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Longlasting insecticidal nets for prevention of *Leishmania donovani* infection in India and Nepal: paired cluster randomised trial

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ABSTRACT

Objective To test the effectiveness of large scale distribution of longlasting nets treated with insecticide in reducing the incidence of visceral leishmaniasis in India and Nepal.

Design Paired cluster randomised controlled trial designed to detect a 50% reduction in incidence of *Leishmania donovani* infection.

Setting Villages in Muzaffarpur district in India and Saptari, Sunsari, and Morang districts in Nepal.

Participants 13 intervention and 13 control clusters. 12 691 people were included in the analysis of the main outcome (infection), and 19 810 were enrolled for the secondary (disease) end point.

Intervention Longlasting insecticidal nets (treated with deltamethrin) were distributed in the intervention clusters in December 2006.

Main outcome measures Infection was determined by direct agglutination test at 12 and 24 months after the intervention in those who had negative results (titre ‹1:1600) at baseline. The effect estimate was computed as the geometric mean of the risk ratios for seroconversion for each cluster pair (net/no net), with its 95% confidence interval. Formal tests of effect of no intervention were obtained with a paired *t* test.

Results There was no significant difference in the risk of seroconversion over 24 months in intervention (5.4%; 347/6372) compared with control (5.5%; 345/6319 people) clusters (risk ratio 0.90, 95% confidence interval 0.49 to 1.65) nor in the risk of clinical visceral leishmaniasis (0.99, 0.46 to 1.40). Adjustment for covariates did not alter these conclusions.

Conclusions There is no evidence that large scale distribution of longlasting insecticidal nets provides additional protection against visceral leishmaniasis compared with existing control practice in the Indian subcontinent. The observed effect was small and not significant, though the confidence intervals did not exclude a 50% change in either direction.

Trial registration Clinical Trials NCT 2005-015374.

INTRODUCTION

Visceral leishmaniasis is a neglected infectious disease[1] caused by intramacrophage protozoa of the *Leishmania donovani* complex transmitted by phlebotomine sandflies. Only a fraction of people infected develop clinical visceral leishmaniasis, characterised by febrile splenomegaly, pancytopenia, and progressive wasting, which can be fatal if left untreated. Visceral leishmaniasis, also known as kala-azar, affects an estimated 500 000 people annually, mostly in Asia and East Africa,[2] and is especially prevalent in poor communities.[3] In India, Nepal, and Bangladesh, it is transmitted between people by *Phlebotomus argentipes*,[4] a peridomestic species of sandfly whose indoor biting rhythm peaks at night.[5] The current vector control strategy is based on indoor residual spraying with dicophane (DDT) or pyrethroids and has been criticised for being costly, not easily accepted, and not sustainable.[6 7] Bed nets treated with insecticide, specifically longlasting nets treated with insecticide, have been proposed as an alternative to indoor residual spraying.[6] Treated nets proved effective in control of malaria[8] and cutaneous leishmaniasis in Iran and Syria.[6] Current evidence on effectiveness of treated nets for prevention of visceral leishmaniasis is limited to entomological data[6 9-11] and an observational study in Sudan,[12] but the latter cannot be extrapolated to the Indian subcontinent because the vector is different. Longlasting insecticidal nets have potential for control of visceral leishmaniasis in this region given the behaviour of *P argentipes*,[5] the high acceptability of bed nets,[13] the fact that untreated nets seem to provide some degree of personal protection,[14-16] and that village-wide distribution of longlasting insecticidal nets reduced the indoor density of *P argentipes*.[10 11] In 2005, a community intervention trial was set up in India and Nepal to test the effectiveness of comprehensive coverage with longlasting insecticidal nets on the incidence of *L donovani* infection in the current context, where

indoor residual spraying and untreated nets are irregularly used.

METHODS

Study design

We used a pair matched cluster randomised trial design as visceral leishmaniasis has a patchy spatial distribution and clusters strongly within villages at hamlet/neighbourhood (tola) and household level. Hamlets were chosen as the unit of analysis as they would be the unit of intervention for a control programme. Clusters were paired on the basis of incidence of visceral leishmaniasis between 2003 and 2005.

Study population

The trial was conducted in Muzaffarpur district in India and Saptari, Sunsari, and Morang districts in Nepal. The clusters—either a complete hamlet or a ward (that is, the smallest administrative subdivision)—were selected in a two step procedure. In February 2006 we identified 34 clusters with a high number of reported cases of visceral leishmaniasis (22 in India, 12 in Nepal) and conducted a house to house survey to retrospectively estimate the incidence of visceral leishmaniasis between 2003 and 2005. In May 2006, we selected and included in the trial 26 high incidence clusters out of these 34 (16 in India, 10 in Nepal) based on the following criteria: at least one case of visceral leishmaniasis in 2003, 2004, and 2005, indicating continuous *L donovani* transmission; a minimum 0.8% average annual incidence rate of visceral leishmaniasis from 2003 to 2005; a population ranging from 350 to 1500 people; and a minimum distance of 1 km between clusters. A census was conducted in July-August 2006 registering individual and household demographic information. All data were entered into a geo-referenced database. All individuals living for at least six months a year in the clusters were eligible, but blood sampling was restricted to individuals aged over 2 years.

Intervention

Between November and December 2006, longlasting insecticidal nets (PermaNet 2.0, Vestergaard-Frandsen, Denmark; 75 denier, 25 holes/cm^2, with deltamethrin (55 mg/m^2) coated fibres) were distributed in the intervention clusters. Enough nets were provided to protect all household members, taking into account the number of people (median five people/household), their age, sex, and sleeping arrangements (mean 2.5 treated nets/household). Any untreated nets were exchanged for treated nets. Community volunteers promoted the correct use of treated nets by information leaflets and home visits. The control clusters were allowed to continue using any existing conventional strategies for personal protection. They were not provided with treated nets nor was the use of untreated nets promoted. Individual use of nets was monitored during quarterly house to house surveys. For ethical reasons we did not interfere with ongoing indoor residual spraying activities, but we collected and considered information on spraying in the analysis. Free diagnosis and treatment of visceral leishmaniasis was provided in all clusters.

Sample size

The sample size was calculated with the method for pair matched randomised controlled trials described by Hayes and Bennett.[17] We aimed to show a 50% reduction in infection incidence rates in the intervention group compared with control. To determine the number of clusters required per arm we assumed a 2% yearly *L donovani* infection incidence rate; 500 inhabitants per cluster with an anticipated 10% loss to follow-up, 10% aged below 2 years, and 10% serologically positive at baseline; and a coefficient of variation between clusters (κ) of 0.25. Under those assumptions we anticipated a power of 90% with an α risk of 5%. The sample size calculations determined a minimum of 10 clusters per arm. The final number of clusters was increased to 13 per arm because the incidence of visceral leishmaniasis in Nepal during the preparation phase was lower than expected.[18]

Randomisation and allocation

The 26 clusters were stratified by country (16 in India and 10 in Nepal) and population size (six and four, respectively, having over 710 residents) and then paired by previous average incidence rate of visceral leishmaniasis. Clusters in each pair were randomly allocated to group 1 or 2. The random selection of clusters into groups was undertaken in Excel (Microsoft), and the difference in the total number of cases of visceral leishmaniasis reported in the past three years between group 1 and 2 had to be less than 10%. The intervention was then randomly allocated to one of the groups by tossing a coin in the presence of observers.

Outcomes

The primary outcome of the clinical trial was the number of incident *L donovani* infections as measured by seroconversion with the direct agglutination test at 12 and 24 months after the intervention, November-December 2007 and 2008, respectively. Serum samples were diluted from 1:400 to 1:25 600, and the cut off for positivity on the direct agglutination test was set at a titre of 1:1600. This titre, which is lower than the one used for diagnosis of visceral leishmaniasis in clinically suspected cases (1:3200), was chosen to increase the sensitivity to detect *L donovani* infection.[16 19] The sensitivity and specificity of the direct agglutination test to detect visceral leishmaniasis (disease) were estimated as 94.8% and 98.7%, respectively, in endemic communities.[20] Seroconversion was considered only in individuals who had negative results on the direct agglutination test ($\leq$1:800) in the baseline survey (or their first blood sample). A person was considered as a seroconverter if the titre at one of the two subsequent visits was $\geq$1:1600 and at least 2 titres above the baseline value. A minimum of 2 titres difference was

required to take into account the known interobserver variability in direct agglutination test reading (+1/−1 titre).[21]

All individuals aged over 2 years provided a capillary blood sample collected by finger prick on a Whatman No 3 filter paper in November-December 2006 (baseline), 2007, and 2008. Dried filter papers were kept at −20°C until the direct agglutination test was performed as described elsewhere.[22] A training workshop was organised to standardise the test protocol. Filter papers were analysed by survey batches to minimise processing errors, and 10% of the filter papers per survey were re-tested for quality control. No systematic errors were detected, and agreement between laboratories was good.

We assessed the number of incident cases of visceral leishmaniasis as a secondary outcome during quarterly house to house surveys from November 2006 to May 2009. Information about any case in the family was double checked with patients' records. People with fever lasting for two weeks or more were examined by a physician and tested with a rapid diagnostic test for visceral leishmaniasis (Kalazar Detect Rapid Test; InBios International, Seattle, WA).[23] All clinically suspected cases detected during the trial were classified as probable or certain visceral leishmaniasis by a clinician who was blinded to the status of the cluster. Suspected cases not corresponding to case definitions (see appendix on bmj.com) or with a positive direct agglutination test result at baseline were excluded. We excluded from the analysis cases of visceral leishmaniasis with onset of disease within two months after the baseline or more than two months after the final survey (to take into account the minimum incubation period)[24]. Asymptomatic infections were clinically followed up for a minimum of six months. Malaria was diagnosed during quarterly house to house surveys with a rapid diagnostic test (Parascreen; Zephyr Biomedicals, Goa, India)[25] in people with fever. Trained field workers carried out verbal autopsies on all deaths recorded during the trial. Two independent physicians ascertained cause of death.

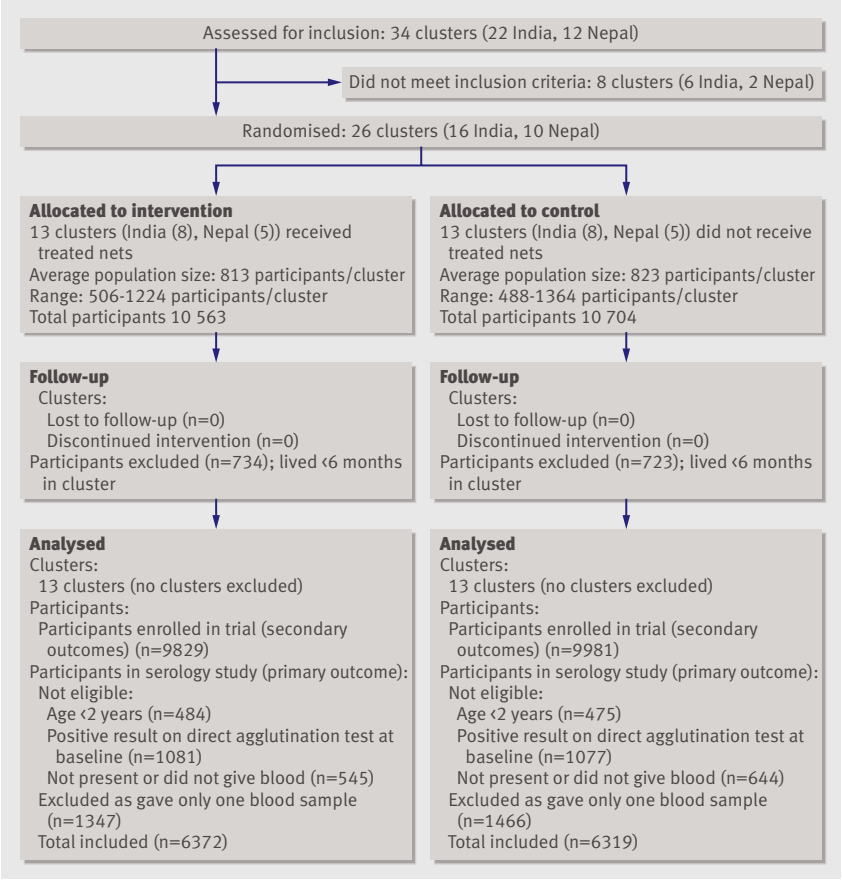

Fig 1 | **Trial profile of clusters and individuals allocated to use of longlasting insecticidal nets or control**

Statistical methods

All data were double entered into an EpiInfo 2000 database (Center for Disease Control and Prevention, Atlanta, GA) and analysed in Stata 11 (StataCorp LP, College Station, TX).

The data were analysed at cluster level, and the overall effect of the intervention was expressed as a risk ratio (treated nets/no treated nets) for *L donovani* infection. This overall estimate was obtained by taking the geometric mean of the risk ratios for seroconversion of each matched cluster pair. Formal tests of no intervention effect and confidence intervals were obtained with an unweighted paired *t* test on the log scale.[26] The confidence interval was computed on the log scale and then back transformed to the original scale by taking the anti-log.

Adjusted analyses were carried out in two stages. Firstly, we used a standard individual level logistic regression model to calculate expected number of events for each cluster ignoring the intervention. This standard model included pair and was simultaneously adjusted for age group, sex, indoor residual spraying, and socioeconomic status. Observed and expected values were used to calculate residuals as the ratio of observed and expected. The adjusted intervention effect was calculated with these residuals in a paired *t* test.

For secondary outcomes (such as visceral leishmaniasis and malaria) and some subgroup analyses (such as *L donovani* infection by socioeconomic status group) where the outcomes were rare and a cluster was left without an event, we calculated the risk ratio as the ratio of the arithmetic mean of proportions in intervention and control arms using the variance suggested by Hayes and Moulton[26] to obtain approximate 95% confidence intervals and test probabilities, thus ignoring the pairing in these situations.

For the adjusted analyses, we calculated a composite index reflecting the socioeconomic status of each household using principal components analysis to aggregate wealth, housing, and demographic characteristics.[3 27] Five equally sized groups were created for household socioeconomic status and age at baseline. The number of times each household was sprayed during the trial was grouped as none, once,

Table 1 | Baseline (November-December 2006) demographic information in intervention (longlasting insecticidal nets) and control groups for total study population. Figures are percentages (numbers) unless stated otherwise

Variable	Intervention	Control
Total		
No of participants	9829 (13 clusters)	9981 (13 clusters)
Mean (SD) age (years)	22.7 (19.2)	23.0 (19.2)
No (%) male	4858 (49.4)	4916 (49.2)
Annual incidence of visceral leishmaniasis 2003-5	1.4 (418/9260)	1.5 (410/8722)
Positive direct agglutination test results at baseline	14.9 (1039/6975)	14.5 (940/6473)
No (range) of households/cluster	1690 (78 to 240)	1803 (88 to 224)
Mean (SD) No of members in household	5.8 (2.7)	5.5 (2.5)
Clusters sprayed before intervention*	6	6
Households with untreated nets (95% CI)†	76.2 (67.2 to 85.1)	78.1 (69.3 to 87.0)
Mean indicator of socioeconomic status‡ (SD)	1.9 (1.4)	2.1 (1.4)
India		
No of participants	5987 (8 clusters)	6207 (8 clusters)
Mean (SD) age (years)	21.9 (19.7)	22.4 (19.6)
No (%) male	2980 (49.8)	3067 (49.4)
Annual incidence of visceral leishmaniasis 2003-5¶	1.51 (279/5677)	1.40 (261/5640)
Positive direct agglutination test results at baseline	19.4 (785/4059)	17.7 (705/3992)
No (range) of households/cluster	967 (78 to 172)	1082 (88 to 224)
Mean (SD) No of members in household	6.2 (3.0)	5.7 (2.7)
Clusters sprayed before intervention*	1	3
Households with untreated nets (95% CI)†	81.4 (70.5 to 92.3)	81.5 (67.0 to 95.9)
Mean indicator of socioeconomic status‡ (SD)	2.0 (1.4)	2.0 (1.5)
Nepal		
No of participants	3842 (5 clusters)	3774 (5 clusters)
Mean (SD) age (years)	23.9 (18.6)	23.9 (18.3)
No (%) male	1878 (48.9)	1849 (49.0)
Annual incidence of visceral leishmaniasis 2003-5	1.3 (139/3583)	1.6 (149/3082)
Positive direct agglutination test results at baseline	8.7 (254/2916)	9.5 (235/2481)
No (range) of households/cluster	723 (103 to 240)	721 (103 to 196)
Mean (SD) No of members in household	5.3 (2.2)	5.2 (2.3)
Clusters sprayed before intervention*	5	3
Households with untreated nets (95% CI)†	67.8 (49.6 to 86.0)	72.8 (62.7 to 82.9)
Mean indicator of socioeconomic status‡ (SD)	1.9 (1.4)	2.1 (1.4)

*July 2005 to November 2006.
†At least one untreated net observed by study teams. Percentage estimated from survey in 25 households per cluster conducted in August-September 2006.[13]
‡Composite index calculated for each household with principal components analysis to aggregate wealth, housing, and demographic characteristics.[27] Household indexes categorised in five equally sized groups.
¶Incidence in nine clusters (four in intervention) calculated from 3.5 years.

and twice or more. Similarly, we tested whether the intervention effect was the same in India and Nepal.

Ethical considerations

Communities were informed about the purpose of the trial and consent for inclusion was sought from village leaders. Written informed consent was obtained from head of households and from each individual or their guardian for those aged under 18. For ethical reasons, we conducted an interim analysis 12 months after the intervention, with a pre-planned rule to stop the trial and distribute treated nets in the control clusters if the 95% confidence interval indicated a 30% or greater reduction in the incidence rate of visceral leishmaniasis in the intervention group compared with the control group.

RESULTS

Participants flow

Out of 15 504 eligible seronegative people, we included 12 691 (82%) in the analysis of the main outcome (infection) and considered 19 810 of the 21 267 (93%) initially enrolled for the secondary (disease) end point. Figure 1 shows reasons for exclusion and the range and number of households and people per cluster and allocation group. The proportion of people lost to follow-up (not present or with one or no blood sample) was slightly higher in the control group (21%, (644 +1466)/9981) than in the intervention group (19%, (545+1347)/9829). But the characteristics of the participants lost to follow-up in both groups were similar (mean age 22 *v* 23, males 62% *v* 63%, mean socioeconomic status 2.0 *v* 2.2, in intervention and control groups respectively). Intervention and control groups were well balanced at individual and cluster levels, but the prevalence of positive results on the direct agglutination test at baseline in India was almost twice as high as in Nepal, despite the previous annual incidence of visceral leishmaniasis being similar (table 1). Participants in the group in which the infection end point was measured—that is, those with negative results on direct agglutination test at baseline—were slightly older and more were female than the general population, but, again, this was well balanced between study groups (table 2). Vector densities at baseline were comparable in intervention and control clusters.[11] During the two year follow-up, 14 clusters (six intervention and eight control) were sprayed under the national control programme with limited or no effect on vector density.[11] In intervention clusters, 8920/9829 (91%) of the individuals slept regularly (that is, over 80% of the nights) under a treated net. Those observations were confirmed by an additional acceptability survey (V Vanlerberghe, personal communication, January 2010). The use of untreated nets in the control group was variable; 7012/9981 (70%) used a bed net at least once during the trial but only 2978/9981 (30%) used it regularly throughout the year as most of the households did not have enough nets for all their members.

Outcomes and estimation

Primary outcome

The number of individuals who seroconverted ranged from 5 to 60 per cluster, and the incidence of infection was variable among cluster pairs (fig 2). The risk of seroconversion during the two year follow-up was significantly different between countries: 7.2% in India (529/7368) and 3.1% (163/5323) in Nepal. The overall risk of seroconversion in the intervention (5.4%, 347/6372) and control (5.5%, 345/6319) groups was similar. At cluster level, the risk of infection was reduced by 10% in the intervention clusters compared with control clusters, but this effect was not significant (risk ratio 0.90, 95% confidence interval 0.49 to 1.65). The results were similar in the model adjusted for age, sex, indoor residual spraying, and socioeconomic status (0.89, 0.48 to 1.64) (table 3). Longlasting insecticidal nets seemed to have an opposite effect on seroconversion according

Table 2 | Baseline demographic information in intervention (longlasting insecticidal nets) and control groups for eligible subgroups observed for main outcome (seroconversion on direct agglutination test)

Variable	Intervention	Control
Total		
No of participants	6372 (13 clusters)	6319 (13 clusters)
Mean (SD) age (years)	23.6 (19.1)	23.3 (19.0)
No (%) male	2882 (45.2)	2809 (44.5)
India		
No of participants	3568 (8 clusters)	3800 (8 clusters)
Mean (SD) age (years)	22.9 (19.8)	22.4 (19.3)
No (%) male	1570 (44.0)	1669 (43.9)
Nepal		
No of participants	2804 (5 clusters)	2519 (5 clusters)
Mean (SD) age (years)	24.5 (18.3)	24.7 (18.4)
No (%) male	1312 (46.8)	1140 (45.3)

to the direct agglutination test result in India (1.09, 0.58 to 2.04) and Nepal (0.66, 0.12 to 3.56), but the interaction was not significant (P=0.40). Similar results were obtained in the adjusted model: risk ratio 1.09 and 0.57 in India and Nepal, respectively (table 3).

Secondary outcomes

A total of 168 suspected cases of visceral leishmaniasis (129 in India and 39 in Nepal) were identified during the trial. The case ascertainment procedure reduced this number to 77 confirmed cases; 62 in India and 15 in Nepal (fig 3). The overall risk of visceral leishmaniasis during the two year follow-up was 0.38% (37/9829) and 0.40% (40/9981) in the intervention group and control group, respectively. The cluster level analysis showed that longlasting insecticidal nets reduced the risk of visceral leishmaniasis by 1% (risk ratio 0.99, 95% confidence interval 0.46 to 1.40), but the effect was not significant. The country specific and adjusted results were similar and not significant (table 3). We obtained analogous results when we analysed all suspected cases and when we used a six month incubation period (see appendix on bmj.com).

A total of 225 cases of malaria were identified, mostly in India (n=220) and mostly non-*P falciparum* malaria (n=208); there were 12 cases of *P falciparum* malaria and five undefined species. There was a non-significant reduction in cases of malaria in intervention clusters (risk ratio 0.63, 0.29 to 1.36), but the adjusted effect was stronger and significant (0.46, 0.28 to 0.77). In India, where most malaria cases were reported, the risk ratio was 0.60 (0.38 to 0.94) in the adjusted model (table 3). Similarly, the total number of deaths was reduced in intervention clusters (0.75, 0.50 to 1.13), but the effect was not significant (table 3). None of the deaths during the trial was attributable to visceral leishmaniasis.

DISCUSSION

In this large scale randomised controlled trial of the effectiveness of longlasting insecticidal nets in preventing visceral leishmaniasis, we found the risk for *L donovani* infection was reduced by 10% in intervention clusters. This was a small and non-significant effect, though its wide confidence interval does not rule out a potential beneficial effect. The findings in the primary (serological) and secondary (clinical visceral leishmaniasis) end points were consistent as there was no difference in the incidence of visceral leishmaniasis between intervention and control clusters. Use of the treated nets in intervention clusters was high in all seasons and significantly reduced the risk of malaria, especially in India, where 98% of all malaria cases in this trial were reported.

Strengths and limitations

The incidence of *Leishmania* infection in study clusters could have been lowered by the active detection and treatment of cases of visceral leishmaniasis implemented during the trial but did not fall below the assumption of 2% annual incidence made when we calculated the sample size. If we assume a constant intervention effect over all pairs, the observed coefficient of variation for *L donovani* infection was 0.56. This value can be of use for the planning of future intervention trials. While our sample size calculation was based on a lower κ than the one finally observed, the power of the study was increased by adding six extra clusters. The impact of migration on the estimation of effect was limited as the number of individuals excluded was similar in both groups. Follow-up rates during the trial were high, with 82% and 93% for the primary and secondary outcome, respectively. The proportion lost to follow-up was slightly higher in control (21%) than in intervention (19%) clusters, but we do not expect this to affect the estimations as the characteristics of these people were similar. We used a cluster level model to analyse the trial data as this was the analytical model specified for the sample size formula[17] and is the method recommended by Hayes and Moulton[26] for pair matched trials with fewer than 15 clusters per arm. The trial results were not significantly modified when we used a multi-level model (random effects logistic regression) (see appendix on bmj.com).

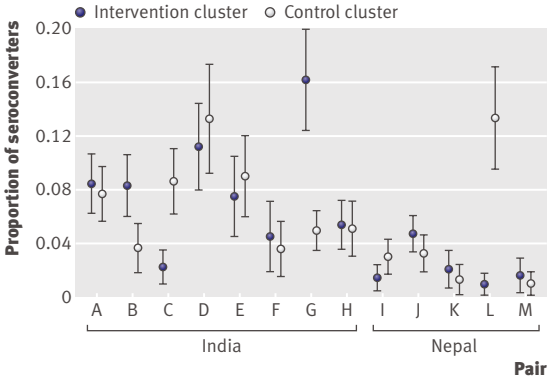

Fig 2 | Observed proportion of seroconversions according to result of direct agglutination test by cluster and pair with 95% confidence intervals

Table 3 | Effect of longlasting insecticidal nets on *L donovani* infection measured by direct agglutination test with cut off 1:1600 and minimum of two titres difference and secondary outcomes: visceral leishmaniasis, malaria cases and all causes of death. Overall and country specific unadjusted and adjusted risk ratios for intervention compared with control from cluster analysis

Variable	Intervention	Control	Risk ratio (95% CI), P value*	
			Unadjusted	Adjusted†
Overall				
No in serology study	6372 (13 clusters)	6319 (13 clusters)	—	—
No of seroconversions (%)	347 (5.4)	345 (5.5)	0.90 (0.49 to 1.65), 0.71	0.89 (0.48 to 1.64), 0.68
Total No of participants	9829 (13 clusters)	9981 (13 clusters)	—	—
Visceral leishmaniasis (%)‡	37 (0.38)	40 (0.40)	0.99 (0.46 to 2.16), 0.99	1.15 (0.61 to 2.16), 0.64
Malaria (%)‡	88 (0.90)	137 (1.37)	0.63 (0.29 to 1.36), 0.21	0.46 (0.28 to 0.77), 0.01
All causes of death (%)	124 (1.26)	167 (1.67)	0.75 (0.50 to 1.13), 0.15	0.78 (0.56 to 1.10), 0.15
India				
No in serology study	3568 (8 clusters)	3800 (8 clusters)	—	—
No of seroconversions (%)	276 (7.7)	253 (6.7)	1.09 (0.58 to 2.04), 0.76	1.09 (0.58 to 2.05), 0.75
Total No of participants	5988 (8 clusters)	6207 (8 clusters)	—	—
Visceral leishmaniasis (%)‡	31 (0.52)	31 (0.50)	1.00 (0.41 to 2.44), 1.00	0.94 (0.44 to 2.02), 0.85
Malaria (%)‡	87 (1.45)	133 (2.14)	0.64 (0.36 to 1.13), 0.11	0.60 (0.38 to 0.94), 0.03
All causes of death (%)	80 (1.34)	121 (1.95)	0.62 (0.32 to 1.19), 0.13	0.72 (0.44 to 1.19), 0.17
Nepal				
No in serology study	2804 (5 clusters)	2519 (5 clusters)	—	—
No of seroconversions (%)	71 (2.5)	92 (3.6)	0.66 (0.12 to 3.56), 0.53	0.57 (0.11 to 2.97), 0.40
Total No of participants	3842 (5 clusters)	3774 (5 clusters)	—	—
Visceral leishmaniasis (%)‡	6 (0.16)	9 (0.24)	0.96 (0.13 to 7.39), 0.96	1.55 (0.17 to 14.18), 0.57
Malaria (%)‡	1 (0.03)	4 (0.11)	0.18 (0.00 to 14.38), 0.23	—§
All causes of death (%)	44 (1.15)	46 (1.22)	1.02 (0.67 to 1.55), 0.93	1.06 (0.69 to 1.64), 0.73

*Test for no intervention effect.
†Adjusted for age group, sex, times sprayed, and socioeconomic status.
‡Rare outcomes: some clusters did not record any event during study period. Risk ratio calculated as ratio of arithmetic mean of proportions in intervention and control arm with variance (see appendix on bmj.com).
§Too few cases of malaria to adjust for covariates.

We used the direct agglutination test as a marker for infection, as in previous epidemiological studies,[16 19 28-30] because the low incidence of visceral leishmaniasis precluded its use as primary outcome. We found no published estimates on the sensitivity and specificity of the direct agglutination test to test for infection in humans, but we have shown that recent seroconverters are at a substantially higher risk of developing visceral leishmaniasis in this setting.[31] The trial outcome was not modified when less (that is, seroconversion based on a 1 titre difference) or more (that is, higher direct agglutination test cut offs) specific criteria were used (see appendix on bmj.com). Though others have used the leishmanin skin test as a marker of exposure, we could not do so in our trial because of unsatisfactory performance in India.[32]

Interpretation

As adherence to use of the treated nets was high during the trial, the lack of effect could be explained by biological factors. World Health Organization pesticide evaluation scheme (WHOPES) standard susceptibility tests showed that *P argentipes* is susceptible to deltamethrin in the study clusters.[33] Moreover, indoor density of *P argentipes* was reduced by 25% in the study clusters that used treated nets compared with control clusters.[11] This reduction, however, might be insufficient to have an impact on immunological and clinical outcomes if, in contrast with previous data,[5] transmission of *L donovani* occurs partly outside the house, as suggested by recent studies.[9 34 35] This trial measured the effectiveness of distribution of treated nets in a real life context, where some clusters were sprayed and some people in control clusters used untreated nets. The use of untreated nets before the trial did not prevent all transmission in these communities. Analysis showed that protection provided by untreated nets was similar to that from treated nets; whereas both treated and untreated nets seemed to provide some degree of protection compared with not using nets (adjusted models $P<0.06$ and $P<0.09$, respectively) (see appendix on bmj.com). This is supported by entomological data from the study clusters.[36]

The fact that 14 of the 26 study clusters were sprayed as part of the visceral leishmaniasis national control programmes during the trial had a limited impact on vector density, as reported elsewhere.[11] Moreover, the effect of treated nets on *L donovani* infection was not significantly modified in the adjusted models, which included indoor residual spraying as a variable. Under controlled conditions, indoor residual spraying effectively reduces the indoor density of *P argentipes*.[10] When applied as part of the national vector control programmes, however, indoor residual spraying was

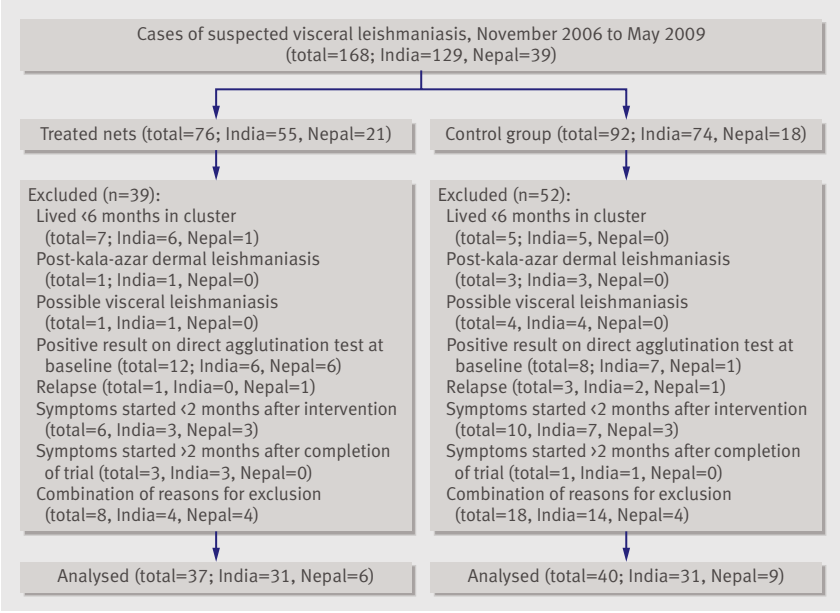

Fig 3 | **Case ascertainment for visceral leishmaniasis and reasons for exclusion**

shown to have a limited effect or no effect on indoor density of *P argentipes* in villages in Bihar with endemic visceral leishmaniasis[9] and, more generally, on control of visceral leishmaniasis.[6] The poor quality of routine indoor residual spraying operations for visceral leishmaniasis in the region is the main reason for this failure[37] and has contributed to the development of DDT resistance in districts with endemic visceral leishmaniasis[6] and in our study areas in particular.[33] Similarly, the variability in incidence observed in some study clusters—notably, pairs G and L (see fig 2)—does not seem to be explained by the effect of indoor residual spraying as both clusters in pair G were sprayed once and neither cluster was sprayed in pair L. The differences could be better explained by heterogeneity in the transmission dynamics (because of, for example, heterogeneity in population susceptibility and vector infectivity). This variability does not seem to affect the trial outcomes, as the effect of treated nets on *L donovani* infection was not significantly altered when we repeated the analyses excluding the clusters with exceptionally high rates (outliers) (see appendix on bmj.com).

WHAT IS ALREADY KNOWN ON THIS TOPIC

Longlasting insecticidal nets are among the major breakthroughs in the control of malaria in recent years, and expectations were raised about their potential to prevent other vector-borne diseases

Observational evidence in East Africa and Asia suggested that people sleeping under nets were protected against visceral leishmaniasis

WHAT THIS STUDY ADDS

Village-wide distribution of insecticide treated nets in India and Nepal did not confer protection against *Leishmania donovani* infection or visceral leishmaniasis when compared with existing preventive practice (irregular insecticide residual spraying and use of untreated nets)

Our results are in contrast with those of an observational study in Sudan, which reported a 27% reduction in cases with a smaller mesh size.[12] The mesh size used in this trial (2 mm) would not be a perfect physical barrier for sandflies, but the insecticide coating of the fibres should prevent their passage.[38]

Implications

Our results show that the distribution of longlasting insecticidal nets did not confer additional protection against *L donovani* infection and visceral leishmaniasis in the current context in endemic communities in India and Nepal when compared with existing control practices (that is, irregular indoor residual spraying, use of untreated nets, and treatment of visceral leishmaniasis). Also distribution of treated nets did not reduce the annual incidence of visceral leishmaniasis below 18.8 per 10 000, which by far exceeds the elimination target in the region.[7] The most biologically plausible explanation for our results is that a substantial fraction of *L donovani* transmission occurs outside the house, where any nets would have less impact on preventing sandfly-human contact. Nonetheless, insecticide treated nets should not be dismissed as a potential intervention for visceral leishmaniasis prevention on the Indian subcontinent, as more research on vector behaviour and implementation of such nets is warranted.

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Contributors: AP, SPS, SR, SS, FC, VV, CRD, and MB designed the trial. AP, SPS, BO, FC, SU, KG, RK, PS, BK, ISP, and MLD collected data. AP, SPS, SR, SS, BO, FC, JCD, MR, VV, EWA, CRD, and MB analysed and interpreted data. All authors wrote the paper. MB is guarantor.

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Ethical approval: Communities were informed about the purpose of the trial and consent for inclusion was sought from village leaders. Written informed consent was obtained from head of households and from each individual or their guardian for those aged under 18. Clearance was obtained from the ethical committees of Institute of Medical Sciences (Banaras Hindu University), B P Koirala Institute of Health Sciences, the

London School of Hygiene and Tropical Medicine, and the University of Antwerp.

Data sharing: Technical appendix, statistical code, and dataset available from the corresponding author at mboelaert@itg.be. Participants' consent for data sharing was not obtained but the presented data are anonymised and risk of identification is low.

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