

Original Research Article

Analysing host preference behaviour of *Phlebotomus argentipes* (Diptera: Psychodidae) under the impact of Indoor Residual Spray.

ABSTRACT

Background: Present scenario of Bihar and neighboring Indian states reveals DDT being an insecticide of choice for controlling the vector of Indian Visceral Leishmaniasis i.e., *Phlebotomus argentipes*, that had reported to attain resistance/tolerance against it leading to the behavioral transition including host preference & selection by them.

Objective: To analyze host preference transition via blood feeding behavior of *Phlebotomus argentipes* under the impact of Indoor Residual Spray.

Methods: For this, the engorged sand flies that were collected before & after 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: Through the study, it was observed that higher percentage (i.e., 56.05%) of sand flies fed on cattle blood over the human blood (i.e., 30.35%) before IRS while a significant increase for cattle blood index (i.e., 79.17%) was observed in contrast to significant drop in human blood index (i.e., 9.43%) post IRS. Result indicates cattle potentially served as preferred host for sand flies in contrast to other available hosts. The lowered value of FR during pre- & post-IRS respectively for human (0.77 & 0.24) as compared to the cattle (1.89 & 2.67) indicates cattle host being selectively preferred by the *P. argentipes* also corroborate with the results of BMI.

Conclusion: Instead of being killed by IRS, *P. argentipes* has attained resistance against it. Under the impact of IRS, *P. argentipes* has changed its behavior from endophilic to exophilic. It migrate from the range of IRS & sheltered at much safer zone, i.e., deserted houses, nearby gardens, bushes, etc for their survival & fed 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 & hence menace caused by them.

Keywords: Visceral Leishmaniasis, *Phlebotomus argentipes*, Host Feeding Index (HFI), Forage Ratio (FR), Blood Meal Identification (BMI), Endophilic, Exophilic.

1. INTRODUCTION

Phlebotomus argentipes (Diptera: Psychodidae) lodged with the matured protozoan parasite *Leishmania donovani* [1], serve as vector for the lethal disease Visceral Leishmaniasis in Indian subcontinent. Earlier studies conducted regarding VL vector reported that *P. argentipes* bears seasonal [2,3], endophilic [4], endophagic [2] and zoophilic nature [5,6]. Female sand fly being hematophagy entails blood to nourish its eggs leading to progeny emergence & hence continuation of its generation. VL transmission occurs when a sand fly lodged with mature parasite that it had collected from the infected host during the blood meal, inject them into the healthy individual [7]. Characterization of vector movement as well

as their feeding preferences is crucial parameter for identifying & success of natural transmission cycles of Leishmaniasis and hence for developing efficacious control strategies regarding vector control programmes [8]. *Phlebotomus argentipes* prefers different host blood in order to maintain its life cycle as well as disease transmission. However, the evaluation of anthropophilic index of the sand fly species is an imperative structure for understanding the epidemiology of the disease. *P. argentipes* of northern Bihar were reported to be predominantly zoophilic [5] while 17.2% of them were observed to be fed on human also. Earlier it was documented that *P. argentipes* are mainly zoophilic (62.8%) and also the multiple blood feed in West Bengal is prevalent [6]. It shows that *P. argentipes* is changing its behavior which may also influence its host seeking behavior. It has been observed that *P. argentipes* being an opportunistic feeder [9] re-appears even in 15 days of spraying in the DDT sprayed houses. In recent time due to regular adoption of faulty IRS for controlling the vector leads to subsequent development of tolerance against the dichlorodiphenyltrichloroethane or DDT in *P. argentipes*, which further might cause some changes in the vector behavior as well as choices of host preferred by them. In this context, it has become pertinent to explore the host preference of *P. argentipes* in the kala-azar endemic states of India, particularly in Bihar, Jharkhand and West Bengal. Perusal of literature reveals that there is still little documentation on the host preferences of *P. argentipes*. Also study of host preference behavior under the insecticidal influence will be helpful to understand the host preference & selection during the parasite transmission dynamics among sand fly.

2. MATERIAL AND METHODS

2.1 Study area

Historically, VL is focally concentrated in the districts of north-eastern Indian states viz., Bihar, Jharkhand, and West Bengal. Hence, for studying the host preference behavior of *Phlebotomus argentipes* the Indian VL vector, selection of highly endemic districts of the targeted states were made on the basis of DDT spray report indicating frequent DDT spraying all over the year for reducing vector density. Thus, Darwa Village (Samastipur district, Bihar), Suderpahari Basti (Godda district, Jharkhand) and Tapshar village (Maldah district, West Bengal) were selected as study site.

2.2 Study Period

The Indoor Residual Spray (IRS) had been conducted at the Darwa village of Samastipur (Bihar), Sunderpahari Basti of Godda (Jharkhand) & Tapshar village of Maldah (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 fly collection was done during the month of September-October 2012 from the same villages of the study site.

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 aspiration technique 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 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

70 post indexing its species, sex & feeding status using morphological characteristics. The
71 samples were hoard at -20 °C for anaesthetizing purpose till the blood meal analysis for
72 each collected specimen was done.

73 2.4 Census of vertebrate hosts

74 Census of humans for each study sites during the period of IRS were obtained from the
75 Primary Health Centre (PHC) of village of study-site while the domestic living assets i.e.,
76 cattle, goats, etc., owned by each households of study site was done by door-to-door
77 interviewing the occupants of each residence. If the number of vertebrate animals at a site
78 changed (i.e. due to death), the site was no longer used for this study. Site was only used if
79 the number and species composition of animals remained constant during the study period.
80 Due to practical constraints, the numbers of non-domesticated animals temporally and
81 spatially associated with each study site were not estimated & categorised as 'Unknown'.
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83 2.5 Blood meal Identification

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85 The Blood Meal Identification for 1,326 *Phlebotomus argentipes* (Diptera: Psychodidae),
86 collected from the endemic focus of visceral leishmaniasis in India states during pre- and
87 post- IRS was performed by Dot Elisa Assay technique described earlier [10]. For this, the
88 sand flies were dissected to remove its wings and appendages. The collected blood fed
89 abdomen samples of *Phlebotomus argentipes* were absorbed on Whatman filter paper no.
90 1® and then were stored at -20° C for further blood meal analysis. Sand fly's blood samples
91 were eluted individually into 100µl of 1 X Phosphate Buffer Saline (PBS) (pH 7.4) using
92 sterile micro-pestle overnight at temperature 4° C. After 24 hrs, 2µl of each elute were
93 transferred to small piece of Nitrocellulose paper inside flexible polyvinyl chloride ELISA
94 plate and incubated overnight at 4°C. Serum dilutions in PBS pH (1: 500) from selected host
95 were used as positive control. The plates were blocked with 150µl of 5% BSA and incubate
96 for 1hrs, after that it washed three times with PBS, 0.05% Tween 20. The anti-IgG host
97 specific peroxides conjugates were diluted in 0.05% Tween 20 according to previously
98 determined ratio mixed with 50µl of each heterologous serum to reduce the cross reactivity
99 and to enhance specificity, incubated for 1hr at 37°C and washed three times with PBS
100 Tween 20. Subsequently, peroxidase substrate; di-aminobenzidine (DAB) was added and
101 incubated in the dark for 30 minutes. The reactions were stopped by washing with distilled
102 water. The samples were considered as positive due to appearance of definite blue purple
103 spot on the specific antigen dot.
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105 2.6 Host Feeding Index

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107 The Host Feeding Index (HFI) is an observed proportion feeds on one host with respect to
108 another divided by the expected comparative proportion of feeds on the two hosts was
109 calculated by using undernoted formula [11].

$$110 \quad \quad \quad \text{HFI} = \frac{111 \quad N_x/N_y}{112 \quad A_x/A_y}$$

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114 Here Nx and Ny are the mean numbers of blood meals taken from hosts x and y per study
115 site, respectively, and Ax and Ay are the mean numbers of hosts x and y per study site,
116 respectively.

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

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

2.7 Forage Ratio Analysis

The Forage Ratio (FR), quantifying host selection by the vector in contrast to the available hosts [12] 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 [13,7].

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

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 & 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 & Jharkhand respectively.

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

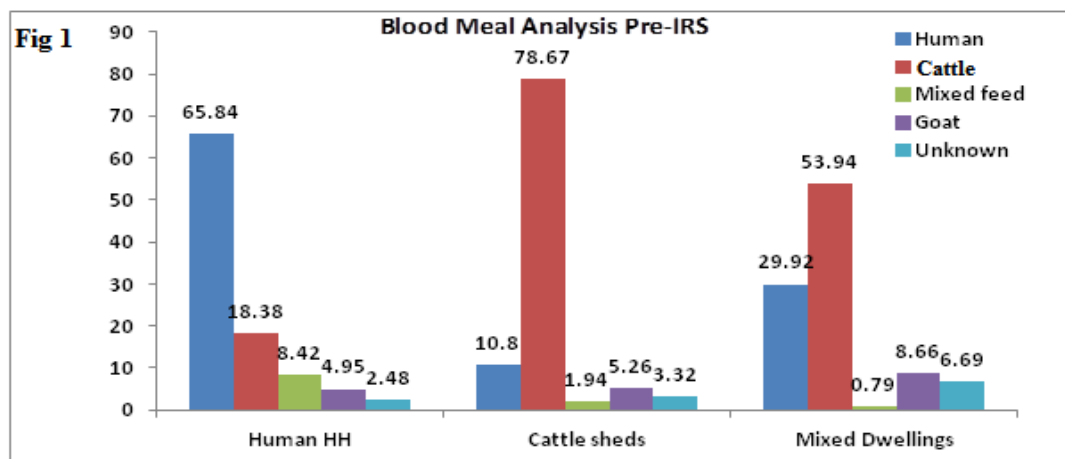
Table 1: The results of Blood Meal Identification of *P. argentipes* collected from the study site during Pre-IRS session¹.

Habitats	Total SF collected	Pre- IRS Treatment				
		Human	Cattle	Mixed Feed	Goat	Unknown
HH	202	65.84%	18.38%	8.42% H+G	4.95%	2.48%
CS	361	10.80%	78.67%	1.94% C+G	5.26%	3.32%

MD	254	29.92%	53.94%	0.79%	8.66%	6.69%
				H+G		
Total	817	30.35%	56.05%	3.18%	6.24%	4.16%

151 [†] HHs – Households, CS – Cattle shed, MD – Mixed dwelling, H – Human, G – Goat, C-Cattle

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Figure 1. Graph illustrating the result of Blood Meal Identification of *P. argentipes* collected from the study site during Pre-IRS session.

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 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 positive for human, cattle, goat and unknown respectively. Also 8.42% & 1.94% blood meal index was identified as multiple blood meals from human-goat & cattle-goat respectively.

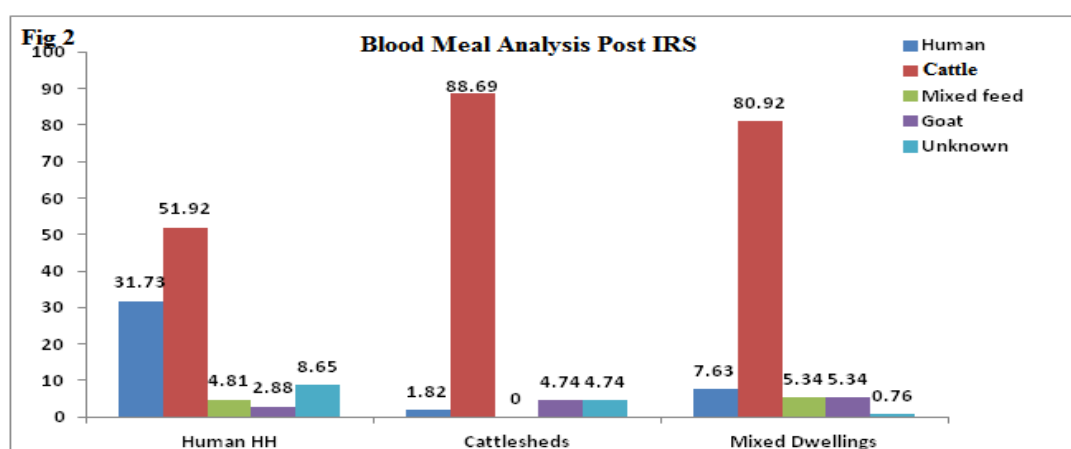
Accordingly, during post-IRS session at the study site, a total of 509 sand flies were analyzed for the suspected host through blood meal analysis. Data are illustrated in Table 2, Figure 2.

Table 2: The results of Blood Meal Identification of *P. argentipes* collected from the study site during Post-IRS session².

Habitats	Total SF Collected	Post- IRS Treatment				
		Human	Cattle	Mixed	Goat	Unknown

				Feed		
HH	104	31.73%	51.92%	4.81%	2.88%	8.65%)
				C+G		
CS	274	1.82%	88.69%	-----	4.74%	4.74%
MD	131	7.63%	80.92%	5.34%	5.34%	0.76%
				H+C		
Total	509	9.43%	79.17%	2.35%	4.51%	4.51%

174 [†] HHs – Households, CS – Cattle shed, MD – Mixed dwelling, H – Human, G – Goat, C–Cattle



175 **Figure 2. Graph illustrating the result of Blood Meal Identification of *P. argentipes***
176 **collected from the study site during Post-IRS session.**
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179 Out of 509 collected samples 79.17% (n=403) insect's blood meal was observed to be
180 positive for cattle host over the human host i.e., 9.43% (n=48). Results showed that out of
181 104 samples collected from HHs 31.73%, 51.92%, 2.88% and 8.65% samples were positive
182 for human, cattle, goat and unknown respectively. Of the CSs, 274 samples were analyzed,
183 and results showed 1.82%, 88.69%, 4.74% and 4.74% samples were positive for human,
184 cattle, goat and unknown respectively. Of 131 samples collected from MDs, 7.63% fed on
185 human, 80.92% on cattle, 5.34% on goat, 0.76% on unknown host. Multiple blood feeding
186 was also identified that included 5.34% from human-cattle as well as 4.81% from cattle-goat.
187 The data comparing the result of host blood meal for each *P. argentipes* collected before &
188 after IRS at the districts of the study sites are illustrated in Figure 3.
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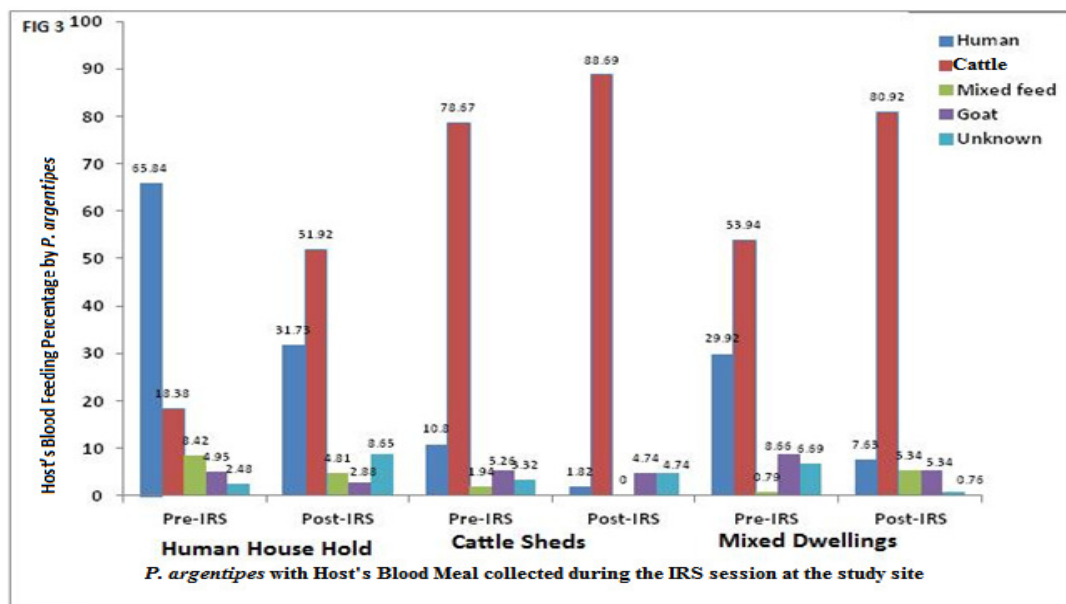


Figure 3. Results of Comparative analysis of Host's Blood Meal Identification of *P. argentipes* collected before & after IRS session.

Result of our analysis showed that regardless IRS, cattle feedings were pre-dominated by *P. argentipes* which prefer cattle as their blood meal source as compared to any other vertebrate hosts available. 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.

3.2 Host feeding Indices & 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 & 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*.

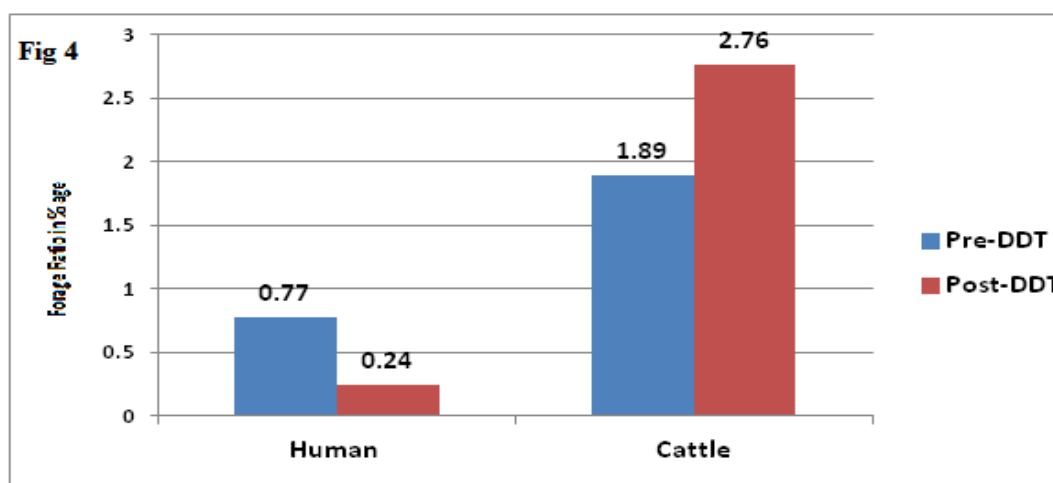
The forage ratio (FR) for *P. argentipes* fed on the blood of human hosts and cattle hosts before and after IRS was calculated. The data comparing the FR of *P. argentipes* before and after IRS is represented in Table 3 & Figure 4.

Table 3: Forage ratio analysis of *P. argentipes* collected from the study site during pre- & 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	Total cattle population in village of study site	%of female insect feeding on cattle host	Forage ratio

					site			
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

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Figure 4. Graphic illustration for the Forage ratio analysis of *P. argentipes* collected from the study site during pre- & post-IRS session.

From 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, the total census for the human and animal host at the villages of the study-site during the study period was recorded as 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 on the human host before IRS while a significant drop in percentage i.e., 9.43% was observed after the IRS. In case of cattle host, 56.06% female insects fed on cattle hosts before IRS and in contrast significant increase in percentage i.e., 79.17% was observed after IRS at the study site. From the study we also observe that the FR for human is lower (0.77 & 0.24) as compare to the cattle (1.89 & 2.67) during both pre and post IRS.

The study reveals that sand flies being anthropophagi as well as zoophagy prefer humans & animals blood for feeding purpose are selectively biased for the cattle host during the pre- & post- IRS session. We observe the host selection tendency for cattle host by *P. argentipes* 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, etc. 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.

Hence, in nutshell, we sum up our study with inference that IRS doesn't cause much change in the anthropophilic behavior of *P. argentipes*.

4. DISCUSSION

As there were very few reporting on the host-meal analysis concerning vector's behavioral adaptation enforced by the continuous application of Indoor Residual Spray (IRS), present study dealing with the evaluation of host preference and selection of *P. argentipes*, under the impact of Indoor Residual Spray (IRS) in Indian states viz., Bihar, Jharkhand and West Bengal were conducted through estimating Host Feeding Index (HFI) and Forage Ratio (FR) by estimating Blood Meal Identification (BMI) from each collected samples of *P. argentipes*.

The result of our analysis from different biotopes of kala-azar endemic regions of India showed that *P. argentipes* is somewhat anthropophilic, but had taken a significant proportion of blood meals from cattle (i.e., 51.81%, 58.18% and 58.27% for the villages of Bihar, West Bengal and Jharkhand respectively during pre-IRS session). One of the inference can be drawn from the study is that the sand fly basically prefers cattle blood and it takes the human blood incidentally; however, the sand flies of the Malda district are more zoophilic in comparison to that of the Bihar and Jharkhand. The previous workers [5,6] also indicated the zoophilic nature of *P. argentipes* of Bihar and West Bengal. 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 & 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 & mixed dwellings after IRS in contrast to same before IRS reveals that though having hosts choices for *P. argentipes* i.e., humans & cattles at CSs and MDs, vectors tends to incline towards their preferred host & 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 liveliness 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 [14,15] 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 by the early authors [16]. Studies reporting 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 [17].

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

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 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.

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.

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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382 ACRONYMS AND ABBREVIATIONS

383

384 IRS: Indoor Residual Spray

385 DDT: Dichlorodiphenyltrichloroethane

386 HFI: Host Feeding Index

387 FR: Forage Ratio

388 BMI: Blood Meal Identification

389 VL: Visceral Leishmaniasis
390 HH: Human Household
391 CS: Cattle shed
392 MD: Mixed Dwelling
393 PHC: Public Health Centre

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