enlarged spleen, and in the positive cases, they performed the rK39 rapid test with Kala-azar Detect (InBios, Seattle, WA, USA). All persons positive for rK39 were referred to the subdistrict, district, or medical college hospital with a case referral form for further confirmation of splenomegaly and VL diagnosis. A case of VL was defined in accordance with the national kala-azar elimination program fever for >2 weeks, enlarged spleen, and rK39 rapid test positivity in a person from a VL-endemic area. The FRAs also conducted in-depth interviews with household heads by using a structured questionnaire in every 11th household and in households where they found past and present VL cases.

After the baseline survey, all 8,287 households (31,442 persons) in the Deopara union were invited to participate in the bed-net impregnation program. This was to simulate an eventual implementation of a VL vector control program by the national program in the union. There were 2,512 households from Deopara (11,426 persons) that were surveyed for VL incidence at baseline that constituted the study intervention area and a total of 3,143 households (14,021 persons) from VL-endemic villages in the other 4 unions without intervention that constituted the study control area.

### **The Intervention**

During February–March 2008 a bed-net impregnation program with KO-Tab 1-2-3 was implemented in the Deopara union according to standard operational procedures, provided by the manufacture (Bayer Environmental Science, Bayer [Ply] Ltd., reg. no. 1968/011192/07, Isando, South Africa, CODE 05682036 C). Details about the dipping program can be found elsewhere (1). Briefly, the research team numbered all the households, collected information including the numbers of existing bed-nets in every household, and prepared a log against which number of dipped bed-nets was checked. The research team also trained public health personnel of the health system in bed-net dipping procedures, safety, and precautions. In each village, a village committee named “Kala-azar Nirmul Committee (village committee for VL elimination)” was formed. The committee selected the volunteers and bed-net dipping points. The public health personnel trained village volunteers how to educate villagers about bed-net dipping and how to conduct the dipping of bed-nets. Public health personnel informed villagers by house-to-house visits about the need for washing the nets before bringing them for dipping, about the procedures

of dipping, safety measures, and subsequent drying of the dipped nets in a horizontal position in a shaded area.

### **Follow-up Survey after 18 Months**

In December 2009 and January 2010, a follow-up survey for active VL cases and past VL cases in the previous 12 months was conducted by using the procedures described above in all 72 villages.

### **Estimation of VL Incidence**

Incidence per 10,000 was calculated by the number of VL cases (newly found during the survey plus those reported in the survey for the preceding 12 months) divided by the total population in the surveyed households multiplied by 10,000.

VL incidence at household level is expressed as number of VL-affected households per 1,000 households.

### **Sample Size Calculation**

Sample size was calculated by assuming VL incidence per 10,000 people was 0.27%, expecting a 50% reduction of VL in the intervention area after intervention; setting the power of the study and the confidence limit of the estimation, respectively, at 80% and 95%. The required number of persons to be screened for active VL cases was 9,493 persons in each study area with a total sample size of 18,986. However, we surveyed a total population of 25,447 at baseline and follow-up, which gave sufficient power to our study.

### **Data Management and Statistical Analysis**

A data entry program was developed by using Epi Info version 3.2.2 software (Centers for Disease Control and Prevention, Atlanta, GA, USA). Data were cleaned and checked for duplicates. Descriptive statistics were applied. Bivariate association was analyzed by using Pearson  $\chi^2$  or Fisher exact test where applicable. Z test was used to compare the estimated proportion between the intervention and control arm. Because the baseline statistics for outcome measurement differed significantly between intervention and control arm, we adapted a regression model to compare the rate of VL incidence and VL affected household. Comparative analyses were made at the population level as well as at the household level.

Effect of intervention (EI) was assessed by percentage reduction of VL incidence per 10,000 persons and VL-affected household per 1,000 households.

The EI was calculated on the basis of difference in differences analysis by using the following formula:

Effect of intervention (EI):  $(B-A) - (D-C)$

where A = baseline value for VL incidence per 10,000 people/VL-affected households per 1,000 households in the intervention group; B = postintervention value for VL incidence per 10,000 people/VL-affected households per 1,000 households in the intervention group; C = baseline value for VL incidence per 10,000 people/VL-affected households per 1,000 households in the control group; D = postintervention value for VL incidence per 10,000 people/VL-affected households per 1,000 households in the control group.

The EI was negative or positive if the VL incidence per 10,000 people/VL-affected households per 1,000 households was decreased/increased after intervention and the effect was 0 if the VL incidence per 10,000 people/VL-affected households per 1,000 households was the same as at baseline. The percentage reduction of VL incidence per 10,000 people/VL-affected households per 1,000 households attributable to the intervention was calculated as  $(EI/[A]) \times 100$  and p value was calculated by using Z statistic as follows:

$Z = D/SE$  where  $D = RD2 - RD1$  (RD1 and RD2 pre- and post-rate difference, respectively, for control and intervention areas); standard error,  $SE = \sqrt{S}$  ( $S = \text{no. of event (VL cases or VL-affected HHs)}/\text{square unit (number of population}/10,000) \text{ or (number of households}/1,000) \text{ for each of 4 categories}$ }).

#### **Simple (Unadjusted) Model at Population and Household Level**

The main outcome variables were “VL case” and “VL-affected households” before and 18 months after intervention. The outcome variable categorized as binary response (1 for VL case/VL-affected household and 0 for person without VL/household, not affected by VL). Based on the nature of the outcome variable, the longitudinal logistic regression model was used at population as well as at household levels to see whether the intervention significantly reduced the number of VL cases and VL-affected households, respectively. In the model, an interaction



term of being in the intervention arm at follow-up was included to estimate the effect of the intervention. The basic structure of the difference in differences regression model was:

$$\text{Outcome} = \text{Intercept} + a \times \text{Bed-net impregnation} + b \times \text{Time} + c \times \text{Interaction} + \text{error} \dots (i)$$

where bed-net impregnation is 1 if it is the intervention area and 0 if it is the control area; Time is 1 if follow up and 0 if baseline; and interaction is 1 for intervention group at follow up.

#### **Full (Adjusted) Model at Household Level**

Within the sample of 5,655 households, a representative subsample of 556 households was used to collect the household socioeconomic and VL awareness data by using systematic random sampling. Variables with  $p < 0.20$  in the bivariate analysis on the subsample were considered as possible confounders and were extended to the 5,655 households database to develop the full model. It was found that the binomial distribution fitted the data on subsample for the confounding variables. Therefore, extended sample for only confounding variables were made through the Bernoulli trial with only 2 possible random outcomes by using the probability (proportion) parameter estimated from the subsample (Technical Appendix Table). The outcomes were mutually exclusive and exhaustive. Then the extended sample was merged to the 5,655 household's database to develop a full model by using the same model structure (i) including the confounders for adjustment. The following variables were adjusted to determine how the intervention affected the household level: family size, household head occupation, housing condition (precarious house), household head knowledge on VL symptoms and VL transmission, having bed-net and use of bed-net.

In the table, odds ratio (OR) (95% CI) and its p value are given. Protection from VL disease were estimated as  $(1 - \text{OR}) \times 100$  if  $\text{OR} < 1$ . Significances are stated at 5% level, and 95% CIs are given. For the data analysis, we used Stata 10.1 (Stata Corp LP, College Station, TX USA).

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Technical Appendix Table. Sociodemographic characteristics and knowledge and practice about VL of HH heads in intervention and control areas, Bangladesh, 2006–2010

Indicator	Observed results, N = 556				Results after extension, N = 2,512			
	Intervention area, n = 254	Control area, n = 302	Total, N = 556	p value	Intervention area, n = 2,512	Control area, n = 3,143	Total, N = 5,655	p value
Mean age, y (SD)	42.1 (12.6)	43.3 (12.4)	42.8 (12.5)	0.22	42.7 (13.3)	42.7 (13.0)	42.7 (13.1)	0.95
Male respondents	242 (95.3)	290 (96.0)	532 (95.7)	0.66	2,337 (93.0)	2,934 (93.4)	5,271 (93.2)	0.63
Family size ≤5 persons	374 (68.3)	199 (65.9)	374 (67.3)	0.45	1,848 (73.6)	2,375 (75.6)	4,223 (74.7)	0.09
HH head without any education	134 (52.8)	155 (51.3)	289 (52.0)	0.74	1,318 (52.5)	1,609 (51.2)	2,927 (51.8)	0.34
HH head occupation, labor	73 (28.7)	108 (35.8)	181 (32.6)	0.08	719 (28.6)	1,146 (36.5)	1,865 (33.0)	<b>&lt;0.0001</b>
HH head without any knowledge about VL symptoms	124 (48.8)	175 (57.9)	299 (53.8)	0.03	1,207 (48.0)	1,852 (58.9)	3,059 (54.1)	<b>&lt;0.0001</b>
HH head without any knowledge about VL transmission	160 (63.0)	228 (75.5)	388 (69.8)	0.001	1,567 (62.4)	2,383 (75.8)	3,950 (69.8)	<b>&lt;0.0001</b>
Have bed-net at home	247 (97.2)	296 (98.0)	543 (97.7)	0.55	2,439 (97.1)	3,072 (97.7)	5,511 (97.5)	0.13
Use of bed-net to protect against mosquitoes	227 (89.4)	274 (90.7)	501 (90.1)	0.59	2,254 (89.7)	2,866 (91.2)	5,120 (90.5)	0.063
Precarious house	231 (90.9)	299 (99.0)	530 (95.3)	<b>&lt;0.0001</b>	2,280 (90.8)	3,115 (99.1)	5,395 (95.4)	<b>&lt;0.0001</b>

\*Values are no. (%) except as indicated. **Boldface** indicates significance. VVL, visceral leishmaniasis; HH, households.