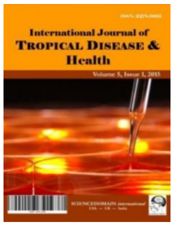

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Investigating Associative Impact of Indoor Residual Spray and Insecticide Treated Nets for Minimizing Visceral Leishmaniasis Vector Population in Bihar (India)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VK, RKD and AK conceptualized idea for designing study protocol and proposal writing. The outdoor-experimental field trials were conducted by authors PSM and RPS while the literature proof-searches, result analysis, preliminary manuscript drafting and periodic editing were done by the author AR. Author NAS contributed to the data computing and statistical analysis. Author PD was involved in project guiding, manuscript editing and providing mandatory approval of draft for final submission. All authors read the final manuscript and approved it for final publication.

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ABSTRACT

Background: Indoor Residual Spraying (IRS) and Insecticide Treated Nets (ITNs) are important tools in the fight against insect vectors of important diseases. In spite of having operational and logistic limitation(s), IRS and ITN are still in practice as conventional tools with mixed results for controlling *Phlebotomus argentipes*, the vector of Visceral Leishmaniasis (VL) in the Indian subcontinent. A combination of both tools might results better for reducing VL vector densities during the attack phase of the VL elimination initiative.

Methods: A comparison-based study was designed for analyzing the field efficacy of a combination strategy of IRS+ITN (PermaNet 3.0®) versus single interventions with IRS and ITNs alone comparing with control respectively. The study was conducted at the villages of Samastipur district of Bihar (India) using aspiration and light traps techniques (i.e., AT and LTT respectively) for collecting sand fly. The numbers of all types of sand flies (male, unfed, fed and gravid female) were recorded during a period of 12 months after the intervention in the three study arms. The bioavailability of insecticide for IRS as well as ITN was also observed at the interval of 3 months with the help of cone bioassay technique established by World Health Organization (WHO).

Results: The observed highest percent-reduction of sand flies (93.59% - 100%) at the sites with combined intervention of IRS+ITN, as compared to the sites without any intervention at control (with 0% reduction) or with single control intervention either with IRS (4.29% - 86.77%) or with ITN (60.18% - 97.07%) followed by the reduction in bioavailability of insecticide in IRS (i.e., 52.38%, 58.33%, 45.45% & 50.00%) and ITN (84.44%, 82.50%, 77.78% & 83.33%) over the period of 12 months since intervention, establishes the success of IRS plus ITN as a combined approach for contaminating vector population. Also, through the house-to-house survey at the end of study period, the acceptance of combined approach for IRS plus ITN by the community was accessed to be highest i.e., 100% as compared to the single-intervention approaches for IRS (87%) and ITN (100%).

Conclusions: Study results advocate the use of the combined strategy during the attack phase of the VL elimination initiative in order to reduce or interrupt disease transmission. It may also be a valid approach during the maintenance phase in hot-spots of VL transmission.

Keywords: Visceral Leishmaniasis (VL) elimination; Phlebotomus argentipes; Indoor Residual Spray (IRS); Insecticide Treated Nets (ITNs); Integrated Vector Management (IVM).

ABBREVIATIONS

VL : Visceral Leishmaniasis
IRS : Indoor Residual Spraying
DDT : Dichlorodiphenyltricholoroethane
ITN : Insecticide Treated Nets
LLIN : Long Lasting Insecticidal Nets
ICMR : Indian Council of Medical Research
NVBDCP : National Vector Borne Disease Control Programme
WHOPES : World Health Organization Pesticide Evaluation Schemes
VCAG : Vector Control Advisory Group
WHO : World Health Organization
DMO : District Malaria Office
CDC : Centers for Disease Control and Prevention

1. INTRODUCTION

Visceral Leishmaniasis (VL), or kala-azar, has been declared as notifiable disease in context to Indian scenario [1] that propagate as well as infect human host by the transmission of protozoan parasite *Leishmania donovani* (Kinetoplastida: Trypanosomatidae) [2,3] followed by the biting of parasite-infected female sand fly *Phlebotomus argentipes*, Annandale and Brunetti (Diptera: Psychodidae) [4]. VL being one of the serious impeding factor responsible for impoverished national health assets, are exclusively followed by the socio- economic conditions of residents at endemic areas and hence referred as a 'disease of the poor' and a 'cause of poverty' in the developing nations. The marginalized, poorer sections mostly rural and tribal with low socio-economic status, with limited access to quality health care, communication, other basic facilities, lack of awareness on protection measures, are often the worst sufferers.

An estimated 500,000 VL cases occur every year worldwide out of which 90% of cases were reported from the developing nations lying in Indian subcontinent, Brazil and Sudan. In India, Kala-azar has become particularly common in the north-eastern states and is currently considered as one of the most severe public health problems [5,6]. The disease is prevalent in 52 districts of Bihar, Jharkhand, West Bengal and Uttar Pradesh and countries adjoining these regions such as Nepal (12 Districts) and Bangladesh (42 districts) [7,8]. The annual incidence of VL in India is approximately 100,000 cases out of which more than 90% cases accounts from the districts of Bihar [9]. The State Bihar alone captured almost 50% out of the total burden in the Indian sub-continent [10,11]. Being a border State, Bihar is highly threatened for Visceral Leishmaniasis (VL) and regarded as a “hot spot” for contaminating its neighboring nations [12]. Bhutan has recently joined in the list of neighboring countries of India (Bihar) affected by VL [13].

In order to meet the challenges for effective control over the Indian VL vector i.e., *P. argentipes*, the National Vector Borne Disease Control Programme (NVBDCP) – the nodal agency for vector borne disease of India has embarked upon new intervention tools such as introduction and regular monitoring of Insecticide Residual Spray (IRS) to check the vector population, scaling up of Long-Lasting Insecticidal Nets (LLIN) and Insecticide Treated Nets (ITNs) in the operational VL control programme in India.

For controlling the instances of VL and other vector-borne diseases including malaria, IRS with Dichlorodiphenyltricholoroethane (DDT) being an insecticide of choice, had remained highlighted since 1953 [14,15]. Rigorous operation of IRS and indiscriminate exploitation of DDT for vector control at endemic areas [14,16,17,18] along with logistic problems associated with sprayers and spray pumps [11,19,20] had contributed the lowered efficacy of IRS as well as in increased no. of survivors followed by the decreased susceptibility of *P. argentipes* towards DDT [21]. In programme mode 5% DDT @ 1 gm/m^2 is being used for the control of kala-azar. The trend shows there is reduction in the cases of kala-azar after DDT spray but the reduction is slow probably due to depletion in efficacy of IRS. In a field trial *P. argentipes* were collected even after 30 days of spray [19]. Decreased susceptibility towards DDT of an IRS evoked increased instances of resistance among *P. argentipes* [22], it is configured as a weakened tool for vector control when applied alone.

Similarly, for containing vector population, Insecticide Treated Nets (ITNs) also provides an alternate protective way for defending vector population [23]. These nets are treated at the manufacturing level with insecticide either incorporated into or coated around fibres, and are resistant to multiple washes. The biological activity lasts as long as the net itself (3 to 4 years for polyester nets, 4-5 years for polyethylene nets). According to World Health Organization Pesticide Evaluation Scheme (WHOPES) a long-lasting insecticidal net should maintain bioefficacy for >95% knockdown and/or >80% mortality against target mosquito vector species for at least 20 serial washings in laboratory and 3 years of continuous use in the field conditions [24]. Fulfilling these criteria, three LNs have been given full recommendations and seven brands of LNs including Permanet® 3.0 net that had received interim recommendations from WHOPES [25] and has a specific claim related to its efficacy with resistant malaria vectors that has been reviewed and accepted by the Vector Control Advisory Group (VCAG) [26].

In this regard, Perma Net® 3.0 is the first insecticide synergist combination net designed for use in areas with pyrethroid resistant malaria vectors. A synergist, piperonyl butoxide, is incorporated with deltamethrin in the roof section of Perma Net® 3.0 because the first point of contact of most mosquitoes approaching a bed net is the roof [27,28,29]. A synergist works by enhancing the effect of the pyrethroid deltamethrin [30,31] by inhibiting the metabolic enzyme defence systems of the mosquito. Perma Net® 3.0 has been recently introduced in India and studies on its wash resistance and bio-efficacy against local malaria vectors have shown good results [32]. Besides being so advantageous and holding positive recommendations, single application of ITN doesn’t guarantees complete protection against vectors nuisance due to its limitations viz, improper implementation, impact of insecticide treatment in the bed-nets, providing protection only to the individual sleeping inside the nets, etc

[33] suggesting futility of intervention for controlling vector population.

Besides having limitation with single intervention with either IRS or ITN alone, these are still in practice as conventional tools producing unsatisfactory result for controlling vector population. In this regard, lack of testimonials regarding comparative assessment of IRS and ITN alone as well as in combination for minimizing the menace, with respect to VL vector at endemic district of Bihar had undoubtedly set goal for our present study.

Taking lessons from successful mosquito control approach [23,34], added benefits of IRS plus ITN were planned to be exploited for controlling VL vector population in Indian Subcontinent. Accordingly, a comparison-based study was designed for accessing the field efficacy trials of IRS and ITN alone as well as in combination, in terms of reduced VL vector density, their changed feeding patterns, perceived effectiveness and adverse events by the residents of VL gripped regime at Bihar.

For the study, PermaNet 3.0 exploited as an ITN at the study sites was manufactured by Vestergaard Frandsen®, Switzerland and gifted to the Rajendra Memorial Research Institute of Medical Sciences (ICMR), Patna (Bihar), India by the World Health Organization (WHO) in year 2012. The study was approved in year 2013 with title 'Integrated vector management for the VL control vis-a-vis case study – A Pilot study' by the Scientific Advisory Committee as well as Institutional Ethical Committee of RMRIMS (ICMR), Patna.

2. MATERIALS AND METHODS

2.1 Hypothesis and Study Plan

Efficacy of IRS and ITN when applied alone, gets depleted over the time, and hence produces unsatisfactory result. If ITNs and IRS possess additive or synergistic effects when applied in combination, then a strategy of combined IRS plus ITNs may be an effective way to drive VL transmission to very low levels. This served as hypothesis for our present study and hence study plan was designed accordingly to achieve our goal.

2.2 Study Sites and Study Period

The Samastipur district (coordinated around the latitude 25.8500° N and longitude 85.7811° E of globe), bordered by Vaishali district in west and course of river Ganges at the south in Bihar state of India, covering an area of 2904 sq. kms with total population of 425 million inhabitants is endemic for VL. Here the annual density peaks for VL vector was observed around May and October [35]. On the basis of report obtained from the District Malaria Office (DMO) of Samastipur district, four villages i.e., Mirzapur, Sahnitola, Nifsy were selected for IRS, ITN, IRS+ITN treatment respectively, and Bisanpur was allocated as Control site that remained untouched from any chemical treatment. Each village contained at least 100 households that were randomly selected. Therefore, overall 400 HHs were targeted for the study. The study period was from October 2014 to October 2015, including a baseline interview survey upon socio-demographic information and sand fly density observation at the selected study sites, conducted just 7 days prior (i.e., in September 2014) to the implementation of interventions and observatory phase during the month of October 2014 to October 2015. The observatory phase as a post intervention session were conducted for getting the trends of efficacy of intervention over the VL vector density at regular interval following the monthly assessment of entomological surveys along with a household interview survey performed at the end of study session.

2.3 Baseline Interview Survey

At the beginning of the study, door-to-door surveys were conducted at the 4 villages by the trained interviewers using a questionnaire sheet. The questions were designed for obtaining information regarding the socio-economic status of the households and the protective measures taken against nuisance of insects.

2.4 Baseline Survey on Sand Fly Density

In order to get an optimal assessment over vectors density sand fly collections were done using Aspiration Technique (AT) [36] as well as Light Trap Technique (LTT) [37] as described below.

2.4.1 Aspiration Technique (AT)

The diurnal resting sand flies were collected from cattle sheds, dark and damped corners of households during 06:00 am – 08:00 am, by the team of expert Insect Collectors and Research Assistant who had been imparted hands-on-training with the help of suction tubes and hand operated LED flashlights. The Borosil® glass test tubes were used for keeping captured sand flies. A cotton wool soaked in glucose solution was plugged on the mouth of these test tubes. These tubes were wrapped in semi-wetted cotton cloth and carefully transported to the laboratory [22].

Here, captured sand flies were released into the wooden-framed, wire meshed, cloth-lined Barraud cage (42×36×36 cm) for their adjustment to the new environment of insectary at Vector Biology and Control Division of RMRIMS (ICMR), Agamkuan, Patna, Bihar, India. After releasing captured sand flies, the *P. argentipes* were sorted out with the help of an identification key [38].

2.4.2 Light Trap Technique (LTT)

Nocturnal indoor sand flies were collected with a battery (John W. Hock Company, Model no. 300-6-220V) operated blower type miniature CDC light trap (John W. Hock Company, Model no. 512, developed by the Centers for Disease Control and Prevention, U.S.). These CDC light traps were properly installed and adjusted during the day time, at the height of 15 cm away from the ground floor level and 3 cm away from the wall at the corner in the bedroom [39] or room where most of household members at each indexed households used to sleep during the night, purposely for collecting sand flies. The battery was completely charged a day before the collection night, for its proper functioning during the period of 06.00 pm to 06.00 am next day under the supervision of field expert. Next morning, the light traps were de-installed and the dead insects collected in its collecting reservoir unit were transferred to the labeled Borosil® glass test tube, mouth tightly plugged with cotton ball. The insect samples were transported carefully to the laboratory where *P. argentipes* were sorted out from the collections following its identification with the help of an identification key [38].

2.5 Insecticide Treated Nets (ITNs)

Before the start of the trial, community group meetings were organized in the study villages and inhabitants of Sahnitola and Nifsy allocated for intervention of ITN and IRS+ITN respectively were educated on proper and regular use of nets and importance of the study.

2.5.1 Specification of ITN

Perma Net® 3.0 is factory manufactured nets treated with an insecticide deltamethrin at a dose of 4.0 g/kg ± 25% as well as incorporated with piperonyl butoxide (25 g/kg ± 25%) as an insecticide synergists, at its roof section, enhancing the bio-efficacy of insecticide of this section, because roof of bed nets serve as first point of contact for most mosquitoes. The sides of Permanet® 3.0 are made up of deltamethrine treated at a dose of 2.8 g/kg ± 25% specifically treated in its soft, polyester fabric of 75 denier, with 20 holes/ cm^2 and 70 cm lower border to enhance the lifetime of the net [40]. These specialized nets were manufactured by Vestergaard Frandsen®, Switzerland and were procured as gift from the World Health Organisation (WHO) in year 2013 with a production date of October 2012.

The nets were distributed at the Intervention sites of ITN and IRS+ITN during the month of October. The number of nets distributed (Table 1) to each household of interventions was recorded in the register and signatures of the recipients were obtained. 255 and 270 treated nets i.e., Perma Net® 3.0 were distributed in the villages for IRS (Sahnitola) and IRS+ITN (Nifsy) intervention respectively so as to cover entire sleeping spaces of population at these villages. The population-treated net distribution ratio was stabilized as 3:1, keeping in view the comfort of two adults and a child sleeping under a family size net [41]. A village committee consisting of panchayat members (Governing council) and other opinion leaders was constituted in Interceptor and untreated net villages to monitor proper use and maintenance of mosquito nets.

2.6 Indoor Residual Spray (IRS)

RMRIMS at Agamkuan Patna, Bihar being affiliated with Indian Council of Medical Research, New Delhi, India heartedly supported the VL eradication initiative launched by Bihar state government. It actively volunteered campaigning and implementation of Insecticide Residual Spray (IRS) activities in the districts of Bihar during October 2014 using 5% DDT suspension with the help of Hand Compressor Pump. Hand Compressor Pump was used as it provides better coverage with minimum wastage of insecticide [42].

For present study, every corner of each households of village Mirzapur and Nifsy allocated with IRS and IRS+ITN respectively were treated with IRS with suspension of 5% DDT. The IRS campaign was again repeated at the study sites during the month of March-July 2015.

2.7 Post Intervention Sand Fly Density Evaluation

Adult sand flies were collected from the 6 randomly selected, indexed households (one room per house), from each 4 allocated study sites targeted for interventions along with control. The collections were done 7 days prior to the intervention (as a baseline survey i.e.,September 2014) and then once per month upto the period of 13 months following the interventions.The same houses were deployed for collecting sand flies with AT as well as LTT throughout the study duration.

2.8 Feeding Success Rate in *P. argentipes*

Feeding success rate in *P. argentipes* was assessed by the visual observation of their feeding status [1,2,37] collected from randomly selected 6 households from each study sites. Identification of male and female *P. argentipes* and distinction between fed, unfed and gravid sand flies were done by scrupulous observation of abdominal appearance that served result for feeding success of the captured sand flies.

2.9 Assessing Bioavailability of Insecticide in Intervention (BII) by WHO Cone Method

The residual activity of IRS and ITN was measured at each interval of 3 months for estimating the Bioavailability of Insecticide in Intervention (BII) associated with IRS and ITN with the help of cone bioassay technique carried out in accordance with protocol led by WHO [1,24,43] i.e., by exposing newly emerged *P. argentipes* (primarily female ones) directly to the (IRS & ITN) treated surfaces of 6 HHs selected from each study sites for 30 minutes and concluding susceptibility remarks by observing knockdown (after 30 minutes of exposure) and mortality (after 24 hours of exposure); and adjusting result by Abbot's formula [21,22,43].

2.10 Assessing Percent Reduction Due to Intervention (% RI) in Vector Density

The sand flies (of both sexes i.e., male and female), (fed, unfed, gravid among the female sand flies) were caught with the help of AT as well as through LTT from 4 study sites during the period of 13 months and on that basis of periodic collection of dead as well as living sand flies after intervention of IRS and ITN, % reduction due to intervention (%RI) was calculated for each months of study duration.

Percent Reduction due to Intervention (% RI) was assessed by measuring the magnitude of reduction in sand fly density at indexed intervened households and comparing those with pre-as well as post- treatment densities with the control houses in accordance with Mulla's formula [44].

2.11 Post Intervention Survey for Quantifying Community Acceptance, Effectiveness and Side Effects Perceived by the Interventions

Monthly house-to-house survey was conducted for 12 months for ascertaining the proper usage of distributed nets. Also, physical status of nets at the study sites was observed during the monthly survey session. Using the net master list, all heads of households were interviewed. The questionnaires were used to determine people's perception of the benefits and/or side effects during use of ITNs and ITNs along with IRS. At the end of the study period after the 12th months of intervention, final discussion with the members of households at each intervened village was conducted for obtaining descriptive information on peoples' perception on the use of ITNs and IRS.

2.12 Data Entry and Analysis

The data were analyzed using SPSS (IBM Statistics) Version 21 software and were transformed to obtain a normal distribution and homogeneity of variance. Significances of result were observed at 95% Confidence Intervals following the calculation of Mean Percent Reduction in sand flies due to Intervention (% RI) observed individually for each household from where *P. argentipes* had been collected. In addition Mann-Whitney-U test was applied for comparison between different interventions (IRS, ITN & IRS+ITN) to see the effect of interventions. P-Value <0.05 was considered statistically significant.

3. RESULTS

3.1 Socio-demographic Characteristics of the Study Populations

Vector surveillance for implementation of control intervention was assessed by door-to-door census survey at the households assigned for intervention approaches with IRS, ITN, IRS+ITN along with at control arm that remained untouched from either of any intervention programme. Socio-demographic observation revealed that majority of houses at the study sites i.e., control, IRS, ITN and IRS+ITN were of thatched (38.5%, 47.7%, 55.1% and 44.5%) and mud plastered type (20.4%, 12.8%, 21.7% and 9.2%) serving best habitat and favorable conditions for surviving sand flies as well as disease transmission among human hosts. Sites also covered with households of concrete type (15.6%, 15.6%, 8.9% and 16.4%) as well as brick type (25.3%, 23.8%, 14.1% and 29.8%) that occasionally shelters sand flies.

Among the total population of 2878 collectively at the study site, female population was observed to be lowered (49%, 45% and 43%) as compared to the male population (51%, 55% and 57%) in the study sites to be intervened with IRS, ITN, IRS+ITN respectively, but literacy rate was observed to be higher i.e., 63% and 59% for the villages to be intervened with IRS and IRS+ITN (Mirzapur and Nifsy) respectively. Results reveal that though female population is less, they are much more conscious about their family health and hence greatly affected the acceptance of control interventions during the study period. The expected outcome at the villages of Sahnitola (for intervention with ITN only) was insignificantly hampered due to poor literacy rate i.e., 28% only. At the study sites, about 44.4% people were engaged as a farmer either owing piece of land as a property or serving to the others field. While 30.5% people served as a daily wage laborer.

The ratio of cattle assets to the cattle sheds was observed to be 98:26, 153:57, 66:47 and 101:31 respectively for Bisanpur, Mirzapur, Sahnitola and Nifsy respectively. Among the cattle, about 45.4% households owned cow followed by goat and buffalo i.e., 31.81% and 23.6% as their cattle assets respectively.

Coming to the insect control measures adopted by the people from the disease causing insect-bite before implementation of intervention, overall 19% population at the study site (i.e., 15.73%, 18.27%, 29.44% and 36.54% for Bisanpur, Sahnitola, Mirzapur, and Nifsy respectively) were satisfied with the traditional means viz., smoking, drainage oiling, etc., for controlling insects while only 17% population (i.e., 22.14%, 26.17%, 24.16% and 27.51% for Bisanpur, Sahnitola, Mirzapur, and Nifsy respectively) preferred modern means viz., bed-nets, IRS, repellents' application, for tackling insect bite. However, majority of them i.e., 41% of population (i.e., 5.73%, 23.42%, 14.35% and 26% for Bisanpur, Sahnitola, Mirzapur, and Nifsy respectively) were observed to be accepting both type of means while 23% population (i.e., 56.4%, 32.14%, 32.05% and 10.31% for Bisanpur, Sahnitola, Mirzapur, and Nifsy respectively) rejected either of any means for controlling biting- insects' nuisance. Among the study site, availability and uses of bed nets by the households before the intervention session were observed as 22.14%, 26.17%, 24.16%, 27.51% at Bisanpur, Sahnitola, Mirzapur, and Nifsy; for intervention as control, IRS, ITN, IRS+ITN respectively (Table 1).

Lowest acceptability (5.73%) of either modern or traditional means for controlling biting-insect population and highest instances of rejection (56.4%) of any control intervention at households of Bisanpur served best for its categorization as 'control site'. Highest acceptability of both types of approaches i.e., 26% as well as lowest rejection instances for insect control i.e., 10.31% was observed for Nifsy village. Result exhibited that resident of Nifsy village being more concerned towards their health and doesn't want to take any risk upon it, therefore they accepted both type of measures for controlling insect population and hence serve best for its selection as an intervention-site for implementation of IRS along with ITN for vector control.

3.2 *P. argentipes* Collection with AT and LTT

Of the total number of 679 *P. argentipes*, collected during 12 months from all sites, 33.43% and 66.56% were collected with the AT and LTT respectively. More sand flies were collected at night with the LTT in contrast to AT. During the night almost the same proportions of male and female sand flies were collected with LTT as unfed or gravid females are active at night in contrast to lazy fed females that after their engorgement with blood-meal, they either rest on the wall or shelter in dark crevices for producing eggs via digestion of blood meal. During the day, particularly in early morning hours, more females were collected through the AT being most of them either gravid (42.75%) or fed with blood (44.22%) (Table 2).

Of the overall number of male sand flies, 39.20% were collected with the diurnal AT in contrast to 48.67% at night with LTT whereas, female sand flies' were more frequently collected (i.e., 60.79%) with diurnal AT as compared to the 51.32% recorded with nocturnal LTT. The highest collection of unfed female sand flies (74.13%) was recorded with the nocturnal LTT as compared to the diurnal AT (13.04%). In contrast, the highest percentage of fed and gravid female sand flies (44.22% and 42.75% respectively) were recorded with the AT as compared to those collected with LTT i.e., 6.89% and 18.96% respectively (Table 2). In present study, LTT effectively caught male and unfed female sand flies (i.e., 48.67% and 74.13% respectively) which seems to be active during the night in contrast to the AT capturing more blood-fed female sand flies (i.e., 44.22%) that, after feeding becomes inactive during the night and being caught during the early morning hours, after partial digestion of blood-meal. The findings were in accordance with the previous report [37]. Hence, it can be inferred that both techniques complement each other for trapping both types of sand flies depending upon their nature and it is beneficial to use them together.

3.3 Feeding Success in *P. argentipes*

As the parasite transmission by *P. argentipes* depends upon the feeding success and hence reflects the chances of disease transmission. Therefore, female *P. argentipes* were collected during the post-intervention phase and their feeding status was verified and detailed in a tabular sheet (Table 2).

Out of all 679 sand flies collected from the intervention sites, 77 were fed female sand flies. Only 2 fed females were caught using AT from the village with the combined intervention (IRS and ITN) while the rest (75 fed female sand flies) were collected from the other villages including either of single interventions or control. Likewise only 6 gravid sand flies were collected in the combined intervention village and the other 97 gravid sand flies were collected from the villages with either ITN or IRS interventions (Table 2). In summary, After the intervention, the lowest numbers/proportions of sand flies were collected from villages with the combined approach (IRS plus ITN) as compared to single intervention sites (either IRS or ITN only) or the control site.

3.4 Bioavailability of the Insecticide in Intervention (BII) Established by the WHO Cone Method

Quarterly evaluation of bioavailability of insecticide in intervention (BII) was accessed at intervals of 3 months following implementation of the intervention. Results were paralleled by the resurgence of the sand fly population after some months, probably due to reduction in the bioavailability of insecticidal content in both IRS as well as ITN. However, the reduction of insecticidal content of IRS was faster and more pronounced (exhibiting corrected mortality rate as 52.38%, 58.33%, 45.45% & 50.00%) as compared to ITN (with corrected mortality rate as 84.44%, 82.50%, 77.78% & 83.33%) over the period of 13 months since intervention are illustrated in Table 3.

3.5 Percent Reduction (% RI) of Sand Fly Density Due to Intervention

The Monthly observation of percent reduction (% RI) of sand fly density due to intervention (Table 4) establishes the highest % RI (93.59-100%) at the sites with IRS+ITN as compared to either at the control site (with 0% reduction) or with single intervention of IRS (with 4.29-86.77%) or with ITN (60.18- 97.01%). This revealed the majority of reductions at a high significance ($p<0.01$) that were observed in the IRS+ITN intervention arm as compared to the single intervention arms (IRS and ITN alone). However, due to the reduction in the bioavailability of insecticide, sand flies re-emerged after the initial reduction in sand fly density at the sites with either IRS or ITN alone, (Table 4 and Fig. 1).

At the site with the combined treatment of IRS+ITN, no re-emergence of sand flies was recorded till 13 months following the intervention (Fig. 1) however, overall % RI for IRS+ITN ranged between 93.59-100% throughout the study period (Fig. 2).

3.6 Perceived Effectiveness and Side Effects by the Community

The door-to-door survey was conducted among heads of the 400 targeted households along with additional 535 neighboring houses of intervened houses at the 4 study arms purposely for assessing the collateral effectiveness of interventions. All respondents facilitated with ITNs, confirmed the proper usage of provided bed nets as well as the continuous good physical conditions of the nets. This was confirmed by the village committee in-charge of supervising bed net use. Almost the only reported side effect was unpleasant smell, particularly in the 2 arms that included DDT spraying i.e., 83% and 91% in IRS and IRS+ITN respectively. Much less frequent was sneezing (10%, 6% and 12% in villages with either IRS or ITN or both respectively). Skin

Table 1. Demographic and socio-economic conditions of the study populations and protective measures against nuisance of insects

Village name & Id. no	Human population			Education profile				Cattle assets		Measures for controlling insects' nuisance			
					Literacy (%)								
	Total population	Male population (%)	Female population (%)	Illiteracy (%)	Matric	Intermediate	Graduate & Above	Total no. of cattle sheds	Total no. of cattles	Modern means (incl. Bednets, IRS, repellents)	Traditional means (incl. Smoking, oil spilling)	Both	None
Bisan Pur (Control)	814	55	45	58	57.24	33.33	9.43	26	98	22.14	15.73	5.73	56.4
Sahni Tola (ITN)	544	55	45	72	63.15	21.05	15.78	47	66	26.17	18.27	23.42	32.14
Mirza Pur (IRS)	857	51	49	37	68.25	26.98	4.76	57	153	24.16	29.44	14.35	32.05
Nifsy (IRS+ITN)	663	57	43	41	76.47	17.64	5.88	31	101	27.51	36.54	26	10.31
Total	**2878**	**---**	**---**	**---**	**---**	**---**	**---**	**161**	**418**	**---**	**---**	**---**	**---**

Table 2. Entomological data (in terms of collection technique, sex, feeding status and gravidity of female *P. argentipes*) observed during the period of 12 months at the studied sites

	Aspiration technique (N=227; 33.43%)				Light trap technique (N=452; 66.56%)			
	Male N=89; (39.20%)	Female N=138; (60.79%)			Male N=220; (48.67%)	Female N=232; (51.32%)		
		Fed N=61; (44.22%)	Unfed N=18; (13.04%)	Gravid N=59; (42.75%)		Fed N=16; (6.89%)	Unfed N=172; (74.13%)	Gravid N=44; (18.96%)
Bisan Pur (Control)	30.30%	44.26%	22.22%	54.23%	43.78%	50%	51.16%	36.36%
Sahni Tola (ITN)	37.93%	18.03%	38.88%	28.81%	46.83%	25%	22.67%	25%
Mirza Pur (IRS)	48.83%	34.42%	38.88%	10.68%	31.31%	25%	23.83%	34.09%
Nifsy (IRS+ITN)	73.91%	3.27%	00.00%	6.77%	87.67%	00.00%	2.32%	4.54%

Table 3. Result for bioavailability of insecticides in intervention (BII) during the study period

Months	No. of sets	Indoor residual spray				Insecticide treated nets			
		No. of sand fly tested	% test mortality	% control mortality	Corrected mortality rate (in%)	No. of sand fly tested	% test mortality	% control mortality	Corrected mortality rate (in%)
(Baseline) October 2014	4	80	30.00	0.00	30.00	80	91.25	0.00	91.25
January 2015	3	45	55.56	6.67	52.38	45	84.44	0.00	84.44
April 2015	4	60	58.33	0.00	58.33	40	82.50	0.00	82.50
July 2015	3	36	50.00	8.33	45.45	36	77.78	0.00	77.78
October 2015	3	30	50.00	0.00	50.00	60	83.33	0.00	83.33

Table 4. Percent Reduction (% RI) of sand fly density due to Intervention with different intervention types in single and combined form

Name of the study villages	Mirzapur		Sahani Tola		Nifsy		P-Value
Type of intervention	IRS (a)		ITN (b)		IRS+ITN (c)		Mann Whitney test (U-test)
	Mean (95% CI)	% RI	Mean (95% CI)	% RI	Mean (95% CI)	% RI	*c vs a; c vs b
IRS and ITN implementation on 12th- 14th October 2014							
Oct 2014	81.75 (70.6-92.8)	83.41	97.18 (93.4-100.9)	95.07	100 (100)	100	p<0.01; p<0.01
Nov 2014	82.40 (72.7-92.0)	83.34	97.00 (91.1-102.8)	97.07	98.9 (96.8-101.0)	98.44	p<0.01; p<0.05
Dec 2014	9.02 (-51.9-69.9)	26.06	87.71 (68.5-106.9)	87.73	96.7 (90.4-103.1)	93.59	p<0.01; p<0.05
Jan 2015	-65.33 (-279.6-148.9)	4.29	79.56 (59.5-99.5)	62.98	100 (100)	100	p<0.01; p<0.01
Feb 2015	14.97 (-20.0-50.0)	26.65	52.93 (5.08-100.7)	60.18	100 (100)	100	p<0.01; p<0.01
Mar 2015	82.65 (68.4-96.8)	86.77	89.68 (80.4-98.9)	87.48	97.84 (93.6-102.07)	98.14	p<0.05; p<0.05
Apr 2015	82.54 (70.2-94.8)	79.89	91.45 (79.9-102.9)	91.15	100 (100)	100	p<0.01; p<0.01
May 2015	83.88 (69.2-98.4)	86.53	85.58 (71.2-99.8)	85.38	100 (100)	100	p<0.01; p<0.01
Jun 2015	66.12 (49.2-82.9)	68.68	88.93 (79.05-98.8)	87.73	100 (100)	100	p<0.01; p<0.01
Jul 2015	62.66 (41.7-83.5)	68.40	87.22 (73.4-100.9)	88.67	100 (100)	100	p<0.01; p<0.01
Aug 2015	65.75 (39.8-91.6)	68.68	81.37 (60.6-102.0)	84.57	100 (100)	100	p<0.01; p<0.01
Sep 2015	73.55 (62.1-84.9)	73.77	82.65 (64.7-100.5)	85.76	100 (100)	100	p<0.01; p<0.01
Oct 2015	58.02 (12.2-103.8)	67.17	80.18 (53.5-106.8)	86.59	100 (100)	100	p<0.01; p<0.01

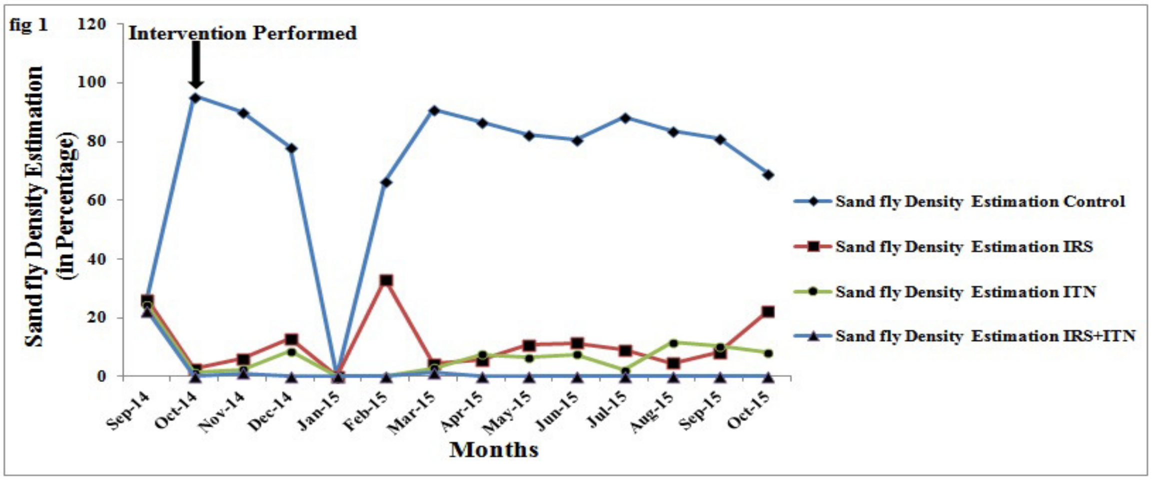

Fig. 1. Graphical representation of sand fly density during Pre- and Post-intervention phase in the study villages

irritations were reported only in the villages which involved DDT spraying i.e., 8% and 11% in IRS and IRS+ITN respectively. The perception of added benefits (mainly reduction in nuisance of insects) was highest in villages where ITNs were involved (i.e., 97% against sand fly and mosquito bite and only in these sites, the disappearance of insects of other class viz., head lice, ants, cockroaches and house flies were also reported. Overall satisfaction was achieved in the villages involving ITNs as compared to the village with IRS as single intervention with 87% acceptability as illustrated in Table 5.

4. DISCUSSION

By rewinding the past experiences, it was observed that IRS or spraying of DDT was undertaken by the government following flare-up of cases at VL endemic region(s) of Indian subcontinent leading to vast devastation from health as well as national economy perspect. Here, DDT being an insecticide of choice since 1977, it has been exploited extensively for controlling *P. argentipes* [45,46] that it became resistant against it and instead of being killed, it manipulated its behaviour from endophilic to exophilic nature [47]. Another remarkable point is that DDT itself fails to hold efficacy for longer duration, resulting in increased lethal time (upto 420 minutes) making it non-effective for killing indian VL vector [21]. Besides these factors, operational facets incorporated by logistic problems [19,20] configures decreasing susceptibility or increasing resistance among *P. argentipes* [21]. In that situation, contaminating vector population by maintaining IRS becomes 'insignificant', 'exhaustive', 'time-taking' and 'cost-defective' as it always demands repetition for avoiding instances of 'zero-efficacy'.

As another approach for containing vector nuisance, ITNs also plays significant role for providing protective way against malaria and mosquitoes [23] but cannot give complete protection against mosquitoes due to its limitations viz, improper implementation of ITN, impact of insecticide treatment in the bed-nets, providing relaxation to insects concealing away from its range as well as protection only to the individual sleeping inside the nets, etc [33].

Due to depletion in chemical constituent over the period of time, as a single approach, neither IRS nor ITNs provide satisfactory result for controlling vector nuisance, and hence strongly corroborate with invasion of insecticide resistance among these populations. In that situation, combined strategy of IRS plus ITN proved to be successful and provided an effective way for driving mosquito population [23,34]. But till date it hasn't been explored and validated in case of VL vector population of Indian subcontinent. Therefore for assessing combo-effect of IRS and ITN for containing *P. argentipes*, the comparision based study was conducted by deploying IRS and ITN alone as well as combined manner at the villages of Samastipur district of Bihar (India). Over here, *P. argentipes* were reported to develop resistant, prior to which it remained susceptible for DDT [12].

From the present study, lowest collection of all types of sand flies i.e., male, unfed, fed or gravid

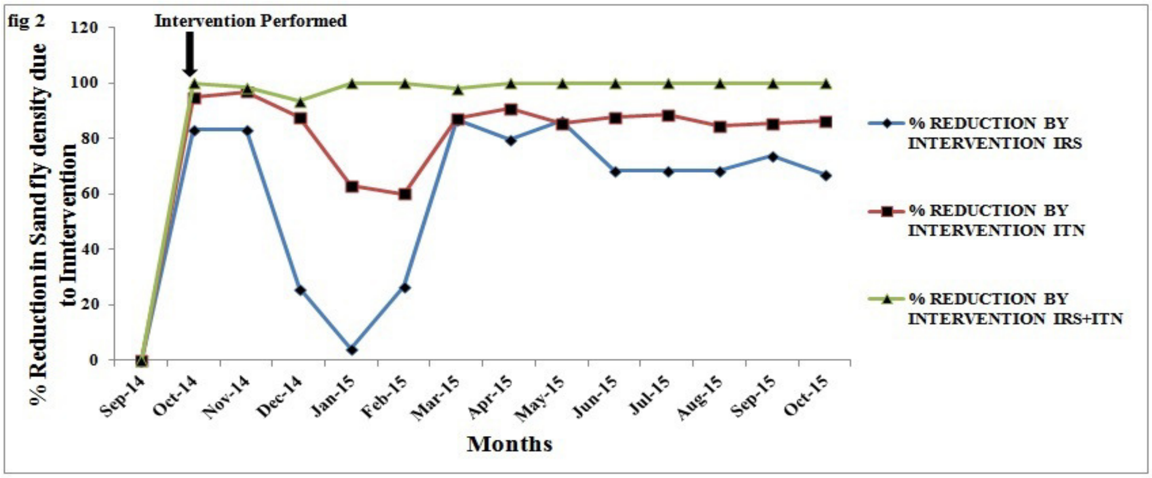

Fig. 2. Comparative assessment of percent reduction (% RI) of sand fly density due to intervention of IRS, ITN and IRS + ITN during the period of 12 months, post intervention phase at the villages of Samastipur district, Bihar, India

Table 5. Household survey after 12 months of Intervention for assessing peoples' perceptions of the interventions according to type of intervention received

Serial no.	Questions	Proportion of users (%)		
		IRS (N=857)	ITN (N=544)	IRS+ITN (N=663)
1	Do you sleep inside the provided nets?	NA	100	100
2	Do you felt any abnormality or suffocation while sleeping under nets?	NA	0	2
3	Did you suffer any of the following during the study period?			
a	Unpleasant Smell	83	76	91
b	Dizziness	0	2	0
c	Running nose	1	0	2
d	Fever	0	0	1
e	Headache	0	5	0
f	Sore Eyes	0	0	0.03
g	Skin Irritation	8	0	11
h	Coughing	0	0	0
i	Vomiting	0	0	2
j	Sneezing	10	6	12
k	Sleeplessness	0	0	0
4	Perceptions of additive benefits of Interventions			
a	Reduction in mosquito/ sand fly bites	39	83	97
b	Reduction in nuisance due to head lice.	0	80	83
c	Reduction in nuisance due to ants, cockroaches and house flies.	0	21	71
5	Will you continue sleeping under provided nets?	NA	100	100
6	Are you satisfied with the intervention?	87	100	100

(were recorded during post intervention session, from the sites intervened with IRS+ITN as compared to either sites without any intervention (i.e., control) or with single control intervention (either IRS or ITN only) as illustrated in Fig. 1 and Table 2. Therefore results embarked from the referred testimonials corroborates with findings of present study that establishes the efficacy of IRS and ITN when applied alone, gets depleted over the period of time (Table 3) producing unsatisfactory result for driving VL vector population as well as competing the instances of increased VL cases. Whereas combined strategy provides additional protection for combating the insects' menace (Fig. 2, Table 4) as well as for driving VL transmission at a lowest level.

As an outcome of any intervention(s) depends upon the compliance rate and mass acceptability for interventions, therefore door-to door survey was conducted periodically during the study period for assuring of proper usage of bed nets at intervened sites. From the results it was observed that regardless having highest rate of complaints in form of unpleasant smell, sneezing, skin irritation, etc. major section of population perceived additional benefits of IRS plus ITN in form of relief from other household pests viz., mosquito, head lice, ants, cockroaches, house flies, etc. The additional benefits of IRS plus ITN perceived by the population was observed to be so advantageous and eco-friendly that it nullified the negative impact of intervention(s) and hence resulted into absolute satisfaction and acceptability (i.e., 100%) for intervention(s) as illustrated in Table 5. This undoubtedly helped in reducing and maintaining the the reduced population of targeted insect at minimal level (Fig. 1). Cent percent population at sites intervened with ITN and IRS+ITN assured with continual practicing of combined intervention(s) in future. Hence, results advocate the efficacy and acceptability for intervention with positive reduction in VL vector population (Table 5) assuring the total control in VL epidemiology following the vector control in future.

5. CONCLUSIONS

Therefore in nutshell, The limitations with IRS and ITNs as single interventions can be overcome by a combined approach that should be rigorously collected through randomized controlled trials. This combined approach is particularly important during the attack phase of the elimination initiative in order to reduce or interrupt the disease transmission in VL endemic villages as well as new emerging foci (i.e. villages with new VL cases which were previously VL free). It may also be a valid approach during the maintenance phase in hot-spots of VL transmission.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This work had been performed under the project entitling 'Integrated vector management for the VL control vis-a-vis case study – A pilot study' approved by the Scientific Advisory Committee as well as Institutional Ethical Committee of Rajendra Memorial Research Institute of Medical Sciences (ICMR), Government of India in year 2013 with study reference no. INT 98 VBC 2013.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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