

Original Research Article
Analyzing host preference behavior of
***Phlebotomus argentipes* (Diptera:**
Psychodidae) under the impact of Indoor
Residual Spray.

Author details:

Vijay Kumar^{1*}, Lokesh Shankar², Aarti Rama³, Shreekanth Kesari⁴, Diwakar Singh Dinesh⁵, Gouri Shankar Bhunia⁶, Pradeep Das⁷

1, Scientist-D, Head of Department, Department of Vector Biology and Control, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Agamkuan, Patna – 800 007, Bihar, India.

2,3,4,5,6, Department of Vector Biology and Control, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Agamkuan, Patna – 800 007, Bihar, India.

7 Director, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Agamkuan, Patna – 800 007, Bihar, India.

Email: vijayrnagar@hotmail.com.

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 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**

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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.

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 ~~heamatophagy~~ *haematophagy* entails blood to nourish its eggs leading to progeny emergence and hence continuation of its generation. [3, 4, 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 [4,5,6]. 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 ~~were~~ *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, 17, 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 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.

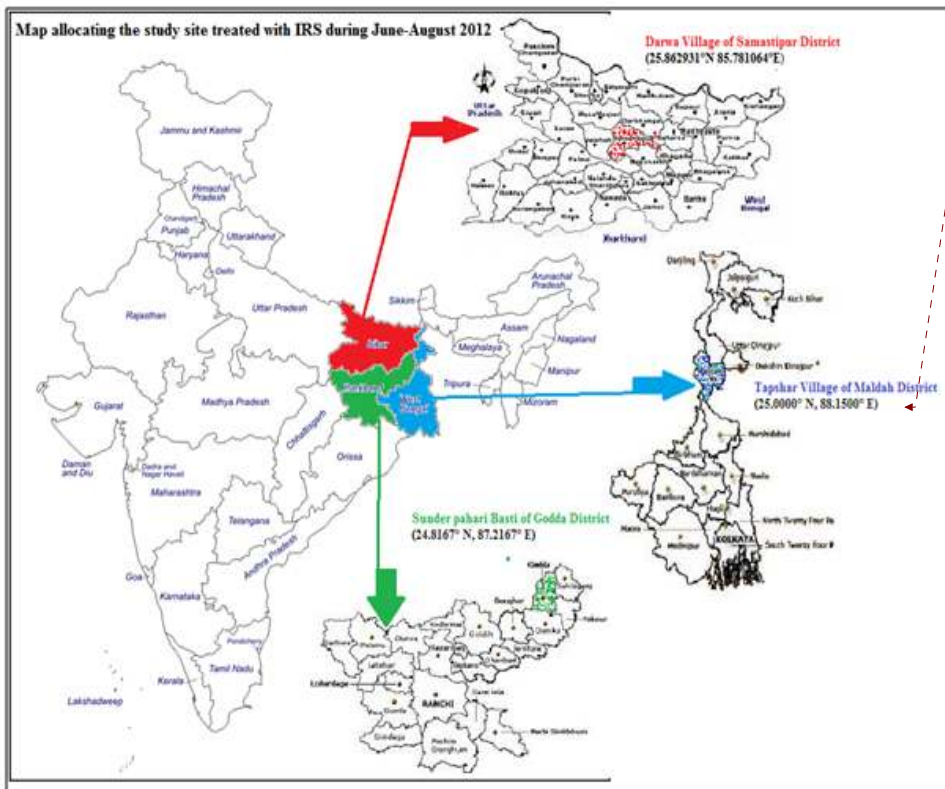
2. MATERIAL AND METHODS

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 Figure 1.

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

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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 ~~i.e., such as number of pets, cattle, goats, etc.,~~ 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~~ (i.e. due to any reasons like migration, death etc.) the site was no longer used for ~~this 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 100µl of 1 X Phosphate Buffer Saline (PBS) (pH 7.4) using sterile micro-pestle overnight at temperature 4° C. After 24 hrs, 2µl 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 150µl of 5% BSA and incubated for 1hrs, after that it washed 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].

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

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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. 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 i.e., 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, Figure 12.

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 H+G	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% H+G	8.66%	6.69%

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Total	817	30.35%	56.05%	3.18%	6.24%	4.16%
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¹ HHs – Households, CS – Cattle shed, MD – Mixed dwelling, H – Human, G – Goat, C-Cattle

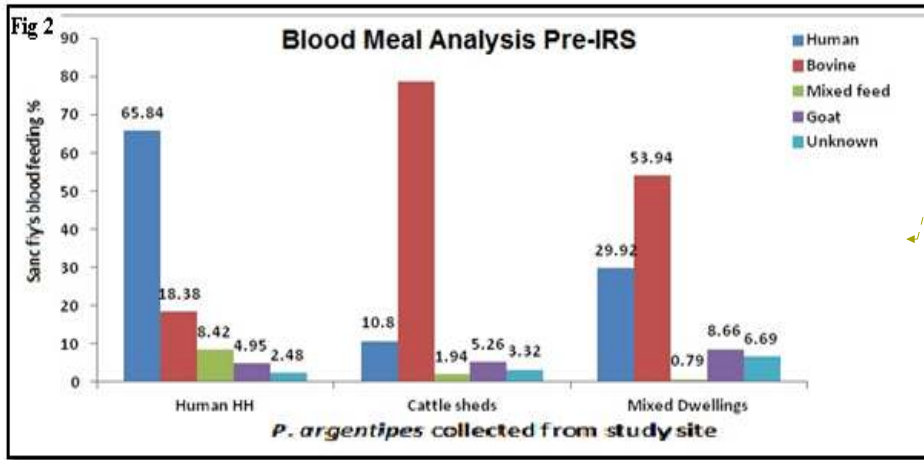
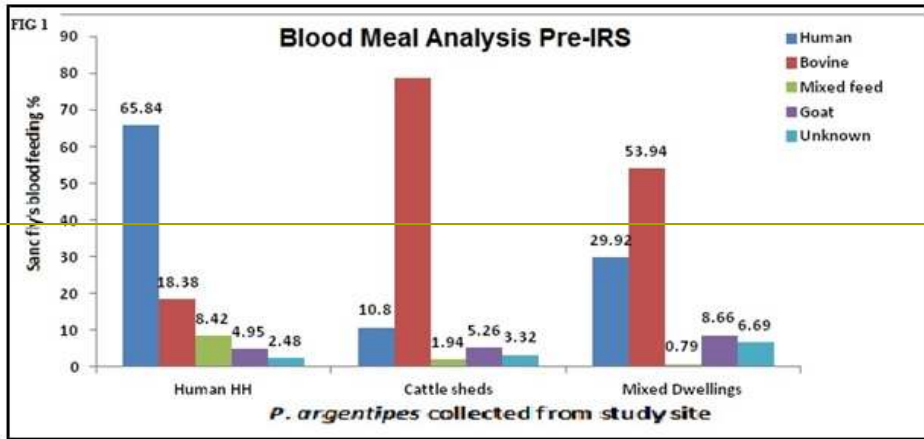


Figure 2. Graph illustrating the result of Blood Meal Identification of *P. argentipes* collected from the study site during Pre-IRS session.

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 observed to be positive for human, cattle, goat and unknown hosts respectively. Out of 361

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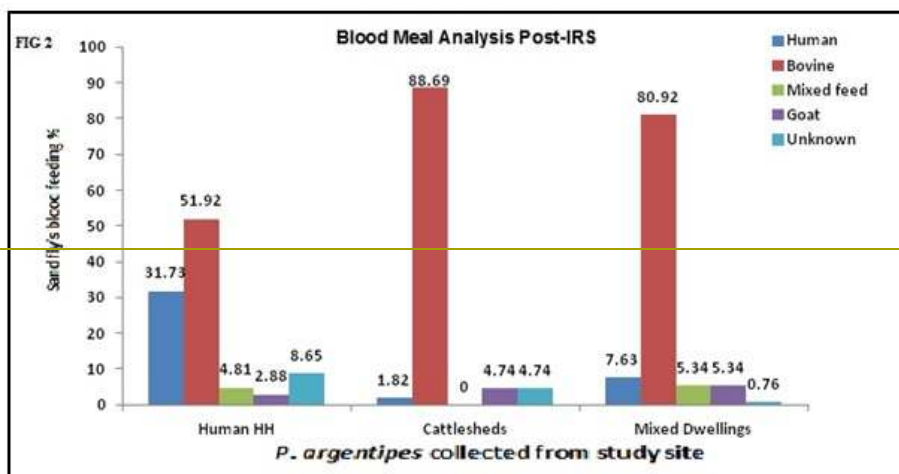
sand flies collected from cattle sheds (CSs), 10.80% were human, 78.67% were cattle, 1.94% mixed feeding, 5.26% were goat and 3.32% were unknown respectively. From the mixed dwellings (MDs), a total of 254 samples were analyzed, and of which 29.92%, 53.94%, 8.66% and 6.69% samples were observed to be 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 suspected host that it fed to sustain its life, through the blood meal analysis technique. Data are illustrated in Table 2, Figure 23.

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 Feed	Goat	Unknown
HH	104	31.73%	51.92%	4.81% C+G	2.88%	8.65%)
CS	274	1.82%	88.69%	-----	4.74%	4.74%
MD	131	7.63%	80.92%	5.34% H+C	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



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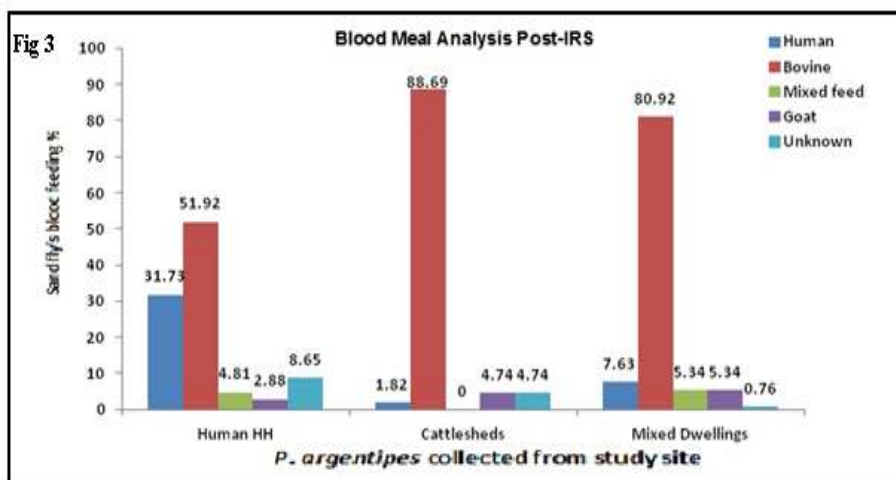


Figure 3. Graph illustrating the result of Blood Meal Identification of *P. argentipes* collected from the study site during Post-IRS session.

Figure 2. Graph illustrating the result of Blood Meal Identification of *P. argentipes* collected from the study site during Post-IRS session.

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 Figure 34.

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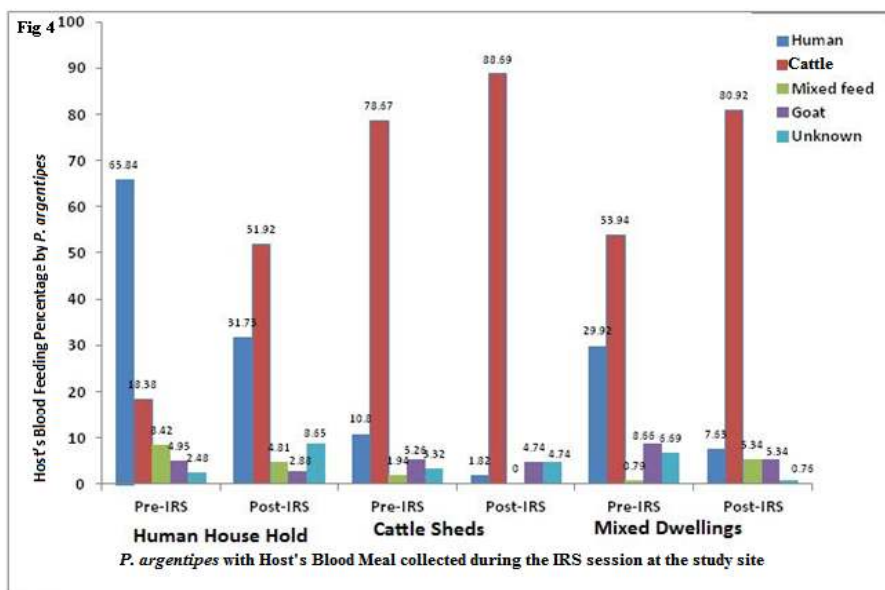
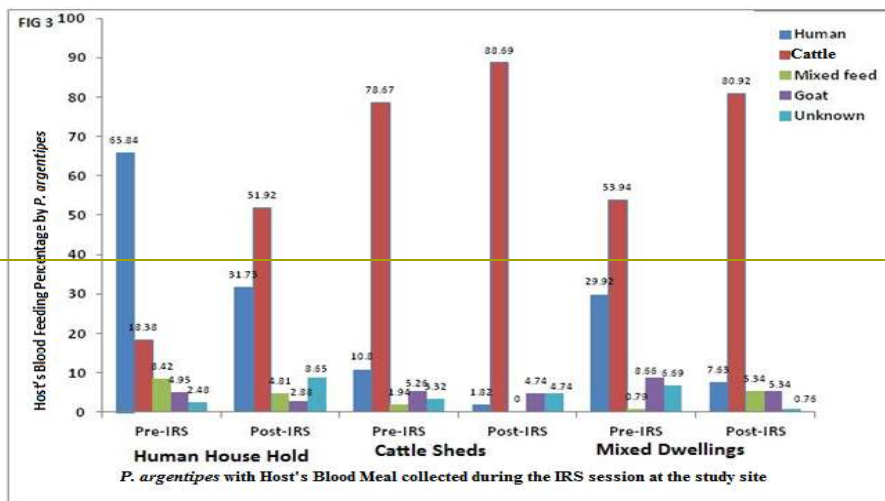


Figure 4. Results of Comparative analysis of Host's Blood Meal Identification of *P. argentipes* collected before and after IRS session.

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

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

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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 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 Figure 45.

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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

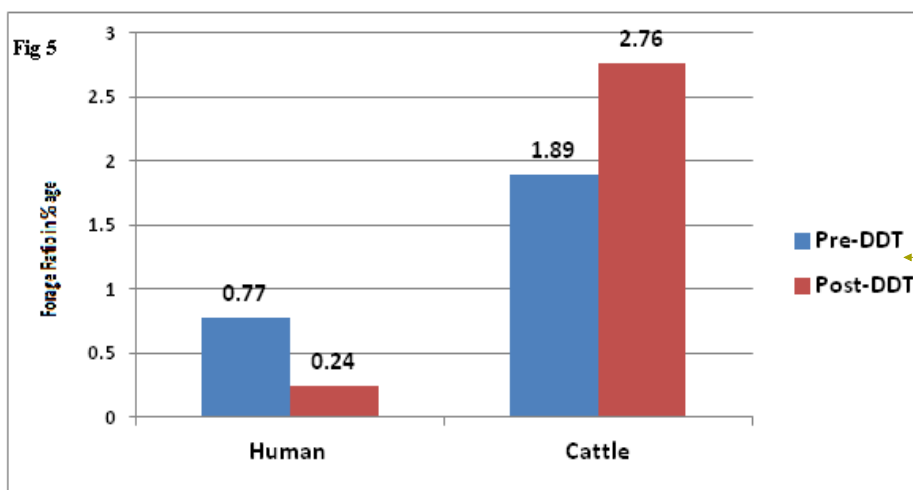
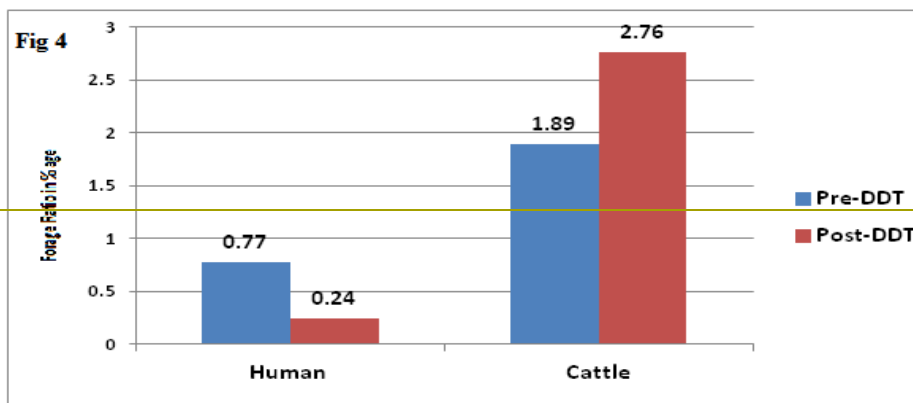


Figure 55. Graphic illustration for the Forage ratio analysis of *P. argentipes* collected from the study site during pre- and post-IRS session.

Figure 4. Graphic illustration for the Forage ratio analysis of *P. argentipes* collected from the study site during pre- and post-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 on the human host before IRS while a significant drop in feeding percentage i.e., (9.43%) was observed after the IRS. In case of cattle host, 56.06% female insects fed on the 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 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.

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

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variation between them. Thus, The study demonstrated that *Phlebotomus argentipes* did not have an equal preference for each host species during pre-DDT spray ($pP < 0.05$) and post DDT spray ($pP < 0.005$). 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 results also revealed maximum numbers of blood meals were counted-obtained from the bovine population in comparison to goat ($pP < 0.003$) and mixed feeding ($pP < 0.004$) 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, 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.

4. DISCUSSION

Limited study and searsescarce documentation confine our knowledge regarding the host preference and selection behavior of *P. argentipes*. Very few reports on the host-meal analysis concerning vector's behavioral adaptation, enforced-followed by the continuous application of exposure of insects against Indoor Residual Spray (IRS) were available reported. 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, 17, 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] The result of previous report from different biotopes of kala-azar endemic regions of India showed that *P. argentipes* is somewhat anthropophilic [10] but had also taken a significant proportion of blood meals from cattle (51.81%, 58.18% and 58.27% for 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 study-report is that the sand fly being zoophilic [10], prefers cattle blood over the human blood, however. 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 shows-showed the nature of Opportunistic-opportunistic anthropophilic nature i.e., 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.*

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336 *argentipes* i.e., humans and cattles at CSs and MDs, vectors tends to incline towards their
337 preferred host and exclusively fed their blood, regardless host availability. One of the
338 inference can be drawn from the study is that the sand fly prefers cattle blood and takes the
339 human blood incidentally.

340 The possible reason behind the above inference could be the ~~liveliness~~ of IRS, which is
341 limited within the range of human habitat. Human households are mainly targeted under the
342 protocol of IRS which significantly led to the development of tolerance against DDT [21, 22,
343 23] encourages the behavioral transition and host preference. Under the impact of IRS,
344 insects migrate from the periphery region of the sprayed houses towards the nearby
345 unsprayed localities viz., bushes, cattle sheds, mixed dwellings etc, for taking rest and
346 shelter during the day time. After the dusk, when insect approaches host for feeding purpose
347 it encounters cattle i.e., their preferred host, lying in the unsprayed area rather than the
348 human host at the sprayed area. However, the sand fly collected from HH before IRS
349 showed the higher degree of preference for human blood in comparison to the sand flies
350 collected in the post IRS era in the same locality. This indicates higher HBI during pre IRS as
351 reported ~~by the early in previous authors studies~~ [24]. Study reports ~~of the~~ high degrees of
352 anthropophily of sand flies often refer to indoor collections of sand flies, where the sand flies
353 are most likely to encounter only humans. Thus, a strong bias for a particular host can occur
354 when circumstances limit host choice. In addition, the species has an inherent opportunistic
355 feeding behavior, small number of *P. argentipes* collected from the periphery of the
356 households after IRS shows tremendous suppression of their anthrophilic behavior and
357 expression of zoophilic nature under the impact of IRS.

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

364 The further analysis of our result showed during the pre-IRS, multiple feeding was observed
365 from human-goat and cattle-goat only whereas during the post-IRS, multiple feeding was
366 found from the human-cattle and cattle-goat in the biotopes of study site. It may be due to
367 the migration of sand flies towards outside after the insecticide spraying. ~~This-The~~ finding
368 suggests for the better control of the vector, the periphery area surrounding the ~~human-host~~
369 dwellings should also be sprayed with insecticide ~~properly in order to get supportive results~~.
370 FR values gave significant results with a very high value of cattle (i.e., cattle and goat) and
371 relatively low values for humans. This information suggests that they are preferred cattle in
372 respect to humans. Our data seem to confirm the previous observations on the attraction of
373 *P. argentipes* to cattle. The enhancing host selection tendency and host avoidance tendency
374 of *P. argentipes* for cattle and human hosts respectively under the influence of IRS indicate
375 the probable reason for the ~~the~~ deviation and migration of insects from the human hosts at
376 the periphery area of sprayed houses towards the cattle-sheds in search of their preferred
377 hosts.
378

Comment [c20]: Meaning?

Comment [s21]: Over here 'liveliness' refer to the effectivity. If suits unsuitable, Can be replace with term 'effectivity'.

Comment [c22]: Meaning?

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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Comment [c23]: Your conclusion should be based on your own findings

Comment [v24]: We had performed the Susceptibility & resistivity test by following the WHO protocol for testing resistivity & sensitivity [WHO, Report of WHO informal consultation, WHO, Geneva, Switzerland, 1998] of *P. argentipes* & through the test we had observed 40-60% mortality at the study site during the post IRS session. Unpublished data of Kumar et al. Its result had not been cited in the present report. Also availability of sand flies at the IRS treated places also supplements & confirms the resistance development among the sand flies. Thus, we can conclude the development of resistance in *P. argentipes*.

Comment [s25]: New References were cited supporting the statements

Comment [s26]: New References were cited supporting the statements

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474

475 ACRONYMS AND ABBREVIATIONS

476

477 IRS: Indoor Residual Spray

478 DDT: Dichlorodiphenyltrichloroethane

479 HFI: Host Feeding Index

480 FR: Forage Ratio

481 BMI: Blood Meal Identification

482 VL: Visceral Leishmaniasis

483 HH: Human Household

484 CS: Cattle shed

485 MD: Mixed Dwelling

486 PHC: Public Health Centre

487