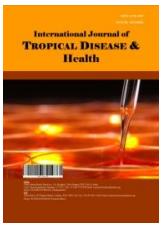

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Understanding Behavioural Temperament of *Phlebotomus argentipes* Under the Influence of DDT-IRS Versus SP-IRS for Scoping New Approaches for Maximum Control Over the VL-Vector Population in Bihar

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Authors' contributions

This work was carried out in collaboration among all authors. Authors VK and PD designed the whole work plan and experimental set-up for the research to be carried out in field as well as laboratory. Experimental observations as well as field activities were performed by authors VK and RM. Author AR helped in drafting manuscript. Authors PD and VK involved in project guiding as well as reviewing manuscript. All authors read the final manuscript and approved it for final submission.

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ABSTRACT

Background: After the decades of Dichlorodiphenyltrichloroethane (DDT) use, *Phlebotomus argentipes* reportedly developed resistance against it affecting every aspect of vector control at grass-root level. Although DDT based Indoor Residual Spray (IRS) has been replaced with Alphacypermethrine-a Synthetic Pyrethroid (SP) based insecticide, since 2016 but its successful implementation at the Visceral Leishmaniasis (VL) endemic regime of Bihar doesn't cause much effect upon VL vector density. Furthermore, the outcomes of existing operational research works, it

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had been observed that VL vectors are continuously changing its behavior under the pressure of insecticides.

Methods: For validating the hypothesis, present study has been carried out at Vaishali and Patna being highly and semi-endemic sites respectively for quantifying the oriental behavior among VL vectors persuaded by the IRS and enforce them to remain alive and get trapped in light trap even after changed chemical composition of IRS i.e., SP-IRS from routine DDT-IRS.

Results: Following results, a significant reduction in sand fly density (i.e., 33.09% and 29.16%) was observed for outdoor and indoor caught sand flies, collected with light trap and aspirator respectively. Significant higher no. of sand fly collection in terms of per light traps per night was recorded from the outdoor sites than those from indoor habitat for each village of Vaishali and Saran district of Bihar. Higher no. of male sand flies than to that of female ones were collected from outdoor sites and only unfed female sand flies (i.e., 100%) were caught following SP-IRS from each study villages of Vaishali and Saran districts of Bihar.

Conclusions: The results of higher no. of sand flies collection from the outdoor sites as compared with the indoor habitat validate the hypothesis of gradual shifting of habitat of VL vectors from endophilic to exophilic which is undoubtedly followed due to the fact of developed resistance among them against chemical constituent of IRS. Results provide very useful information about the sand fly dynamics under the impact of IRS and accordingly, advocates the combined approach of IRS along with insecticidal fogging together at a same time that could be an effective dividend for maximum VL vector control along for negotiating VL cases at par for longer duration during the maintenance phase at the VL foci.

Keywords: Dichlorodiphenyltrichloroethane (DDT); phlebotomus argentipes; Indoor Residual Spray (IRS); Synthetic Pyrethroid (SP); alphacypermethrine; Visceral Leishmaniasis (VL); endophilic; exophilic.

ABBREVIATIONS

VL : Visceral Leishmaniasis;
IRS : Indoor Residual Spraying;
DDT : Dichlorodiphenyltrichloroethane;
SP : Synthetic Pyrethroid;
PHC : Public Health Center;
CDC : Centre for Disease Control;
EVM : Environmental Management;
LLIN : Long Lasting Insecticidal Net;
WHO : World Health Organization;
NVBDCP : National Vector Borne Disease Control Programme;
ICMR : Indian Council of Medical Research
SAC : Scientific Advisory Committee;
RMRIMS : Rajendra Memorial Research Institute of Medical Sciences;

1. INTRODUCTION

Female *Phlebotomus argentipes* (Diptera: Psychodidae) are the obligatory blood-feeders [1, 2] and as a consequences of their blood-feeding lifestyle, it becomes pertinent to harbour *Leishmania donovani* (Kinetoplastida: Trypanosomatidae) within itself so as to deliver the lethal-most disease Visceral Leishmaniasis (VL) among the human hosts of Indian Subcontinent [3,4,5]. For controlling the disease expansion at VL hotspots, vector control strategies stays foremost and guaranteed way elucidating prevention option to be far better than that to cure and treatment.

In this regard, the Indian VL vectors being endophilic [6] and endophagic [7] often gets jeopardized by Indoor Residual Spraying (IRS) incorporated with Dichlorodiphenyl-tricholoroetahne (DDT) - a chlorine based insecticide, validated simple and cost-effective way for restricting man-vector contact by baiting indoor-resting sand flies [8,9]. Hence, remained highlighted as a first line VL vector control chemical option since 1953 [10,11]. After the decades of extensive application of DDT, *P. argentipes* had attained tolerance/ resistance against it [12,13] which is continuing till date [14, 15,16,17]. Due to this, flaring-up of VL cases with diminishing effect of DDT spray was observed [11] that supported the non-decrement in vectorial population too (Kumar et al. Personal communication). Moreover, instead of getting killed by IRS, *P. argentipes* had developed exophilic habitat behavior [18] following resurgence within just 15 days of DDT-spray [19] verifying the development of strong resistance among these vector species with per cent corrected mortality ranging between 40-61.54% [15]. The increased no. of survivors indeed is a resultant of either malpracticing associated with

IRS [9,19,20] or the changed behavior of vectors under the influence of IRS [18]. Though there are piles of literature dealing the former one, but today also we have very limited information upon behavioral changes among VL vectors persuaded by IRS. Therefore, in the present section, extensive investigatory attempts have been made for assessing and comparing the changed mood of this vector specie with respect to its habit and habitat influenced under the effect of IRS.

2. MATERIALS AND METHODS

2.1 Grouping of Selected Sites and Spray Intervention

Initially, the pre- and post-IRS comparative study upon the sand fly density was conducted following the first round of SP-IRS i.e., February-March in year 2016, at Patepur block also referred as Public Health Center (PHC) of Vaishali district (25.6838ºN, 85.35500ºE) and DDT-IRS at Dhanarua PHC of Patna district (25.5941ºN, 85.1376ºE) of Bihar. It was selected as previous reports dealt about Vaishali and Patna being highly and semi-endemic sites respectively for VL. But, after getting encouraging results of sand fly availability at highly endemic villages of Vaishali districts even after the SP-IRS, the study was extended in other 2 villages (Chandpura and Jurawanpur) of Raghopur PHC at Vaishali district (25.6838ºN, 85.35500ºE) and 2 villages i.e., Rahardiyar and Nazarmira of Sonepur PHC at Saran district (25.8560ºN, 84.8568ºE) of Bihar during the second round of SP-IRS (i.e., June-July) for the same year. Saran was included for study as previous reports along with ongoing IRS research trials at these districts revealed Vaishali and Saran to be very much prone towards VL transmission as per the VL cases recorded for last three consecutive years (Kumar, et al. personal communication). This time, extensive study upon sand-fly exploration from VL endemic districts post SP-IRS was carried out only with CDC light traps installed indoor as well as outdoor sites of selected villages during the month of June-July of same year and data were recorded in terms of sand fly collection per light traps per night.

2.2 IRS Schedule and Frequency of Sand Fly Survey

Sand flies were collected from the selected sites for a month before and after the first round IRS (i.e., February-March) and repeated similarly for the second round of IRS (i.e., June-July) of the same year 2016. The indoor dwelling sand flies were manually collected with mouth aspirators and hand operated flashlight for thrice in a week for a month, whereas outdoor resting sand flies were mechanically captured with the help of battery operated CDC light traps. Over all 60 light traps i.e., 6 light traps in a week equalising 30 light traps were installed in a month before and after the first round of IRS in year 2016 for recording and comparing pre- and post- sand fly density at outdoor regime of each selected sites of semi VL endemic (Patna) and highly VL endemic (Vaishali) districts of Bihar. Also, in total 96 light traps i.e., 24 light traps were installed purposely for sand-fly exploration at each selected village of Vaishali (Raghopur) and Saran (Sonepur) districts of Bihar i.e., VL endemic districts for 4 nights following second round of SP-IRS carried out during the month of June-July 2016.

2.3 Sand Fly Collection

The traps utilized for sand fly collection were 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, U.S.); installed at favorable outdoor habitat lying within the periphery of 100 meters from the human dwelling places, comprising different clustered vegetation viz., bamboo plant, banana plant, mango, date and nearby heap of bricks. These CDC light traps were properly installed by the trained insect collectors and adjusted during the day time, at the height of 15 cm away from the ground floor level [9,15], verified by the research personnel of survey team. From the experienced expectancy of vector arousal as well as for safe and undisturbed habitat observation at the outdoor location, sand fly collection with both techniques were performed for an hour only, during the period of dusk (5.00-6.00 PM) and dawn (5.00-6.00 AM) followed by the site selection done one day before the CDC light trap installation. The captured sand flies were carefully transferred to the labeled Borosil® glass test tube, mouth tightly plugged with semi-moist cotton ball and transported to the laboratory of Vector Biology and Control Division of RMRIMS (ICMR) for sorting and confirm microscopic identification.

3. RESULTS

3.1 Pre and Post-IRS Comparative Study upon the Sand Fly Density

For the pre- and post-IRS comparative study upon sand fly density reveals that overall 206 and 71 sand flies were collected before and after first round of DDT-IRS respectively from Dhanarua PHC of Patna district of Bihar. Out of collected 206 sand flies before DDT-IRS, 107 (comprising 54.20% male and 45.79% female) sand flies were collected from the outdoor (with the help of CDC light trap) while 99 indoor sand flies (comprising 56.56% male and 43.43% female) were caught with the help of aspirator. Following the DDT-IRS spray, sand fly density were abruptly found to be declined by 35.15% and 11.60% for outdoor sand flies (collected with CDC light trap) as well as indoor sand flies (collected with aspirator) respectively. After first round DDT-IRS, out of 71 sand flies, 58 (comprising 55.17% male and 44.82% female) sand flies were collected with CDC light trap, while collection of 13 (including 46.15% male and 53.84% female) sand flies were recorded with the help of aspirator.

Similarly, for the Vaishali district of Bihar, treated with insecticide 5% Alphacypermethrine during the trial session of pyrethroid based insecticide for the first ever time, at the seven PHC Vaishali district during the first round of SP-IRS, overall 290 and 135 were collected before and after first round of SP-IRS respectively. Out of collected 290 sand flies, 188 (including 62.76% male and 37.23% female) sand flies were collected during the dusk and dawn period from the outdoor vegetation, with the help of CDC light trap installed for an hour. A significant reduction in sand fly density (i.e., 33.09 % and 29.16%) was observed for outdoor and indoor caught sand flies, collected with CDC light trap and aspirator respectively. After first round of SP-IRS, out of collected 135 sand flies, 93 (including 73.11% male and 26.88%) female sand flies were caught with the help of CDC light traps while 42 sand flies (comprising 71.42% male and 28.57% female) sand flies were recorded from indoor habitat with the help of an aspirator as illustrated in Table 1.

3.2 Extensive Study upon Sand-Fly Exploration from VL Endemic Districts Post SP-IRS

While recording the sand fly density data of villages of endemic districts viz., Vaishali and Saran districts of Bihar after the second round of SP-IRS, it was observed that out of collected 595 sand flies, 131 and 464 sand flies were collected from Vaishali and Saran district respectively. At Raghopur PHC of Vaishali district, of collected 131 sand flies, 63 sand flies were collected from Chandpura village while 68 sand flies were collected from Jurawanpura village with the help of 24 light traps installed for 4 consecutive nights after SP-IRS. In collected 63 sand flies from Chandpura village, 87.3% sand flies were recorded from outdoor vegetation, while 12.7% sand flies were collected from indoor sites. Also, out of collected 68 sand flies, 75% and 25% sand flies were recorded from outdoor and indoor habitat respectively of Jurawanpur village of Raghopur PHC of Vaishali district. Therefore, sand fly collection in terms of per light traps per night recorded for outdoor sites were significantly higher (i.e., 2.2) than those for indoor habitat (i.e., 0.3) of Chandpura village and similar trend were recorded at Jurawanpur village of Raghopur PHC of Vaishali district with sand fly collection per light traps per night 2.1 and 0.7 for outdoor and indoor respectively as illustrated in Fig. 1.

The descriptive microscopic analysis of collected sand flies reveals the record percentage of male sand flies collected from outdoor with 78.18% as well as from indoor with 87.5% as compared to that of just 12.5% and 21.81% female sand flies caught from indoor resting sites as well as outdoor vegetations respectively of Chandpura village. At other village, i.e., Jurawanpur (Raghopur, Vaishali), recorded with 76.47% and 82.38% male sand flies caught from outdoor as well as indoor sites respectively were observed to be significantly much higher than that of either outdoor female sand flies (23.52%) or indoor resting female sand flies (17.64%) exhibiting similar trends as to that of Chandpura village of Raghopur PHC of Vaishali district as illustrated in Fig. 2.

At Sonepur PHC of Saran Districts, in total 464 sand flies were collected with the help of CDC-light traps out of which 290 sand flies were collected from Rahardiyar village while 174 of them were collected from Nazarmira village with the help of 24 light traps installed for 4 consecutive nights following second round of SP-IRS. Of collected 290 sand flies from Rahardiyar, 63.1% sand flies were recorded from outdoor vegetation while rest 36.89% was collected from inside the periphery of households. At Nazarmira village also, higher no. of sand flies collection

were recorded from outdoor sites (53.44%) as compared with indoor habitat (i.e., 46.55%) resulting in higher no. of sand fly collections per light traps per night from outdoor sites (i.e., 3.8) as compared with indoor habitat (i.e., 3.3) revealing similar trend as at Rahardiyar village i.e., 7.6 and 4.4 for outdoor and indoor habitat respectively of Sonepur PHC of Saran district as illustrated in Fig. 3.

While detailing the gender specificity of collected sand flies from Saran district, it was observed that out of 183 sand flies collected from outdoor resting sites of Rahardiyar village of Sonepur PHC, 61.74% were male and 38.25% were female sand flies whereas of recorded 107 indoor dwelling sand flies, 73.83% were found to be male in contrast to 26.16% female sand flies. Similar typed trends were observed for Nazarmira village of Sonepur PHC, recorded with 61.29% male sand flies and 38.7% female sand flies of 93 sand flies collected from outdoor habitat in contrasts to 51.85% male and 48.14% female sand flies of 81 sand flies captured from indoor resting sites during the study period as illustrated in Fig. 4.

While, following feeding status of caught sand flies post-IRS from each study villages of Vaishali and Saran districts, it was observed that only unfed female sand flies (i.e., 100%) were caught in comparison with either fed or gravid ones.

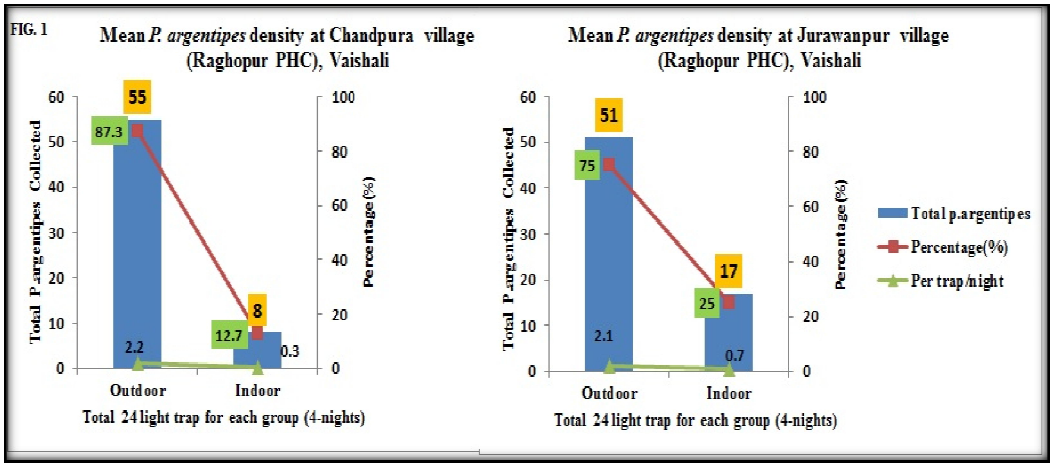

Fig. 1. Sand fly collection recorded from two villages of Raghopur PHC of Vaishali district of Bihar following second round of SP-IRS in year 2016

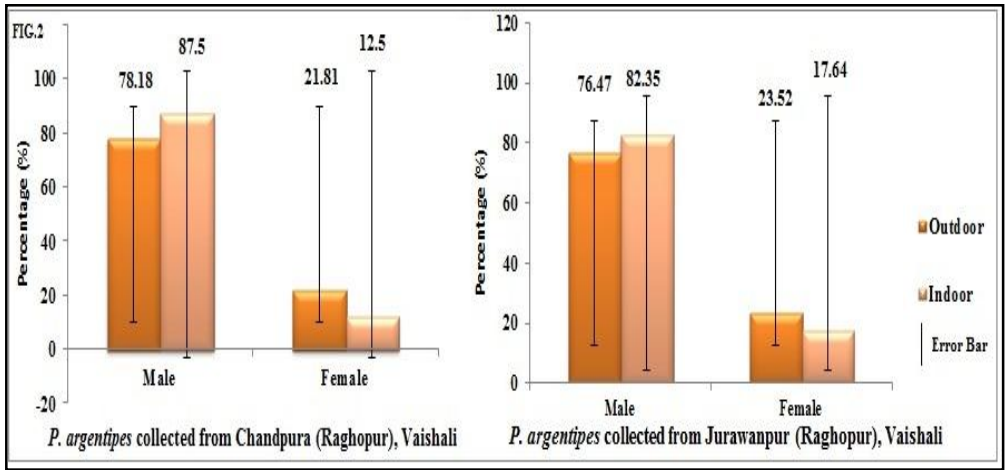

Fig. 2. Detailed sand fly collection recorded from indoor and outdoor habitat of Raghopur PHC of Vaishali district of Bihar following second round of SP-IRS in year 2016

Table 1. Pre- and post sand fly collection data recorded from the village of highly and semi VL endemic district of Bihar during the first round of IRS in year 2016

Place	Collection months	Outdoor collection with CDC light Trap				Indoor collection with aspirator			
		Total no. of CDC-LT installed	Sand fly collection		Total no. of sand flies collected	Total no. of expeditions	Sand fly collection		Total no. of sand flies collected
			Male (P. argentipes)	Female (P. argentipes)			Male (P. argentipes)	Female (P. argentipes)	
Dhanarua/ Patna (DDT-IRS)	Before 1st Round of IRS (2016)	6 Light traps installed in a week for month = 30 Light traps	58 (54.20%)	49 (45.79%)	107	Thrice in a week for a month	56 (56.56%)	43 (43.43%)	99
	After 1st Round of IRS (2016)		32 (55.17%)	26 (44.82%)	58		6 (46.15%)	7 (53.84%)	13
Patepur/ Vaishali (SP-IRS)	Before 1st Round of IRS (2016)	6 Light traps installed in a week for months = 30 Light traps	118 (62.76%)	70 (37.23%)	188	Thrice in a week for a month	31 (30.39%)	71 (69.60%)	102
	After 1st Round of IRS (2016)		68 (73.11%)	25 (26.88%)	93		30 (71.42%)	12 (28.57%)	42

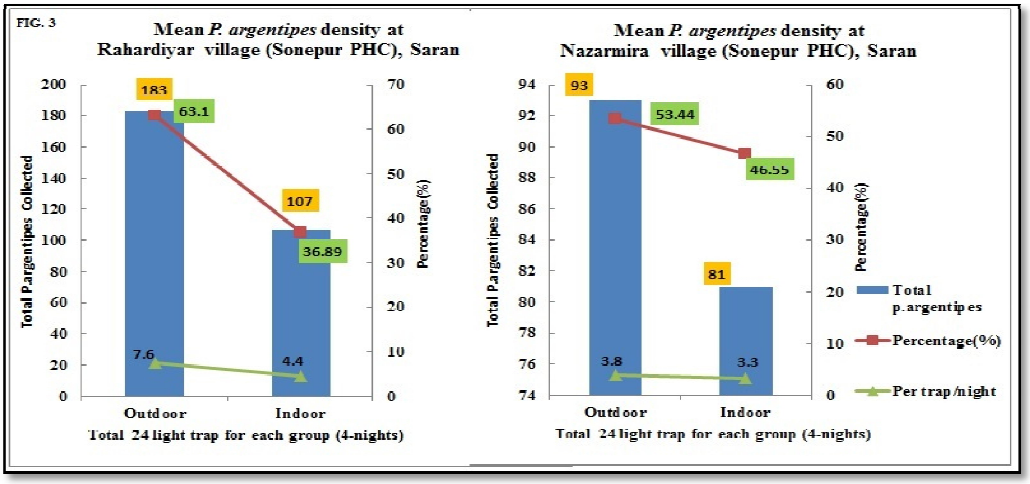

Fig. 3. Sand fly collection recorded from two villages of Sonepur PHC of Saran district of Bihar following second round of SP-IRS in year 2016

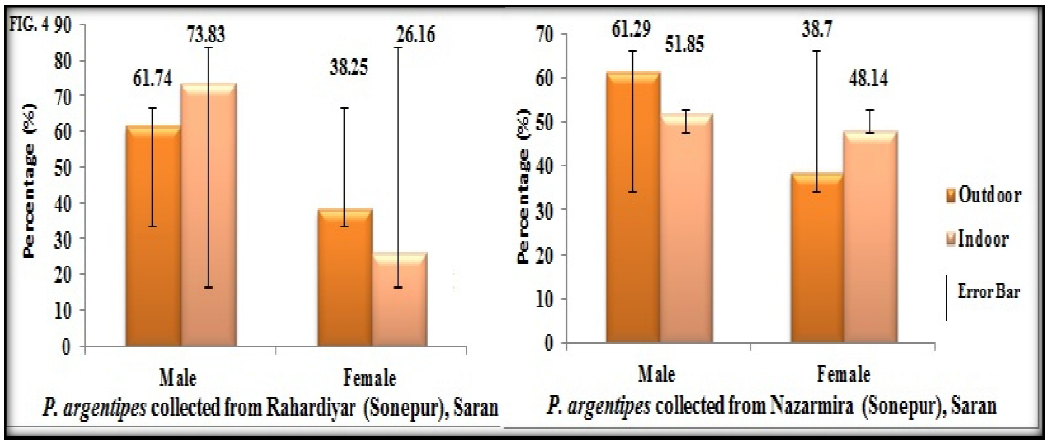

Fig. 4. Detailed sand fly collection recorded from indoor and outdoor habitat of Sonepur PHC of Saran district of Bihar following second round of SP-IRS in year 2016

4. DISCUSSION

Previous report well-adjudicate the districts of Bihar state (25.0961ºN, 85.3131ºE) to be virulent for anthroponosis VL infection transmitted by phlebotomine sand flies [9,21] and for controlling them, Indoor Residual Spray (IRS) with the insecticide of choice i.e., Dichlorodiphenyltrichloroethane (DDT) remained very popular during 1960-70 [22]. After the decades of extensive application of 50% WP emulsified suspension of DDT @ 1 g/m^2 or 100 mg/ft^2 in a programme mode for controlling VL vectors [8], *P. argentipes* has developed the capacity to tolerate its toxicity to such an extent that instead of getting killed, it attained tolerance/ resistance against it [12,13,14] followed with depleting efficacy of DDT-IRS [19,23] also sought to be the reason for flaring-up of cases at these places.

After observing continued unsatisfactory results of VL vector control with DDT-IRS, under trial session during the first round of IRS (i.e., February-March) in year 2016, insecticide spray intervention was carried out with Synthetic Pyrethroid (SP) i.e., Alphacypermethrin 5% at the 7 PHCs of Vaishali district (Kumar et al. personal communication) whereas for Patna district being semi-endemic for VL, IRS continued with DDT during this period. However, after getting promising results in terms of reduced VL vector density, later on the base insecticide i.e., DDT, had been totally replaced with SP for whole 16 PHCs of Vaishali districts during the second round of IRS (i.e., September-October). And

since then, in order to clutch the increasing instances of DDT-resistance, the routine organo-chlorinated insecticide i.e., DDT @ 5% has been totally wrapped-up by the Synthetic Pyrethroid (SP) i.e., Alphacypermethrin 5% @ 0.025 gm per m^2 for implementation of biannual IRS under the National IRS Policy, paving very hopeful way towards the milestones of Kala-azar elimination via vector control from the endemic districts of Bihar. The IRS program had been successfully implemented by the Government of Bihar, under the sponsorship of National Vector Borne Disease Control Programme (NVBDCP), Government of India, co-supervision by Rajendra Memorial Research Institute of Medical Sciences (Indian Council of Medical Research, New Delhi) Agamkuan, Patna-800007, Bihar (India).

With this national initiative, though mass mortality triggered by VL has satisfactorily been controlled but failed to take charges of reduction over either the VL cases [24] or VL vector densities (Kumar et al. personal communication).

Knowing the nature of Indian VL vector as an endophilic [6], endophagic [7], the ongoing practice of IRS targeting indoor walls along with peridomestic sites had undoubtedly transcripted as an effective tools for destroying probable territories of indoor resting sand flies much efficiently, since very long. Also, DDT constituted IRS probed a serious impact over the sand fly density [15,18,23] and accordingly upon year-to-year VL cases too, that remained sessile with DDT-IRS activation got flared-up each time with cessation of DDT-IRS [11] and its continuous perusal furthermore overruled its effect very soon ultimately leading to surging-up of sand fly density at sprayed sites. Hence, it became very difficult to conclude whether the sand fly resurgence was a single effect of DDT withdrawal [11] or the combo effect along with developed resistance among sand flies against DDT insecticide [8,14,15,25,26].

The present study was conducted to quantify the oriental behavior among VL vectors persuaded by the IRS that led them to remained alive and get trapped in light trap even after changed chemical composition of IRS i.e., SP-IRS.

With comparison based results of our present study, indoor and outdoor sand flies collected with hand operated aspirators and CDC light traps respectively, installed at both highly endemic villages of Patepur (Vaishali) and semi endemic villages of Dhanarua (Patna) following SP-IRS and DDT-IRS respectively, it was observed that though the sand fly density reduced significantly under the influence of chemical based insecticide, but it failed to take-up the charge of either absolute or long lasting control over the vector population. This resulted in positive sand flies collection, not only from the IRS treated households of study sites but also from the outdoor habitats within a week of IRS. Results definitely validated the previous report of sand fly resurgence within just 15 days of DDT-spray [19] corroborating the findings of earlier work may be due to the poor IRS quality. Previous reports configuring *P. argentipes* to be endophilic [6], endophagic [7] and zoophilic [22, 27] is now appears to turn up to transit its behaviour purposely for coping up and competing with environmental condition driven by the chemical complexities for their survival.

After getting the satisfactory results in terms of sand fly collection just within a week of IRS, the comparison based exophilic versus endophilic sand fly density was extended at the villages lying within the regime of Saran along with Vaishali i.e., highly endemic for Kala-azar. The data exhibited a major chunk of *P. argentipes* collected from the outdoor sites as compared with the indoor habitat validating our hypothesis of gradual shifting of habitat of VL vectors from endophilic to exophilic which is undoubtedly followed due to the fact of developed resistance among them [14,17,26] against chemical constituent of IRS. Under the influence of developed resistance against insecticide, insects instead of getting killed, they duly modify their behavior to cope the toxicity of that insecticide that further rewards them with survival [17]. But their survival is manifested with “excito-repellent” effect i.e., spatial repellency with 'irritancy' (contact excitation) and toxicity together referred to as an under the influence of insecticide composition of DDT [28] that further causes them to migrate from the periphery of sprayed households and to take shelter at nearby bamboo bushes, banana plants as well as crevices in the heap of bricks [18]. Here they can easily procure the favorable components needed for their survival as well as for propagating their generations. Though the cent-percent collection of unfed female sand flies as well as non-availability of either fed or gravid sand flies from the outdoor regime signifies the dormant feeding habit of outdoor resting female sand flies under the impact of IRS and somehow relieves the fear of disease transmission. As these outdoor resting sand flies are enough potent to harbor infection

and transmit them to the next vulnerable hosts i.e., humans, there exists a great chances of reversal of their reduced density and feeding dormancy anytime at the condition of availability of favorable situation, likely to re-invade the corners of house and target inhabitants effectively.

5. CONCLUSION

Concluding the study, it is a high time to re-think upon the ongoing strategies of VL vector control tools especially with the IRS. In order to uphold the reduced VL cases along with controlled VL vector population during the maintenance phase of VL elimination target affixed by WHO, it is needed to upgrade the efficacy of IRS and for this, it is very urgent to weaponise each and every tools of vector control competed with transitioned vector behavior.

The comparision based findings of sand fly collection from indoor and outdoor regime of sprayed the presented in this study does provide very useful information about the sand fly dynamics under the impact of IRS and accordingly, the combined approach of IRS (especially targeting in-house resting sand flies) along with insecticidal fogging, (covering probable shelters occupied by 'migrated' sand flies) together at a same time following quantification of 'vector mood and behavior' could provide an effective dividend for maximum VL vector control along with reduced VL cases at par for longer duration. It could be validated as an effective approach for negotiating and maintaining the reduced sand fly density during the maintenance phase at the VL foci.

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kumar V, Kesari S, Kumari K, Krishnakumari B, Venugopalan R, Das P. Comparison of *In vivo* host animals as blood-feeding source for laboratory rearing of the sand fly vector *Phlebotomus argentipes* (Diptera: Psychodidae). Ann Entomol Soc Am. 2011;104(3):429-433.
2. Malaviya P, Hasker E, Picado A, Mishra M, Van Geertruyden JP, Das ML, et al. Exposure to *Phlebotomus argentipes* (Diptera, Psychodidae, Phlebotominae) sand flies in rural areas of Bihar, India: the role of housing conditions. PLOS one. 2014;9(9):e106771. Available:https://doi.org/10.1371/journal.pone.0106771
3. Swaminath CS, Shortt HE, Anderson LAP. Transmission of Indian kala-azar to man by the bite of *P. argentipes* Annadale and Brunetti. Indian J Med Res. 1942;30:473-477.
4. Kumar V, Bimal S, Kesari S, Kumar AJ, Bagchi AK, Akbar MA, et al. Evaluation of a dot-immunoblot assay for detecting leishmanial antigen in naturally infected *Phlebotomus argentipes* (Diptera: psychodidae). Ann Trop Med Parasit. 2005;99(4):371-376.
5. Sharma U, Singh S. Insect vectors of Leishmania: Distribution, physiology and their control. J Vector Borne Dis. 2008; 45:255–272.
6. Ghosh KK, Das S, Hati AK. Studies on seasonal man sandfly (*Phlebotomus argentipes*) contact at night. Journal of Indian Association of Communicable Diseases. 1982;5:14-18.
7. Dinesh DS, Ranjan A, Palit A, Kishore K, Kar SK. Seasonal and nocturnal

landing/biting behaviour of *Phlebotomus argentipes* (Diptera: Psychodidae). Ann Trop Med Parasitol. 2001;95(2):197-202.

8. Kishore K, Kumar V, Kesari S, Bhattacharya SK, Das P. Susceptibility of *Phlebotomus argentipes* against DDT in endemic districts of North Bihar, India. J Comm Dis. 2004;36(1):41-44.
9. Kumar V, Kesari S, Kumar AJ, Dinesh DS, Ranjan A, Prasad M, et al. Vector density and the control of kala-azar in Bihar, India. Mem Inst Oswaldo Cruz 2009;104:1019-1022.
10. Joshi A, Narain JP, Prasittisuk C, Bhatia R, Hashim G, Jorge A, et al. Can Visceral leishmaniasis be eliminated from Asia? J Vector Borne Dis. 2008;45:105-111.
11. Muniaraj M. The lost hope of elimination of Kala-azar (visceral leishmaniasis) by 2010 and cyclic occurrence of its outbreak in India, blame falls on vector control practices or co-infection with human immunodeficiency virus or therapeutic modalities? Trop Parasitol. 2014;4:10-19.
12. Mukhopadhyay AK, Sexena NBLK, Narasimhan MVVL. Susceptibility status of *Phlebotomus argentipes* to DDT in some Kala-azar endemic districts of Bihar, India. Indian J Med Res. 1990;91:458-460.
13. Singh R, Das RK, Sharma SK. Resistance of sandflies to DDT in Kalaazar endemic district of Bihar, India. Bull. World Health Organ. 2001;79:793.
14. Coleman M, Foster GM, Deb R, Singh RP, Ismail HM, Shivam P, et al. DDT-based indoor residual spraying suboptimal for Visceral leishmaniasis elimination in India. Proc Natl Acad Sci. 2015;112:8573-8578.
15. Kumar V, Shankar L, Kesari S, Bhunia GS, Dinesh DS, Mandal R, et al. Insecticide susceptibility of *Phlebotomus argentipes* & assessment of vector control in two districts of West Bengal, India. Indian J Med Res. 2015;142(2):211-215.
DOI: 10.4103/0971-5916.164260
16. Rama A, Kumar V, Kesari S, Singh VP, Das P. Monitoring susceptibility status of *Phlebotomus argentipes* (Diptera: Psychodidae) at Bihar (India) for the procurement of homozygous DDT resistant colony. J Trop Dis. 2015;3(4):170.
Available:http://dx.doi.org/10.4172/2329-891X.1000170
17. Rama A, Kesari S, Das P, Kumar V. Studying DDT susceptibility at discriminating time interval focusing maximum limit of exposure time survived by DDT resistant *Phlebotomus argentipes* (Diptera: Psychodidae): An investigative report. Jpn J Infect Dis. 2017;70(4):437-441.
18. Kumar V, Shankar L, Rama A, Kesari S, Dinesh DS, Bhunia GS, et al. Analysing host preference behavior of *Phlebotomus argentipes* (Diptera: Psychodidae) under the impact of indoor residual spray. International Journal of Tropical Disease & Health. 2015;7(2):69-79.
19. Chowdhury R, Huda MM, Kumar V, Das P, Joshi AB, Banjara MR, et al. The Indian and Nepalese programmes of indoor residual spraying for the elimination of Visceral leishmaniasis: performance and effectiveness. Ann Trop Med Parasitol. 2011;105:31-35.
20. Huda MM, Mondal D, Kumar V, Das P, Sharma SN, Das ML, et al. Toolkit for monitoring and evaluation of indoor residual spraying for Visceral leishmaniasis control in the India subcontinent: application and results. J Trop Med. 2011; 876742.
21. Desjeux P. Leishmaniasis: Current situation and new perspectives. Comparative Imm Micro Infect Dis. 2004; 27:305–318.
22. Kishore K, Kumar V, Kesari S, Dinesh DS, Kumar AJ, Das P, et al. Vector control in leishmaniasis. Indian J Med Res. 2006; 123(3):467-472.
23. Kumar V, Rama A, Mishra PS, Siddiqui NA, Singh RP, Dasgupta RK, et al. Investigating associative impact of indoor residual spray and insecticide treated nets for minimizing visceral leishmaniasis vector population in Bihar (India). International Journal of Tropical Disease and Health. 2017;23(4):1-15.
DOI: 10.9734/IJTDH/2017/32540
24. National Vector Borne Disease Control Programme (NVBDCP). Accelerated plan for kala-azar elimination. 2017. Directorate National Vector Borne Disease Control Programme. NVBDCP; 2017.
Available:http://nvbdcp.gov.in/Doc/Accelerated-Plan-Kala-azar1-Feb2017.pdf
25. Dhiman RC, Raghavendra K, Kumar V, Kesari S, Kishore K. Susceptibility status of *Phlebotomus argentipes* to insecticides in districts Vaishaii and Patna (Bihar). J Comm Dis. 2003;35(1):49-51.

26. Gomes B, Purkait B, Deb RM, Rama A, Singh RP, Foster GM, et al. Knockdown resistance mutations predict DDT resistance and pyrethroid tolerance in the visceral leishmaniasis vector *Phlebotomus argentipes*. PLoS Negl Trop Dis. 2017; 11(4):e0005504.
27. Mukhopadhyay AK, Chakravarty AK, Kureel VR. Resurgence of *Phlebotomus argentipes* & *Ph. papatasi* in parts of Bihar (India) after DDT spraying. Indian J Med Res. 1987;85:158-160.
28. Roberts DR, Alecrim WD, Hshieh P, Grieco JP, Bangs M, Andre RG, et al. A probability model of vector behavior: effects of DDT repellency, irritancy, and toxicity in malaria control. J Vector Ecol. Journal of the Society for Vector Ecology. 2001;25(1):48-61.

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