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Comparative study of kala-azar vector control measures in eastern Nepal

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ABSTRACT

This study was conducted to explore the most effective vector control tool among indoor residual spraying (IRS), long lasting insecticidal nets (LLINs) and ecological vector management (EVM) as a part of the regional visceral leishmaniasis elimination initiative. Alpha-cypermethrin as IRS, PermaNet® as LLINs and plastering the inner walls of houses with lime as EVM were the interventions. One baseline and three follow-up entomological surveys were carried out in all arms using CDC miniature light traps (LT) and mouth aspirators. Comparisons were made between intervention arms and control arms with pre-intervention and post-intervention vector densities. Light traps were found more efficient in the collection of *Phlebotomus argentipes* in comparison with aspiration. Vector densities were significantly low in both IRS arm ($p = 0.009$ in LT and $p < 0.001$ in aspirator collections) and LLIN arm ($p = 0.019$ in LT and $p = 0.023$ in aspirator collections) in comparison with control arm. However, in EVM arm, there was no significant difference in *P. argentipes* sand fly density in comparison with control arm ($p = 0.785$) in LT collections in follow-up surveys. Hence, IRS was found most effective control measure to decrease vector density. LLINs were also found effective and can be considered as a promising alternative vector control tool in VL elimination initiative.

1. Introduction

Kala-azar or visceral leishmaniasis (VL) is a major public health problem in Nepal with more than 6.5 million people at risk (Thapa et al., 2009). Kala-azar was considered eliminated from the Indian sub-continent in the 1960s, but it re-emerged, a few years after withdrawal of the extensive DDT spraying for malaria control. Vector control, early case detection and complete treatment are the strategies currently in practice to fight the disease as no vaccine against kala-azar is available till date. *Phlebotomus argentipes* sand fly is the single identified vector species in the Indian sub-continent and only the anthroponotic form is reported (Lawyer, 1992); it is expected that kala-azar can be controlled by reducing vector–human contact through vector control. Indoor residual spraying (IRS) of insecticides are typically long-acting. However, their effectiveness depends on a number of factors including quality of spraying, climate of the area and building materials used in the households. Limitation of IRS is so prominent that even after over a decade of IRS campaign in Nepal kala-azar has not been controlled to its targeted level yet (Bista et al., 2005).

An alternative to IRS, insecticide treated nets (ITNs) were shown over the past two decades as one of the most effective method of reducing man-vector contact and intra- and peri-domiciliary transmission of vector-borne diseases (Kishore et al., 2006). Effectiveness of the ITN is independent of the endophilic or exophilic behavior of the vector, less insecticide is used for impregnation, households can exert control over its use (indoor/outdoor) and the net provides a mechanical barrier against biting insects. These factors make their effect less dependent on the performance of a disease control program. Recently, long lasting insecticidal nets (LLINs) have been introduced; whose insecticidal properties comprise a prolonged effectiveness without need to retreat frequently.

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Plastering of the sand fly breeding sites with aqueous solution of lime could be an effective ecological vector management (EVM) to reduce their density and ultimately reduce the kala-azar transmission rate. In India, liming was found to reduce vector density in a pilot study (Kumar et al., 1995). EVM can be considered to be helpful in destroying the breeding places of sand flies. It is a common cultural practice to paint walls and floor of the household frequently with mud or lime in rural areas of Nepal. Hence it is feasible to encourage people to use lime instead of mud for painting and repairing the cracks and crevices inside the household.

This paper reports a study conducted to explore the most effective vector control tool among IRS, LLINs and EVM as a part of the regional visceral leishmaniasis elimination initiative.

2. Materials and methods

2.1. Study area

The study area included two kala-azar endemic districts in eastern part of Nepal namely Sunsari and Morang (26°40′N 87°30′E) with 24 clusters. All clusters lie in subtropical zone with altitudes ranging from 72 to 349 m and experience three seasons; viz. dry winter (November–February), summer (March–June) and rainy (July–October). Temperature varies from minimum 8 °C in winter to maximum 39 °C in summer. Total annual rainfall received in the study area, in the year 2006 was 1236 mm ranging from 0 mm in the months of January and February to 308 mm in the month of September.

Clusters are surrounded by open paddy fields of alluvial soil. Multi-crop cultivation is adopted in a few places where jute, wheat, sugarcane, maize and vegetables are grown round the year. Paddy rice is always the predominant crop. Bamboo trees, ornamental and mango plantations are found in the vicinity of houses. Cattle are an additional source of income and are also employed in agriculture for ploughing and threshing grains. Most of the villages are inhabited by the indigenous Tharu community. Drainage and ponds nearby the households are used for duck rearing and jute seasoning. These stagnant water reservoirs keep the ground water level high and maintain moist environment inside the houses. The economic status of the people is very low; income is exclusively dependent on agriculture or part-time labor in nearby factories.

2.2. Study period and selection of the study clusters

The study was carried out from November 2006 to April 2007. Based on government records of Sunsari and Morang districts, 24 clusters with high incidence of kala-azar were identified. All of the selected clusters were from the same geographical region and with similar socio-economic status. After stratification of villages on the basis of disease incidence, four groups of six clusters were randomly allotted to the four arms of intervention; LLIN, IRS, EVM and Control. Each cluster had more than 50 houses and the minimum distance between any two clusters was 15 m. All the houses were identified with a unique household number. A questionnaire was completed in each household to collect the information on demographic and socio-economic factors.

2.3. Intervention

Interventions were put into operation in the three arms in the months of November/December 2006 after the completion of the baseline entomological survey in November 2006.

LLIN: Sleeping arrangements of the family members were noted and sufficient nets were provided to cover every member of the household. PermaNet® mosquito-nets (Vestergaard Frandsen, 100% polyester, 25 mesh/cm^2, impregnated with 55 mg a.i. deltamethrin/m^2) of blue colour were distributed according to the choice of community (Das et al., 2007a).

IRS: Synthetic pyrethroids; alpha-cypermethrin (Gharda Chemical Ltd, 5/6 Germansion, Bandra west, Mumbai-400050, India) was used for spraying with a concentration of 25 mg/m^2. Household members were informed about the precautions to be taken before spraying, to avoid possible health risks. According to the government strategy of IRS, spray-men sprayed insecticide to the interior walls of the houses and cattle sheds up to six feet height covering all possible resting and breeding sites of sand flies.

EVM: Lime ($CaCO_3$) was provided to the inhabitants of this arm. The quantity of lime distributed per family was based on the record of the number of rooms with precarious walls in their housing compound. Proper application procedure was explained and demonstrated before and during the distribution of lime so that they could be made conscious for repairing the cracks, crevices and other potential sand fly breeding and resting places. Local volunteers were trained for regular monitoring of the intervention.

Control: In these clusters no intervention was employed, but free transportation to a treatment facility as well as free diagnostic work-up and treatment were provided to the inhabitants.

2.4. Entomological survey

In each cluster two mixed (cattle and human staying under the same roof) houses and three human houses were randomly selected by simple random sampling after stratification of all the households in the cluster into mixed and human houses. Sand fly collection was done in those houses by using Centers for Disease Control (CDC) light traps (LT). In each cluster, five CDC light traps were used for two consecutive nights every month from 6 PM to 6 AM. Each LT was fixed six inches above the ground and one inch apart from the wall in a corner of the main sleeping room. Other five houses (two mixed and three human) adjacent to the CDC LT houses were allocated for the collection of sand flies by mouth aspirator (Castro type). In each house, 15 min active searching was done for aspirator collection for two consecutive mornings. Thus 60 collections were made in 30 houses through CDC light trap and other 60 collections through mouth aspiration method in each arm. Baseline and follow-up surveys were conducted on 2 weeks before and 2 weeks, 4 weeks and 5 months after the interventions. Sand fly identification was based on morphological characters while confirmation of obscure specimens was done by dissection and observation of the spermatheca in females (Lewis, 1978). Species, abundance, gender and physiological status of females were recorded in a pre-set form.

2.5. Ethical considerations

The proposal was approved by the Ethical Review Board of the B.P. Koirala Institute of Health Sciences, Dharan, Nepal. The households were informed about the purposes of the study. Only those households who had given written informed consent were included in the study.

2.6. Data analysis

Data were entered in database prepared in Epi-info-3.3.2. All results are based on Poisson regression analysis with clustering at sample cluster-level using STATA version 9.2 and 10. A robust sandwich estimator of the variances was applied. Significances are stated at 5% level and 95% confidence intervals are given.

Control arm was considered common for all the three intervention arms. For CDC light trap collections, density was calculated as the total count per house per night. For aspirator collections, density was calculated per house per morning aspiration.

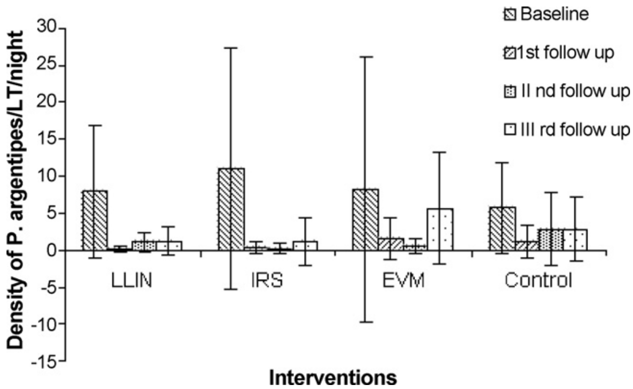

Fig. 1. Density of *P. argentipes* per light trap per night in four arms in four consecutive entomological surveys.

3. Results

The 24 clusters included in four arms comprised 1335 households and the total population covered was 6955 with 36.4% of them below the age of 15 years. Majority of houses (94.7%) had doors; windows were present in only 39% of the houses but were without glass or screen and only 19.5% of total households owned curtains. Houses were generally of low economic status; more than 80% of the houses had bamboo walls plastered with mud, 90.7% had mud floor, and 57.2% had straw roof. Cattle were present in only 47.7% of households. Most of the households (67.4%) had a bicycle for transportation. Radio, TV and motor-cycle were available with 43.1%, 40.6% and 2.2% of the households, respectively.

At the baseline survey, 4661 sand flies were collected comprising 71% by light traps and 29% by mouth aspirators. The vector species, *P. argentipes* occupied 49% of the total sand fly collection and remaining species were *P. papatasi and Sergentomyia* spp. Percentage of *P. argentipes* in the total number of sand flies collected by light traps (60%; 1976 out of 3309) was considerably higher than those collected by aspirators (24%; 333/1352). Statistical analysis of *P. argentipes* sand fly densities revealed no significant difference between intervention and control arms in LT collections. Similar results were obtained in LLIN and IRS arms except EVM arm in aspirator collections.

During three follow-up surveys, a sum of 5280 sand flies were collected (60% by light traps and 40% by aspirators). The vector species, *P. argentipes* occupied 26.6% of the total sand fly collection (36%, 1146 out of 3184 by light traps and 12%, 258 out of 2096 by aspirators) and remaining were other species (*P. papatasi and Sergentomyia* spp.).

3.1. Impact of LLIN

In comparison with the baseline survey, vector density dropped from 7.9 to 0.9 per house per night by LT collections and from 1.8 to 0.5 per house per morning aspirator collections in three follow-up surveys after intervention. Vector density in LLIN arm if compared with that in control arm, there was significant difference in density at follow-up collections; $p = 0.019$ in LT and $p = 0.023$ in aspirator collections (Table 1). A trend of decrease in *P. argentipes* density after intervention is shown in Figs. 1 and 2.

3.2. Impact of IRS

In IRS arm, *P. argentipes* density decreased from 11.0 to 0.6 per house per night by LT collections and from 1.3 to 0.0 per house per morning by aspirator collections in the baseline to follow-up surveys. Comparing follow-up collections in IRS versus control

Table 1
Descriptive and cross-sectional testing based on regression analysis of total *P. argentipes* counts in light traps and aspirators, CI and *p*-values based on exponential (B) and exponential (CI).

Method of collection	Survey	LLIN			IRS			EVM			Control			*p*-Value		
		Mean	CI 95%		Mean	CI 95%		Mean	CI 95%		Mean	CI 95%		LLIN vs Cont	IRS vs Cont	EVM vs Cont
			Lower	Upper		Lower	Upper		Lower	Upper		Lower	Upper			
Light trap	1	7.9	4.9	12.9	11.0	6.0	20.3	8.2	4.3	15.5	5.7	3.6	8.9	0.303	0.070	0.342
	2–4	0.9	0.6	1.3	0.6	0.3	1.3	2.6	1.8	3.7	2.3	1.1	4.9	0.019	0.009	0.785
Aspirator	1	1.8	1.1	3.0	1.3	0.7	2.4	0.6	0.4	1.0	1.8	1.1	2.9	0.978	0.391	0.001
	2–4	0.5	0.3	0.7	0.0	0.0	0.1	0.1	0.0	0.4	0.8	0.6	1.0	0.023	<0.001	0.001

Note: Survey 1 = baseline survey, Survey 2–4 = 3 consecutive follow-up surveys.

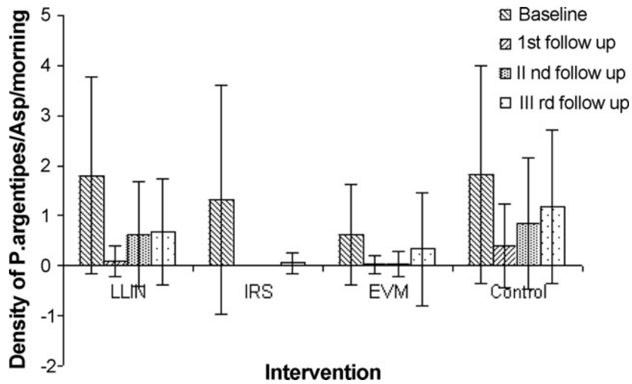

Fig. 2. Density of *P. argentipes* per house per morning by mouth aspirator in four arms in four consecutive entomological surveys.

arm, a significant difference in vector density was observed in LT ($p = 0.009$) and aspirator ($p < 0.001$) collections. The result simply demonstrated that IRS was highly effective for reducing vector density (Table 1). A sharp decrease in *P. argentipes* density after intervention was observed in this arm (Figs. 1 and 2).

3.3. Impact of EVM

In EVM arm, *P. argentipes* density decreased from 8.2 to 2.6 per house per night in LT collections and from 0.6 to 0.1 per house per morning aspirator collections in the baseline to follow-up surveys. No significant difference in vector density while comparing with the control arm ($p = 0.785$ in LT collections) was observed in follow-up surveys. An ambiguous result, significant difference ($p = 0.001$) in *P. argentipes* sand fly density between intervention and control arm, was obtained in baseline and follow-up surveys by aspirator method. As the baseline difference in aspirator collection is significant ($p = 0.001$) which then of course increases the possibility to have a significant follow-up difference, which might be due to some manual error in the method of collection (Table 1).

3.4. Variation in sand fly density

P. argentipes sand fly density decreased in all intervention and control arms in first follow-up survey. In consecutive follow-up surveys, the density was found increasing and highest density was recorded in EVM arm in light trap collections and highest in control arm in aspirator collections. Bar graphs with error bars show the distribution of sand fly densities in sampled houses in each arm. A wide fluctuation in the data can be observed, which is attributed to the seasonal effect as in the winter season density of *P. argentipes* drops down to negligible level (Figs. 1 and 2).

Abundance of *P. argentipes* sand flies at baseline survey was 53.6% in human and 46.4% in mixed dwellings, and 54.5% in human and 45.5% in mixed dwellings in the three consecutive follow-up surveys in light trap collections. In aspirator collections, 55% and 45% at baseline, and 74% and 26% *P. argentipes* in the three consecutive follow-up surveys were collected in human and mixed dwelling, respectively. Above-mentioned figures do not show significant difference in abundance of *P. argentipes* in human and mixed dwellings except in aspirator collections in three follow-up surveys.

4. Discussion

The light traps were found more efficient in the collection of *P. argentipes* in comparison with aspiration. A similar finding was reported by Dinesh et al. (2008a,b) who found LT as most effective in catching vector species. Collections by aspirators might have subjective biases as it depends on the skills and efforts executed by the individual insect collector. Decline in sand fly density in all arms during first follow-up survey might be due to adverse environmental conditions; low temperature and humidity, etc. During the subsequent follow-up surveys, increasing tendency in sand fly density might be the influence of favorable environmental condition and successive loss of effectiveness of the intervention methods; however, effectiveness of the PermaNet® remains for 3–5 years (WHO, 2004) as claimed by the manufacturer.

4.1. LLIN

Baseline count for *P. argentipes* was higher than control arm and the opposite was true at follow-up surveys in LLIN arm. It can be inferred from the result that LLINs were effective on reducing the sand fly density. LLINs may be more effective since deltamethrin has not only insecticidal effect but also repellent effect (Courtenay et al., 2007). Deltamethrin impregnated bed nets with fine mesh provided an effective barrier against vectors or other annoying insects (Alexander et al., 1995). In Brazil the insecticide increased the barrier effect of nets by 35% though there was no evidence of diversion of the sand fly vector onto unprotected hosts (Courtenay et al., 2007). Insecticide impregnated curtains were found effective against sand fly vector in Venezuela (Kroeger et al., 2002). On the contrary, Dinesh et al. (2008a,b) found LLINs fail at household level to reduce abundance of sand fly vector *P. argentipes* in treated houses in Bihar (India).

LLINs were found as a promising alternative to IRS for vector control, but prediction of efficacy of impregnated nets on VL transmission could not be done simply by monitoring the vector density (Jalouk et al., 2007). Tayeh et al. (1997) found no evidence that the use of pyrethroid-impregnated bed nets had an impact on reducing the density of female vector species of cutaneous leishmaniasis in the intervention area. However, significant drop down in the disease incidence was observed in both the studies. Similar study conducted for VL control in Sudan demonstrated the effect of treated nets on reducing the disease by 59% suggesting that the effectiveness of the net depends on the behavioral factors of the communities (Ritmeijer et al., 2007). In Nepal, efficacy of LLINs to reduce VL is under investigation by Kala-net project (www.kalanetproject.org), which will be concluded in late 2009.

High percentages of people (47% and 61%) of this area were found to be having local nets to get protected from mosquito bites (Koirala et al., 1998; Das et al., 2007b); the fact is supportive to compliance of people to LLIN use. Knowledge and practice of proper use (Das et al., 2007b), washing methods, cost of the nets and seasonal factors are the key factors to be considered before distribution of LLINs in every focal area (Binka and Adongo, 1997).

4.2. IRS

Our result emphasized the fact that it was the most effective tool for vector control. DDT is banned in Nepal and the only choice is the use of pyrethroid-based insecticides (Bista et al., 2005). Residual spraying is generally done up to six feet height only because sand fly breeding sites are preferably near to the corners of human dwellings or cattle sheds where high humidity and organic debris present (Das et al., 2008). Unlike mosquitoes, adult sand flies hop for movement and for crossing the distance from breeding sites (floor level) to six feet height on the wall it is exposed sufficient, on residual insecticide, to kill itself. Besides lots of drawbacks during spraying and application procedures, it can be a good tool to reduce the sand fly density, if supervised properly. An extensive study of the same kind also revealed IRS as the most significant on reduction of sand fly density independent of the type of dwelling (Joshi et al., 2009).

4.3. EVM

It was presumed that the exploration of most preferred sand fly breeding places might help to plan the larval control strategy and destruction of the habitat led to the adult sand fly density reduction (Kesari et al., 2000). Kumar et al. (1995) found that lime could remarkably reduce the sand fly density but its long lasting effect was not satisfying. In our study, EVM was found least effective as compared with LLIN and IRS in reducing the sand fly density. Sand fly density in this arm was found to be reduced for the first few months, however, it could not be decided whether it was due to weather or it was due to the chemical ($CaCO_3$) in the lime. Ecological intervention method could not represent itself as long-term vector control method as shown in the result, but it might be the alternative choice as a better non-insecticidal vector control method to be integrated with other intervention methods. Besides, frequent use of lime on walls and its impact on the vector is still a matter of investigation.

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