

Analysing Host Preference Behavior of *Phlebotomus argentipes* (Diptera: Psychodidae) Under the Impact of Indoor Residual Spray

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VK, SK and DSD designed the whole work plan and experimental set-up for the research to be carried out in field as well as laboratory. Experiments as well as field activities were carried out by author LS. Authors AR and GSB helped in drafting manuscript. Author PD 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: Present scenario of Bihar and neighboring Indian states reveals dichlorodiphenyltrichloroethane (DDT) being an insecticide of choice for controlling the vector of Indian Visceral Leishmaniasis (VL) i.e., *Phlebotomus argentipes*, that had reported to attain resistance/tolerance against it, leading to the behavioral transition including host preference and selection by them. The relationship between insecticidal resistance and host preference/ selection

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is not yet well understood.

Objective: Exploring the host preference/selection behavior under the influence of insecticidal pressure in different biotopes of VL endemic regions in India

Methods: For this, the engorged sand flies that were collected before and after Indoor Residual Spray (IRS) were subjected for feeding behavior analysis. The parameter studied were Host Feeding Index (HFI) and Forage Ratio (FR) by analyzing Blood Meal Identification (BMI).

Results: The higher percentage of sand flies were recorded to be fed on cattle host (56.05%) with respect to the human host (30.35%) before IRS while a significant increase in cattle blood index (79.17%) in contrast to significant drop in human blood index (9.43%) was recorded during post IRS session at the study site. It establishes, cattle being potentially served as a preferred host for sand flies in contrast to other available hosts. The lowered value of FR during pre- and post-IRS respectively for human (0.77 and 0.24) as compared to the cattle (1.89 and 2.67) indicates cattle host being selectively preferred by the *P. argentipes* also corroborate with the results of BMI.

Conclusion: Through the study we can conclude that instead of being killed by IRS, *P. argentipes* has attained resistance against DDT. Under the insecticidal pressure the host preference as well as selection tendency of *P. argentipes* for cattle host gets enhanced under the influence of IRS, as abrupt increment was observed in the FR's post-IRS. While, the avoidance tendency of insects from the human hosts in favor of other available hosts viz., cattle, goat, pigs, etc. with slight decrement in the forage ratios for the human hosts during the IRS was also observed. Thus, under the impact of IRS, *P. argentipes* has changed its behavior from endophilic to exophilic and migrated from the human hosts at the periphery area of sprayed houses towards much safer zone, i.e., deserted houses, nearby gardens, bushes, etc for their survival for feeding cattle i.e., preferred host, lying in unsprayed horizon. Therefore, change in control strategy involving the proper management of insecticide resistance is very much needed to tackle the vector outbreak and hence menace caused by them.

Keywords: Visceral Leishmaniasis (VL); *Phlebotomus argentipes*; Indoor Residual Spray (IRS); Host Feeding Index (HFI); Forage Ratio (FR); Blood Meal Identification (BMI); endophilic; exophilic.

ACRONYMS AND ABBREVIATIONS

IRS: Indoor Residual Spray; DDT: Dichlorodiphenyltrichloroethane; HFI: Host Feeding Index; FR: Forage Ratio; BMI: Blood Meal Identification; VL: Visceral Leishmaniasis; HH: Human Household; CS: Cattle shed; MD: Mixed Dwelling; PHC: Public Health Centre.

1. INTRODUCTION

Phlebotomus argentipes (Diptera: Psychodidae), harbouring mature protozoan parasite *Leishmania donovani* serves as a vector for Visceral Leishmaniasis (VL), a lethal disease in Indian subcontinent [1]. Disease transmission occurs when a female sand fly lodged with parasite that it had collected from the infected host during the blood meal, successfully inject them into the healthy individual [2]. Female sand fly being haematophagy entails blood to nourish its eggs leading to progeny emergence and hence continuation of its generation. [3-5] Characterization of vector behavior focusing onto the host selection and host preferences for blood feeding plays a crucial parameter for identifying and success of natural transmission of leishmaniasis as well as allowing vector control

authorities to design & implement efficient strategies for vector control [4-6]. Earlier studies regarding the behavior of VL vector, reports that *P. argentipes* bears seasonal [7,8], endophilic [9], endophagic [7] and zoophilic nature [10,11]. *P. argentipes* from northern Bihar was predominantly reported to be 62.8% zoophilic whereas 17.2% of them were observed to be fed on human also. This suggests that *P. argentipes* to possess an instinct opportunistic anthropophilic behavior [10] that compels them to deviate towards the other host, in absence of preferred hosts for feeding purpose. It shows that *P. argentipes* is changing its behavior which may also influence its host seeking behavior.

Coming on to the control strategies in context to VL endemic Indian states, dichlorodiphenyltrichloroethane (DDT) served as an insecticide of choice for controlling the

menace since 1960-70 [11]. Extensive use of DDT led to the development of resistance/tolerance against it among *P. argentipes* population, at VL endemic areas in Bihar [12,13]. Instead of being killed by the Indoor Residual Spray (IRS) it had attained the behavioral transformation including host preference and host selection by them. *P. argentipes* being an opportunistic feeder [10] re-appears even in 15 days of spraying in the DDT sprayed houses and feed the available host rather than their preferred host [14,15]. In this context, it has become pertinent to explore the host preference of *P. argentipes* under the impact of IRS, in the kala-azar endemic states of India, particularly in Bihar, Jharkhand and West Bengal.

Perusal of literature on host preference and host selection by the Indian VL vector reveals that there is still little documentation on this subject. Host preference and selection behavior using Forage Ratio (FR) and Host feeding Index (HFI) had never been dealt in case of sand flies; however these have been individually reported in case of mosquitoes [16-18]. Along with that HFI and FR via Blood Meal Identification (BMI) with respect to Indoor Residual Spray (IRS) had never been reported earlier in case of any hematophagous insect. Hence, present study conducted to explore host preference and selection behavior of *P. argentipes* for feeding purpose, under the influence of IRS. It will help in understanding the the host preference as well as monitoring vector dynamics under the insecticidal pressure for controlling the menace caused by them and hence proper management of insecticide resistance.

2. MATERIALS AND METHODS

2.1 Study Area

VL is focally concentrated in the districts of north-eastern Indian states viz., Bihar, Jharkhand, and West Bengal providing adequate environment to the VL vectors remain arrested at this place. Hence, for studying host preference behavior of Indian VL vector *P. argentipes*, selection of highly VL endemic districts were made on the basis VL cases report as well as IRS report provided by the authorized governing officials at Public Health Centers (PHCs) Districts level. Comparing the reports, chances of availability of sand flies were estimated in huge amount at Darwa village of Samastipur district (25.862931°N 85.781064°E) at Bihar, Sunderpahari Basti of Godda district (24.8167°

N, 87.2167°E) of Jharkhand and Tapshar village of Maldah district (25.0000°N, 88.1500°E) at West Bengal, that were selected as study site as well as represented in Fig. 1.

2.2 Study Period

The Indoor Residual Spray (IRS) had been conducted in Bihar, Jharkhand and West Bengal during the month of June-August 2012. Hence, the sand flies collection for the pre-IRS session was done during the month of April-May 2012, whereas for the post-IRS session, the sand flies were collected during the month of September-October 2012 from the same village of the study site. The sand fly collection for pre- and post-IRS session was performed during the peak of vector season as reported earlier studies [19,20].

2.3 Sand Fly Trapping

Diurnal indoor as well as outdoor resting wild strain sand flies were collected from the sheltering places viz., Human Households (HH), Cattle sheds (CS), and Mixed Dwellings (MD) with the help of hand-held mouth aspirator during the pre- and post- IRS at the villages of study sites. The sand fly collections were done from the site where the insects' density had been reported to be at peak. Aspirated sand flies were then gently confined to the Hilton's pot and labeled with name, place and date of sample collection. Collected sand flies were brought to RMRI laboratory, Patna. Fully fed female sand flies were separated out from the collections, post indexing its species, sex and feeding status using morphological characteristics. The samples were hoard at -20°C for anaesthetizing purpose till the blood meal analysis for each collected specimen was done.

2.4 Census of Vertebrate Hosts

Census report of human population for each study sites during the period of IRS were obtained from the Primary Health Centre (PHC) of village of study-site while the living domestic assets such as number of pets, cattle, goats-owned by each households at study site was done by door-to-door interviewing the occupants of each residence. If the number of vertebrate animals at a site found to be changed due to any reasons like migration, death etc., the site was no longer used for the study. Site was only used if the number and species composition of animals remained constant during the study

period. Due to practical constraints, the number of non-domesticated animals temporarily and spatially associated with each study site were not estimated and hence categorised as 'Unknown'.

2.5 Blood Meal Identification (BMI)

The Blood Meal Identification for 1,326 *Phlebotomus argentipes* (Diptera: Psychodidae), collected from the endemic focus of visceral leishmaniasis in India states during pre- and post- IRS was performed by Dot Elisa Assay technique described earlier [21]. For this, the sand flies were dissected to remove its wings and appendages. The collected blood fed abdomen samples of *Phlebotomus argentipes* were absorbed on Whatman filter paper no. 1® and then were stored at -20°C for further blood meal analysis. Sand fly's blood samples were eluted individually into 100 µl of 1 X Phosphate Buffer Saline (PBS) (pH 7.4) using sterile micro-pestle overnight at temperature 4°C. After 24 hrs, 2 µl of each elute were transferred to small piece of Nitrocellulose paper inside flexible polyvinyl chloride ELISA plate and incubated overnight at 4°C. Serum dilutions in PBS pH (1: 500) from selected host were used as positive control. The plates were blocked with 150 µl of 5% Bovine Serum Antigen (BSA) and incubated for an hour, after that it was washed for three times with PBS, 0.05% Tween 20. The anti-IgG host specific

peroxides conjugates were diluted in 0.05% Tween 20 according to previously determined ratio mixed with 50µl of each heterologous serum to reduce the cross reactivity and to enhance specificity, incubated for 1hr at 37°C and washed three times with PBS Tween 20. Subsequently, peroxidase substrate; di-aminobenzidine (DAB) was added and incubated in the dark for 30 minutes. The reactions were stopped by washing with distilled water. The samples were considered as positive due to appearance of definite blue purple spot on the specific antigen dot.

2.6 Host Feeding Index (HFI)

The Host Feeding Index (HFI) is an observed proportion feeds on one host with respect to another divided by the expected comparative proportion of feeds on the two hosts was calculated by using undernoted formula [16].

$$HFI = \frac{N_x/N_y}{A_x/A_y}$$

Here N_x and N_y are the mean numbers of blood meals taken from hosts x and y per study site, respectively, and A_x and A_y are the mean numbers of hosts x and y per study site, respectively.

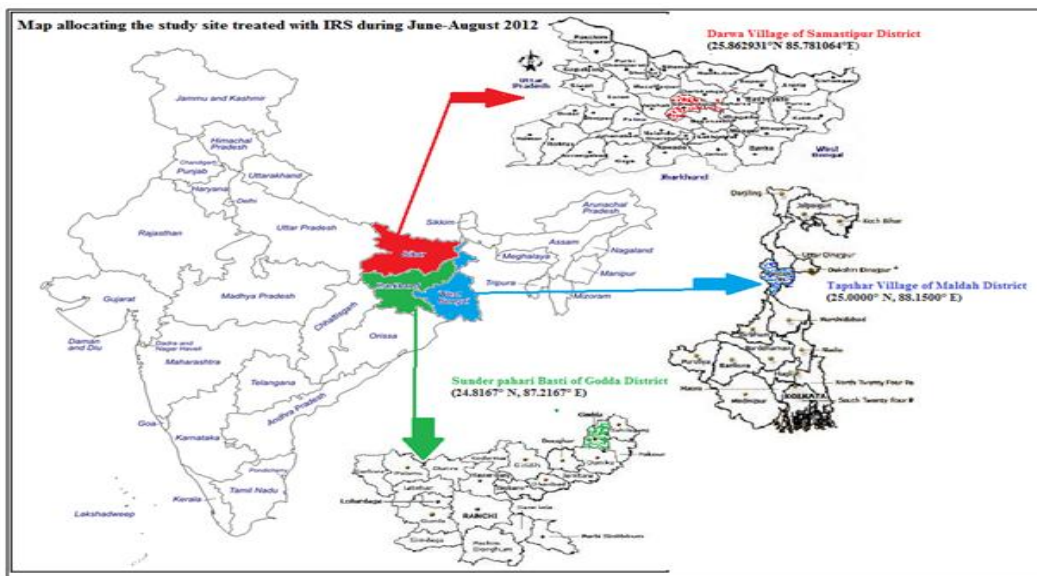


Fig. 1. Map allocating the Study Site for analysing host preference behavior of *P. argentipes* under the impact of IRS

An index of 1.0 indicates equal feeding on the two hosts. Results < 1.0 and > 1.0 indicate a decrease or increase, respectively, in feeding on the first host relative to the second.

For our study HFI were calculated for human and cattle host only.

2.7 Forage Ratio (FR)

The Forage Ratio (FR), quantifying host selection by the vector in contrast to the available hosts [17] was estimated for the available hosts at the study site. Forage ratios (FRs) for understanding the host preference of *P. argentipes* in the surveying sites, were calculated by using undernoted formula [16,18].

FR =

$$\frac{\% \text{ of female insect containing blood of a particular host}}{\% \text{ of total available host population represented by that particular host}}$$

As per method, an FRs significantly >1.0 reveal discerning preferences and values <1.0 divulge evasion in favor of other hosts. However, in our study, the percentage of the FRs was calculated for human and cattle only.

3. RESULTS

3.1 Sand Fly Collection and Blood Meal Identification

A total of 1, 326 sand flies were collected from the different indoor resting habitats, viz., Human household, cattle sheds, mixed dwellings. Of the total sand flies, 26.09%, 41.31% and 32.60% were captured from the districts of Bihar, West Bengal and Jharkhand respectively.

Among the collection, 817 blood-fed sand flies, that were captured from the study-site that is, Bihar, Jharkhand and West Bengal before the Indoor Residual Spray (IRS) session, were subjected for host's blood meal analysis. Data are illustrated in Table 1, Fig. 2.

Through the analysis it was observed that out of 817 samples collected from the study site, 56.05% (n=458) insect's blood meal was positive for cattle host in contrast to the human host i.e., 30.35% (n=248). Out of overall collection, 202 samples were collected from households (HHs), among which 65.84%, 18.38%, 4.95% and 2.48% samples were observed to be positive for

human, cattle, goat and unknown hosts respectively. Out of 361 sand flies collected from cattle sheds (CSs), 10.80% were human, 78.67% were cattle, 1.94% mixed feeding, 5.26% were goat and 3.32% were unknown respectively. From the mixed dwellings (MDs), a total of 254 samples were analyzed and of which 29.92%, 53.94%, 8.66% and 6.69% samples were observed to be positive for human, cattle, goat and unknown respectively. Also 8.42% and 1.94% blood meal index was identified as multiple blood meals from human-goat and cattle-goat respectively.

Accordingly, during post-IRS session at the study site, a total of 509 sand flies were analyzed for the host that it fed to sustain its life, through the blood meal analysis technique. Data are illustrated in Table 2, Fig. 3.

Out of 509 collected samples 79.17% (n=403), insect's blood meal was observed to be positive for cattle host over the human host i.e., 9.43% (n=48). Results showed that out of 104 samples collected from HHs 31.73%, 51.92%, 2.88% and 8.65% samples were positive for human, cattle, goat and unknown respectively. Of the CSs, 274 samples were analyzed, and results showed 1.82%, 88.69%, 4.74% and 4.74% samples were positive for human, cattle, goat and unknown respectively. Of 131 samples collected from MDs, 7.63% fed on human, 80.92% on cattle, 5.34% on goat, 0.76% on unknown host. Multiple blood feeding was also identified that included 5.34% from human-cattle as well as 4.81% from cattle-goat.

The data comparing the result of host blood meal for each *P. argentipes* collected before and after IRS at the districts of the study sites are illustrated in Fig. 4.

Result of our analysis showed that regardless IRS, *P. argentipes* pre-dominantly prefer cattle as their blood meal source in contrast to any other vertebrate hosts available in exophilic situation. In situation of getting host choices in mixed dwelling condition, peaks for the host's blood feeding percentage of collected *P. argentipes* was observed in case of cattle, revealing the cattle to be the host of choice for them. Also, an increasing trend of BMI for cattle host at CS as well as MD, contrast to the decreasing trend BMI for the human hosts at HH after the IRS at the villages of the study site indicate the IRS being effectively carried out in HHs rather than at the CSs or the MDs.

Table 1. The results of blood meal identification of *P. argentipes* collected from the study site during Pre-IRS session

Habitats	Total SF collected	Pre- IRS treatment				
		Human	Cattle	Mixed feed	Goat	Unknown
HH	202	65.84%	18.38%	8.42%	4.95%	2.48%
CS	361	10.80%	78.67%	1.94%	5.26%	3.32%
MD	254	29.92%	53.94%	0.79%	8.66%	6.69%
Total	817	30.35%	56.05%	3.18%	6.24%	4.16%

¹ HHs – Households, CS – Cattle shed, MD – Mixed dwelling, H – Human, G – Goat, C-Cattle

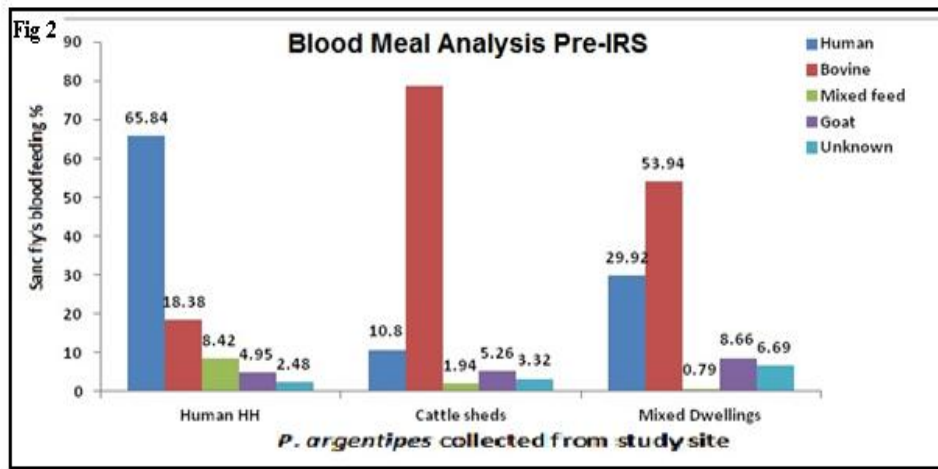


Fig. 2. Graph illustrating the result of blood meal identification of *P. argentipes* collected from the study site during Pre-IRS session

Table 2. The results of Blood Meal Identification of *P. argentipes* collected from the study site during Post-IRS session

Habitats	Total SF Collected	Post- IRS treatment				
		Human	Cattle	Mixed feed	Goat	Unknown
HH	104	31.73%	51.92%	4.81%	2.88%	8.65%)
CS	274	1.82%	88.69%	-----	4.74%	4.74%
MD	131	7.63%	80.92%	5.34%	5.34%	0.76%
Total	509	9.43%	79.17%	2.35%	4.51%	4.51%

¹ HHs – Households, CS – Cattle shed, MD – Mixed dwelling, H – Human, G – Goat, C-Cattle

3.2 Host Feeding Indices and Forage Ratio analysis

Through calculating host feeding indices for human versus cattle host at study-site before and after IRS we observed that proportion of human host feeding and cattle host feeding tends to increase (i.e., 0.60) after IRS when compared to pre-IRS (i.e., 0.13) indicating decrease in the human host feeding relative to the cattle host

during the IRS session. Through the study we inferred that cattle being highly preferred over the human as blood meal source for female *P. argentipes*.

The forage ratio (FR) for *P. argentipes* fed on the blood of human hosts and cattle hosts before and after IRS was calculated. The data comparing the FR of *P. argentipes* before and after IRS is represented in Table 3 and Fig. 5.

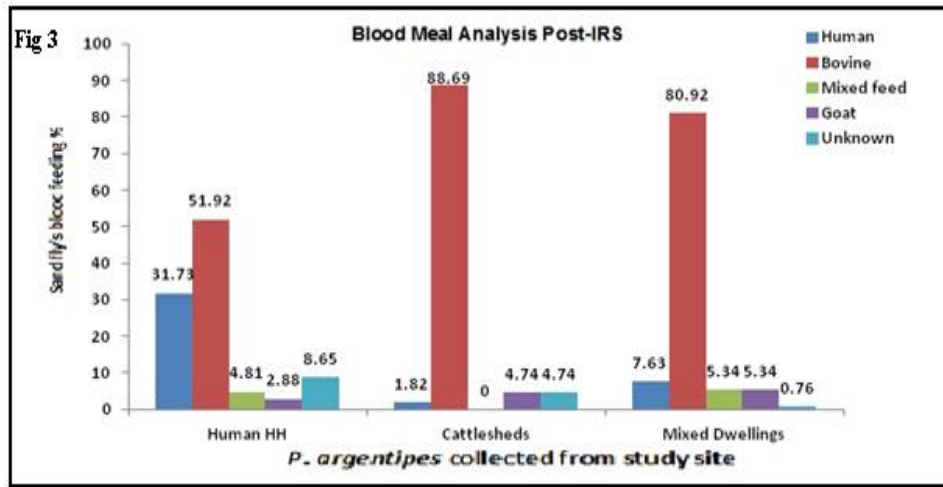


Fig. 3. Graph illustrating the result of blood meal identification of *P. argentipes* collected from the study site during Post-IRS session

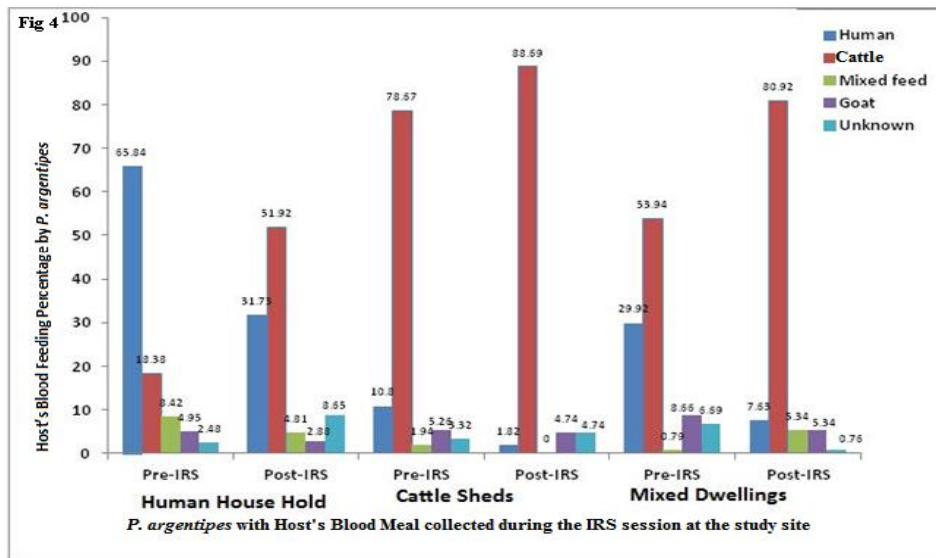


Fig. 4. Results of comparative analysis of host's blood meal identification of *P. argentipes* collected before and after IRS session

The census report obtained from Public Health Center (PHC) as well as door-to-door survey at each households of village at the district of study site reveals that the human and animal host at the villages of the study-site during the study period were 1000 and 438 respectively. For human and cattle host, 39.50% and 29.68% of the total population was studied during the study period. Also, 30.35% female insects fed the human host before IRS while a significant drop in

feeding percentage (9.43%) was observed after the IRS. In case of cattle host, 56.06% female insects fed the cattle hosts before IRS and in contrast significant increase in percentage (79.17%) was observed after IRS at the study site. From the study, we also observed that the FR for human is lower (0.77 and 0.24) as compare to the cattle (1.89 and 2.67) during both pre and post IRS.

Table 3. Forage ratio analysis of *P. argentipes* collected from the study site during pre- and post-IRS session

	Total census in villages of study site	Total human population covered during the study period	% of female insect feeding on human host	Forage ratio	Total animal census in villages of study site	Total cattle population in village of study site	% of female insect feeding on cattle host	Forage ratio
Pre-IRS	1000	(39.50%)	(30.35%)	0.77	438	(29.68%)	(56.06%)	1.89
Post-IRS	1000	(30.50%)	(9.43%)	0.24	438	(29.68%)	(79.17%)	2.67

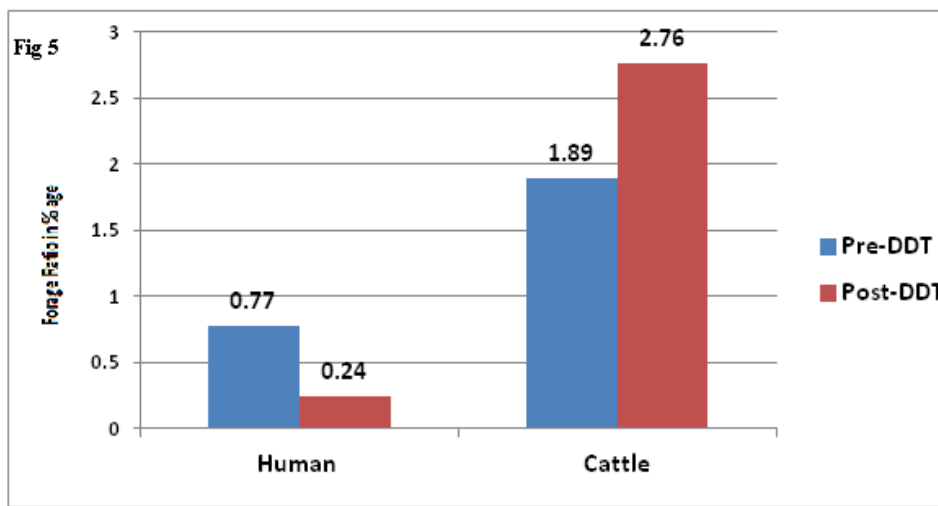


Fig. 5. Graphic illustration for the Forage ratio analysis of *P. argentipes* collected from the study site during pre- and post-IRS session

Applying T-test, at 5% level of significance, the data of human host and cattle host feeding by *P. argentipes* was observed to be statistically insignificant, indicating the presence of variation between them. Thus, study demonstrated that *Phlebotomus argentipes* did not have an equal preference for each host species during pre-DDT spray ($P < 0.05$) and post DDT spray ($P < 0.005$). Sand flies being anthropophagi as well as zoophagy prefer humans and animals blood for feeding purpose are selectively biased for the cattle host during the pre- and post- IRS session. The results of same test conducted for other hosts revealed maximum numbers of blood meals were obtained from the bovine population in comparison to goat ($P < 0.003$) and mixed feeding ($P < 0.004$) during the post DDT spray.

Hence, through the study we can conclude that the host preference as well as selection tendency by *P. argentipes* for cattle host gets enhanced under the influence of IRS, as abrupt increment

was observed in the FR's post-IRS. Along with this, the avoidance tendency of insects from the human hosts in favor of other available hosts' viz., cattle, goat, pigs and birds, with slight decrement in the forage ratios for the human hosts during the IRS was also observed indicating the deviation and migration of insects from the human hosts at the periphery area of sprayed houses towards the cattle-sheds in search of their preferred hosts.

4. DISCUSSION

Limited study and scarce documentation confine our knowledge regarding the host preference and selection behavior of *P. argentipes*. Very few reports-son the host-meal analysis concerning vector's behavioral adaptation, followed by the continuous exposure of insects against Indoor Residual Spray (IRS) are available. Forage Ratio (FR) and Host Feeding Index (HFI) had never been dealt in case of *P. argentipes*. However,

these had been individually reported in case of mosquitoes [16-18] while HFI and FR analysis collectively with respect to IRS, had never been reported in case of any haematophagous insect. Therefore, present study was conducted to compare the change in host preference behavior of *P. argentipes* before and after IRS at the three endemic states of India viz., Bihar, West Bengal and Jharkhand. It was performed by capturing the female sand flies from the study site and estimating FR and HFI through estimating Blood Meal Identification (BMI) from each collected samples of *P. argentipes*. The lowered percentage of sand fly collection during post-IRS session in contrast to the collections of pre-IRS session revealed lowering down of efficacy of IRS rather than total ceasing of IRS impact at the sprayed area ultimately inviting the the situation of of vector resurgence at sprayed area.

Pursuing anthropophilic characteristics [10] previous report from different biotopes of kala-azar endemic regions of India showed that *P. argentipes* had also taken a significant proportion of blood meals from cattle (51.81%, 58.18% and 58.27% from the villages of Bihar, West Bengal and Jharkhand respectively) during pre-IRS session [19,20]. One of the inference can be drawn from the report is that the sand fly being zoophilic [10], prefers cattle blood over the human blood However, the sand flies of the Malda district are more zoophilic in comparison to that of the Bihar and Jharkhand [10,11]. The previous workers [10,14,] also indicated the zoophilic nature of *P. argentipes* of Bihar and West Bengal. Along with that, *P. argentipes* also showed the nature of opportunistic anthropophilic nature means taking human blood incidentally [10] in absence of their preferred hosts.

Our data illustrate that post-IRS, at all the biotopes of study site, proportion of meals by *P. argentipes* with human blood collected from HHs, CSs and MDs decreased dramatically. Consequently, proportion of blood meals from cattle hosts became slightly higher after IRS at HH, CS, and MD respectively at the study site. Moreover, the higher no. of sand flies that were identified with cattle blood collected from the cattle sheds and mixed dwellings after IRS in contrast to same before IRS reveals that though having hosts choices for *P. argentipes* i.e., humans and cattles at CSs and MDs, vectors tends to incline towards their preferred host and exclusively fed their blood, regardless host availability. One of the inference can be drawn from the study is that the sand fly prefers cattle blood and takes the human blood incidentally.

The possible reason behind the above inference could be the effectivity of IRS, which is limited within the range of human habitat. Human households are mainly targeted under the protocol of IRS which significantly led to the development of tolerance against DDT [21-23] encourages the behavioral transition and host preference. Under the impact of IRS, insects migrate from the periphery region of the sprayed houses towards the nearby unsprayed localities viz., bushes, cattle sheds, mixed dwellings etc, for taking rest and shelter during the day time. After the dusk, when insect approaches host for feeding purpose it encounters cattle i.e., their preferred host, lying in the unsprayed area rather than the human host at the sprayed area. However, the sand fly collected from HH before IRS showed the higher degree of preference for human blood in comparison to the sand flies collected in the post IRS era in the same locality. This indicates higher HBI during pre IRS as reported in previous studies [24]. Study reports the high degrees of anthropophily of sand flies often refer to indoor collections of sand flies, where the sand flies are most likely to encounter only humans. Thus, a strong bias for a particular host can occur when circumstances limit host choice. In addition, the species has an inherent opportunistic feeding behavior, small number of *P. argentipes* collected from the periphery of the households after IRS shows tremendous suppression of their anthropophilic behavior and expression of zoophilic nature under the impact of IRS.

Data of our analysis also showed that total blood meals from the unknown species slightly increased during the post-IRS session i.e., 4.51%; while the total blood meals from the goat and mixed feed i.e., 4.51% and 3.18% respectively were observed to be slight decrease during the post-IRS in comparison to pre-IRS. Previously *P. argentipes* was known as a concordant species however the multiple feeding behaviors indicates in the change of behavior resultant in the development of sibling species as also reported [25].

The further analysis of our result showed during the pre-IRS, multiple feeding was observed from human-goat and cattle-goat only whereas during the post-IRS, multiple feeding was found from the human-cattle and cattle-goat in the biotopes of study site. It may be due to the migration of sand flies towards outside after the insecticide spraying. The finding suggests for the better control of the vector, the periphery area surrounding the host dwellings should also be

sprayed with insecticide properly in order to get supportive results.

FR values gave significant results with a very high value of cattle (i.e., cattle and goat) and relatively low values for humans. This information suggests that they are preferred cattle in respect to humans. Our data seem to confirm the previous observations on the attraction of *P. argentipes* to cattle. The enhancing host selection tendency and host avoidance tendency of *P. argentipes* for cattle and human hosts respectively under the influence of IRS indicate the probable reason for the deviation and migration of insects from the human hosts at the periphery area of sprayed houses towards the cattle-sheds in search of their preferred hosts.

5. CONCLUSION

From the study, we conclude that instead of being killed by IRS, *P. argentipes* has changed its behavior and migrated towards the deserted houses, bushes etc, lying in exophilic situation. It is so because it had developed resistance against IRS, as reported in several studies. Present study reveals that sand flies deviate from the range of IRS to the much safer zone, towards cattle i.e., preferred host, lying in unsprayed horizon, for their survival. Therefore, it is high time to consider on change in the control strategy by involving proper management of insecticide resistance as well as regular monitoring the behavioral transition of vector for controlling the menace caused by them.

CONSENT

It is not applicable for this research study.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed for conducting research experiments involving animal. Protocols led by animal ethics committee of Indian Council of Medical Research, Government of India, were also followed for the present research work involving experiments using animal subject. All experiments had been conducted under the guidance of the Institutional ethical committee of RMRI (ICMR), Patna.

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COMPETING INTERESTS

All the authors of the manuscript hereby declare that no competing financial interests exist between us.

REFERENCES

1. Swaminath CS, Short HE, Anderson LAP. Transmission of Indian kala-azar to man by the bite of *P. argentipes*. Indian J Med Res. 1942;30:473-477.
2. Bongiorno G, Habluetzel A, Khoury C, Maroli M. Host preferences of phlebotomine sand flies at a hypoendemic focus of canine leishmaniasis in central Italy. Acta Trop. 2003;88:109-116.
3. Seenivasagan T, Vijayaraghavan R. Chapter 24-oviposition pheromones in haematophagous insects. Vita & Horm. 2010;83:597-630.
4. Rama A, Kumar V, Kesari S, Dinesh DS, Das P. Exploring semiochemical based oviposition response of *Phlebotomus argentipes* (Diptera: psychodidae) towards pre-existing colony ingredients. IJMPS. 2014;4(4):35-46.
5. Rama A, Kesari S, Dinesh DS, Seema K, Das P, Kumar V. Vertebrate excreta based semiochemical influencing oviposition & neonates' survival in *Phlebotomus argentipes*- Visceral Leishmaniasis vector in Indian subcontinent. JEZS. 2014;2(6):172-178.
6. Killick-Kendrick R. The biology and control of phlebotomine sand flies. Clin Dermatol. 1999;17:279-289.
7. Dinesh DS, Ranjan A, Palit K, Kishore K, Kar SK. Seasonal and nocturnal

- landing/biting behavior of *Phlebotomus argentipes* (Diptera: Psychodidae). Ann Trop Med Parasit. 2001;95:197-202.
8. Picado A, Das ML, Kumar V, Dinesh DS, Rijal S, Singh SP, et al. *Phlebotomous argentipes* seasonal patterns in India and Nepal. J Med Entomol. 2010;47:283-286.
 9. Ghosh K, Das KP, Hati AK. Studies on seasonal man sand fly (*Phlebotomus argentipes*) contact at night. J Indian Assoc Comm Dis. 1982;5:14-18.
 10. Mukhopadhyay AK, Chakravarty AK. Bloodmeal preference of *Phlebotomus argentipes* and *Ph. papatasi* of north Bihar, India. Indian J Med Res. 1987;86:475-480.
 11. Kishore K, Kumar V, Kesar S. et al. Vector control in leishmaniasis. Indian J Med Res. 2006;123:467-472.
 12. Mukhopadhyay AK, Saxena NBL, Narasimhan MVVL. Susceptibility status of *Phlebotomus argentipes* to DDT in some Kala-azar endemic districts of Bihar India. Indian J. Med. Res. 1990;91:458-60.
 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. Palit A, Bhattacharya SK, Kundu SN. Host preference of *Phlebotomus argentipes* and *Phlebotomus papatasi* in different biotopes of West Bengal, India. Int J Environ Heal R. 2005;15:449-454.
 15. Addy M, Mitra AK, Ghosh KK, Hati AK. Host preference of *Phlebotomus argentipes* in different biotopes. Trop Geogr Med. 1983;35:343-345.
 16. Richards SL, Ponnusamy L, Unnasch TR, Hassan HK, Apperson CS. Host Feeding Patterns of *Aedes albopictus* (Diptera: Culicidae) in relation to availability of human and domestic animals in suburban landscapes of central North Carolina. J Med Entomol. 2006;43:543-551.
 17. Garrett-Jones C. The human blood index of malaria vectors in relation to epidemiological assessment. B World Health Organ. 1964;30:241-261.
 18. Hess AD, Hayes RO, Tempelis CH. The use of the forage ratio technique in mosquito host preference studies. Mosq News. 1968;28:386-389.0000.
 19. Poché D, Garlapati R, Ingenloff K, Remmers J, and Poché R. Bionomics of phlebotomine sand flies from three villages in Bihar, India. J Vector Ecol. 2011;36(1):106-117.
 20. 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.
 21. Chowdhary 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 Parasit. 2010;105(1):31-45.
 22. Dhiman RC, Raghavendra K, Kumar V, Kesari S, Kishore K. Susceptibility status of *Phlebotomus argentipes* to insecticides in Districts Vaishali and Patna (Bihar). J Comm Dis. 2003;35:49-51.
 23. Singh RK, Mittal PK, Dhiman RC. Insecticide susceptibility status of *Phlebotomus argentipes*, a vector of visceral leishmaniasis in different foci in three states of India. J Vector Dis. 2012;49:254-257.
 24. Garlapati RB, Abbasi I, Warburg A, Poché D, Poché R. Index of Bloodmeals in Wild Caught Blood Fed *Phlebotomus argentipes* (Diptera: Psychodidae) using Cytochrome b PCR and Reverse Line Blotting in Bihar, India. J Med Entomol. 2012;49(3):515-521.
 25. Dines DS, Kishore K, Singh VP, Bhattacharya SK. Morphological variations in *Phlebotomus argentipes* (Diptera: Psychodidae) Annandale and Brunetti. J Comm Dis. 2005;37:35-38.

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