Original Article

Spatial distribution of *P. argentipes* in association with agricultural surrounding environment in North Bihar, India

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Abstract

Introduction: *Phlebotomus argentipes* is considered to be one of the major control measure targets in eradicating visceral leishmaniasis (VL). This study demonstrates the spatial association of *P. argentipes* abundance in relation to agricultural environment in the endemic area of north Bihar.
Methodology: Vector data were collected from the 95 villages of the study area using a handheld aspiration technique. Space technology and ground observationwere made to estimate the environmental characteristics.
Results: A total of 1,663 *P. argentipes* were collected between April and November 2011. Results showed negative and significant association between *P. argentipes* density and proximity to the agricultural land in both the dry ($r = -0.39$) and wet seasons ($r = -0.55$), respectively. A strong and positive association was observed between relative humidity and *P. argentipes* abundance ($r = 0.55$). Results illustrated a very strong positive association between soil moisture and *P. argentipes* abundance ($r = 0.58$). The agricultural land class density and the spatial abundance of *P. argentipes* showed a very strong and positive association ($r = 0.46$).
Conclusions: This study will help in understanding the advantage of agricultural land use practices to delineate of *P. argentipes* habitat suitability, which may strengthen existing control strategies in this endemic area.

Introduction

Visceral leishmaniasis (VL), also known as kala-azar, is caused by the protozoan parasite *Leishmania donovani* in India. It is one of the foremost public health problems in India, especially in Bihar, and has been endemic for several decades. However, in India, more than 90% of cases are reported from the state of Bihar alone [1]. Muzaffarpur district is a predominantly agricultural developed region of Bihar located in the Lower Gangetic plain. During the period of 2004-2008, a total of 15,973 registered cases were reported from the Muzaffapur district, with an annual average of 3,993 cases [2]. The highly affected public health centres (PHCs) are Sahebganj, Motipur, Bochhan, Paroo, and Minapur.

Various programs to control the disease have been unsuccessful despite extensive work having been done on various aspects of the disease [3,4]. The efficient management of leishmaniasis transmission risk is predominantly accomplished through chemical, biological, and/or environmental methods of sand fly control. Earlier studies have suggested that the vector is highly influenced by environmental and socio-economic factors [5-7]. It is reported that agricultural practices are a major determinant of leishmaniasis transmission [8], and could cause proliferation of *Leishmania* spp. [9-11]. Moreover, farming of certain crops generates a seasonal need for transitory agricultural workers, often during or just after the rainy season, when transmission of leishmaniasis is most severe [12,13]. It has been shown that the association of human dwellings with a large scale of cultivated land and excessive irrigation can increase the incident of vector-borne disease [14]. However, there is little information about the habitat of *P. argentipes*. Remote sensing (RS) and geographic information system (GIS) technologies can record and analyze the environmental variables of the earth's surface by its synoptic view and produce digitally processed maps to illustrate sand fly ecology [15].

In this study, we attempted to describe the spatial distribution of *P. argentipes* abundance associated with the surrounding agricultural environment. The outcome of the study may provide important information about the breeding ecology of *P.argentipes* in the Indian subcontinent and also help to strengthen the integrated vector control program in the VL-endemic region of Bihar, India.

Methodology

Study area

This study was conducted in rural areas of 14 PHCs in the Muzaffarpur district in north Bihar (Figure 1). The district lies between the latitudes 25º54'00" N and 26º23'00" N and longitudes 84º53'00" E to 85º45'00" E and covers an area of 3,112 km². The district is bound on the north by Purba-champaran and Sitamarhi districts, on the south by the districts of Darbhanga and Samastipur, and the west by Saran and Gopalganj districts. The total population of the district is 4.78 million per the 2011 Indian decadal census (www.census.india).

The district has three distinct seasons: summer (March-June), winter (November-February), and the rainy season (July-October). January is the coldest (lowest temperature < 5°C) month, while April and May are the hottest months (maximum temperature >42ºC). Rainfall is characterized by a monsoonal regime, with 75%-80% of the total annual rainfall concentrated in the months of July to October. Most of the study area is characterized by intensive agricultural practices; rice, maize, sugar cane, pulse, wheat, and tobacco are the most common crops. Mango, litchi, banana, and bamboo crops are also predominantly disseminated in the study area.

Sand fly trapping

For the present study, samples were collected from the indoor resting sites of *P. argentipes* (human dwellings and cattle sheds) at different distances from the agricultural land. A total of 95 villages were surveyed randomly across the district. The sand flies were collected once every month using a handheld aspiration technique and three-cell torch light between April and November 2011. In each village, ten households were selected randomly for vector collection. In this study, only *P. argentipes* density was considered for the analysis, as it is a proven vector of Indian kala-azar [16]. However, the vector density (man hour density, MHD) was calculated following the formula suggested by Kumar *et al*. [17]. The average value of MHD of ten houses was used to

Figure 1. Location map of the study area and sample collection sites
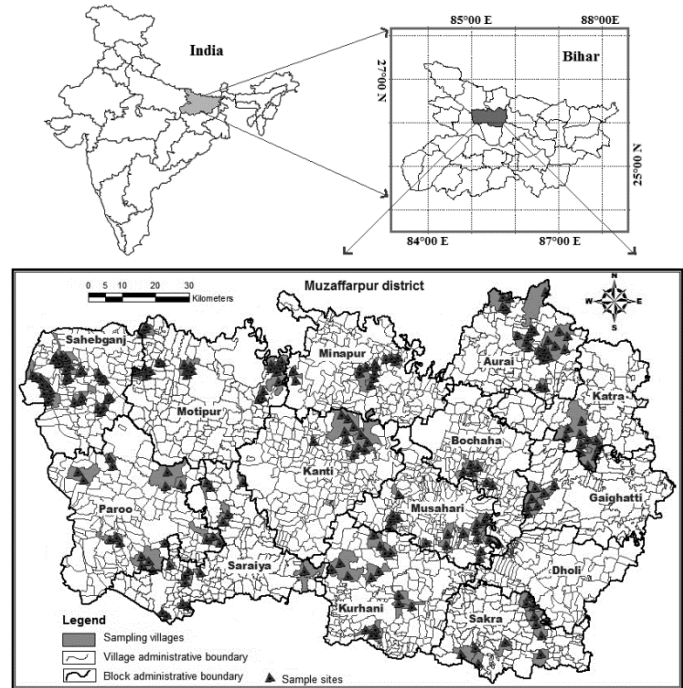
estimate the sand fly density of the respective villages for further analysis.

Climatological data collection

The indoor climatic data (temperature and relative humidity) was recorded simultaneously from each sand fly collection site using Polymeter (Barigo, Mod-305, Germany).

Estimation of soil moisture and soil pH measurement

Soil moisture and soil pH were measured at each vector collection site. Soil moisture was estimated using a digital soil moisture measuring instrument (Model-PMS: 714; range 0-50%; resolution 0.1%) from a depth of three to six centimetres from the earthen floor. To estimate the soil moisture, a minimum of four sites were chosen from each place, and the average value was incorporated for final analysis. To estimate the soil pH, a digital soil pH meter (accuracy ±0.1 pH; range 0.0-14.0 pH) was used, and the soils were collected from a depth of six to ten centimeters from the surface.

Remote sensing data acquisition and analysis

Landsat 5 Thematic Mapper (TM) sensor satellite data (Path/Row 141/042, date of pass 22 October, 2009) with a spatial resolution of 30 meters and seven spectral band with repetivity of 16 days (98.9 minutes

per orbit) and 8-bit quantisation were used. The topographical sheet was also collected from the Survey of India (SOI) office, Patna, Bihar. The topographical sheet was rectified based on the Universal Transverse Mercator (UTM) projection system (zone 45N and datum WGS-84) using a second-order polynomial algorithm and the nearest neighbor re-sampling method.The satellite data and administrative boundary layer was also geo-referenced based on the topographical sheet. To discriminate the area of agricultural land use, a satellite-based digitally processed land use and land cover (LULC) map of Muzaffarpur district was generated. The district was classified into eight LULC classes (Figure 2). ENVI software version 4.7 was used to generate the LULC through a supervised classification technique using the spectral angle mapper (SAM). Classification accuracy was assessed using an error matrix table by estimating the user accuracy and producer accuracy. Kappa statistics were used to measure map accuracy [18-19]. The class density of agricultural land was calculated based on total agricultural area divided by total area of the respective village [20].

Database generation and mapping

The district and village boundary layer was generated based on the heads-up digitizing method using ArcGIS version 9.3. To map the spatial distribution of the *P. argentipes*, the location of villages was geo-referenced with a global positioning system (GPS) (GARMIN 76CSX model) with an accuracy ±10 meters for horizontal and ±15 meters for vertical. Finally, a database was generated on the geographical information system (GIS) platform on the location of the villages based on their respective MHD.

Statistical analysis

Pearson's correlation coefficient test and linear regression analysis was performed to measure the association between the density of sand flies and indoor climate, soil moisture, and soil pH. Descriptive statistics of each environmental parameter were also calculated. All statistical analyses were performed using SPSS software version 16.0 (SPSS Inc, Chicago, IL, USA) at < 0.05 significance level.

Results
Sand fly trapping

A total of 1,663 *P. argentipes* were collected from the 95 villages. Of these, 1,097 (66.0%) were females and 566 (34.0%) were males. A total of 68.8% of

Figure 2. Land use land cover (LULC) map of the study area. The LULC map is derived from Landsat TM satellite data
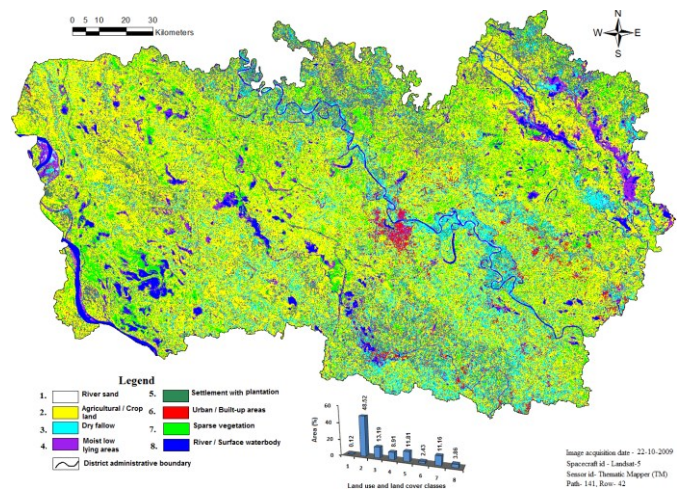
female *P. argentipes* (average MHD 8.7) was recorded from human dwellings; 31.3% was recorded from the cattle shed (average MHD 5.3). The recorded percentage of male *P. argentipes* was almost same in both human dwellings and cattle sheds. The lowest numbers of *P. argentipes* were collected in the month of April (average MHD 2.7). The sand fly population increased between July and September, with the peak occurring in the month of October (average MHD 12.8), and decreasing in the month of November (Table 1). In the dry season (April to July), the *P. argentipes* density in the study villages varied from 0.67 to 14.3 MHD (mean ± standard deviation: 4.3 ± 2.7) and in the wet season (July to November), the *P. argentipes* density ranged from 0.6 to 27.1 MHD (mean ± standard deviation: 8.5 ± 5.7) at the study sites.

Indoor climate vs. P. argentipes distribution

The relative humidity (RH) of the study villages ranged from 46% to 81% (mean ± SD: 58.6 ± 6.6) during the dry season (April to June), and in the wet season (July to November), it ranged between 57% and 84% (mean ± SD: 69.8 ± 5.6). A significant positive association was observed between relative humidity and *P. argentipes* abundance ($r = 0.55$, p < 0.0001). In the study area, the temperature (RT) varied between 27.2°C and 36.2°C (30.3 ± 1.8) in the dry season; in the wet season, temperatures ranged from 24.0°C to 35.8°C (28.3 ± 2.1). A significant and positive relationship was found between room temperature and *P. argentipes* density ($r = 0.14$, p< 0.04). During the sand fly abundance in October, the mean temperature and mean relative humidity was

recorded as 29.1°C (95% confidence interval – 26.8° C to 31.7°C) and 72.6% (95% confidence interval – 62% to 81%), respectively (Table 1). The recorded sand fly abundance was lowest in April, when the mean temperature and relative humidity was 33.8°C (95% confidence interval – 28.3°C to 36.2°C) and 55.7% (95% confidence interval – 46% to 77%), respectively.

Soil factors vs. P. argentipes distribution

Soil moisture of the study area varied from 16.0% to 30.2% (mean ± S.D:24.1 ± 2.8) in the dry season, while in the wet season, the soil moisture ranged between 21.6% and 36.1% (mean ± S.D:27.8 ± 2.2). Pearson's correlation test showed a significant and positive association between soil moisture and abundance of *P. argentipes* ($r = 0.58$, p< 0.0001). Soil pH of the study area ranged from 5.9 to 8.6 (7.2 ± 0.5) in the dry season and 6.5 to 8.9 (7.3 ± 0.4) in the wet season. A significant and positive relationship was found between the soil pH and *P. argentipes* abundance ($r = 0.38$, p < 0.0001). The results also illustrated that the alkaline character of soil (pH value of > 7) was positively correlated with *P. argentipes*

abundance ($r = 0.29$, p < 0.000).

Location of human dwelling at different proximity to agricultural land and P. argentipes distribution

The sand fly abundance was estimated at different distances in relation to agricultural land in both the dry and wet seasons (Table 2). The result of the analysis showed an inverse relationship between *P. argentipes* density and proximity to agricultural land. In both seasons, the recorded sand fly density (5.3 MHD and 13.0 MHD in dry and wet seasons, respectively) was higher at distances of less than 10 meters from the surrounding agricultural land. However, the abundance of *P. argentipes* became nil or very small in indoor resting places within a 50-meter distance from the agricultural land in both seasons. A simple linear relationship was drawn to estimate the role agricultural land in *P. argentipes* abundance. The result of the analysis showed a negative and significant association between *P. argentipes* density and proximity to agricultural land in both the dry season ($r = -0.39$, p = < 0.000) and wet season ($r = -0.55$, p< 0.000), respectively.

Table 1. Monthly abundance of *P. argentipes* and climatic factors (relative humidity and room temperature) recorded during the study period between April and November 2011

Month	Number of *P. argentipes* (%)	Man Hour Density(MHD)	Relative humidity (RH) (%)			Room temperature (RT) (°C)		
			Mean	Maximum	Minimum	Mean	Maximum	Minimum
April	73 (4.4)	2.7	57.1	77	46	32.2	36.2	28.3
May	89 (5.4)	4.0	59	81	51	31.6	34.1	29.5
June	126 (7.6)	5.3	61.6	79	47	30.4	35.5	27.2
July	189 (11.4)	8.9	73.8	80	68	29.7	35.8	27.4
August	295 (17.7)	10.4	69.9	84	66	27.9	31.4	26.6
September	302 (18.7)	10.7	71.4	82	61	28.8	30.5	27
October	416 (25.0)	12.8	72.6	81	62	29.1	31.7	26.8
November	173 (10.4)	7.2	68.0	78	57	27.2	29.1	24

Table 2. Spatial distribution of *P. argentipes* in relation to proximity to agricultural land in dry and wet seasons

Distance (m)	Dry season			Wet season		
	MHD of*P. argentipes* (mean ± SD)	Number ofSand flies (male : female ratio)	Positive site (observed site)	MHD of *P. argentipes* (mean ± SD)	Number of sand flies (male : female ratio)	Positive site (observed site)
<10	1.4-14.3 (5.3 ± 3.0)	124 (1:1.7)	37 (42)	2.9-27.1 (13.0 ± 5.4)	713 (1:1.8)	46 (50)
10-20	1.4-10.1 (4.7 ± 2.5)	108 (1:1.1)	31 (38)	2.8-18.6 (8.5 ± 4.0)	398 (1:1.8)	37 (45)
20-30	1.2-5.7 (3.0 ± 1.6)	39 (1:1.2)	16 (35)	1.32-20.51 (5.8 ± 3.8)	109 (1:1.1)	25 (35)
30-40	1.1-5.8 (2.0 ± 1.6)	14 (1:1.1)	9 (35)	1.6-13.4 (4.7 ± 3.2)	111 (1:0.8)	26 (35)
40-50	0.7-1.3 (1 ± 0.5)	03 (1:0.5)	2 (25)	0.6-6.0 (1.7 ± 1.2)	44 (1:0.9)	21 (25)
>50	0	00.0	0 (25)	0.7-4.2 (1.9 ± 2.0)	4 (1:0.3)	3 (25)

S D= standard deviation; MHD = Man Hour Density

*Agricultural land class density and P. argentipes distribution*

A LULC map was generated from the Landsat TM data (Figure 2). The following LULC classes were considered in this study: river/surface water, sand, cropland, dry fallow, settlement with plantation, urban and built-up area, and sparse vegetation. The results also showed that 48% of the study area was surrounded by agricultural land; however, an unequal distribution of agricultural land was allotted among the study villages. Map accuracy of classified features was calculated and it produced an overall accuracy rate of 90.5%. A Kappa co-efficient value of 0.9 indicates that the classification is 90% better than a classification that resulted from random assignment. The agricultural land class density and the spatial abundance of *P. argentipes* had a significant positive relationship (r = 0.46, p= 0.021). The spatial distribution map of *P. argentipes* density in relation to agricultural land class density also illustrated that the areas covered by high agricultural density were closely associated with higher *P. argentipes* density (Figure 3).

Discussion

Studies on environmental factors in respect to VL occurrence and vector abundance have been conducted by different researchers and scientists [5,21]. Several studies have been conducted about agricultural land use and emergence or re-emergence of vector-borne disease on national and international levels [22,23]. Our results showed a significant association between climatic factor and *P. argentipes* abundance, which had also been found in a previous study [15]. A significant association was established between sand fly density and soil pH of this study region. In an earlier study conducted by Singh *et al.*, [24], *P. argentipes* preferred to breed in neutral or near-neutral soil in households and in alkaline soil in cattle sheds. In our study, 85% of *P.argentipes* was recorded in soil with a pH of more than 7.0. Moreover, high moisture of soil was found to be significantly associated with sand fly density. This finding corroborates that of other studies [5,25].

The Muzaffarpur district of Bihar is an unforested region; most of the area is irrigated agricultural land. The spatial distribution of *P. argentipes* was found among all villages across the district, and female populations were found to be more prevalent than male populations. In our study, we found a significant positive association between the agricultural land density of the villages and the abundance of *P.*

Figure 3. Association between agricultural land and P. *argentipes* density
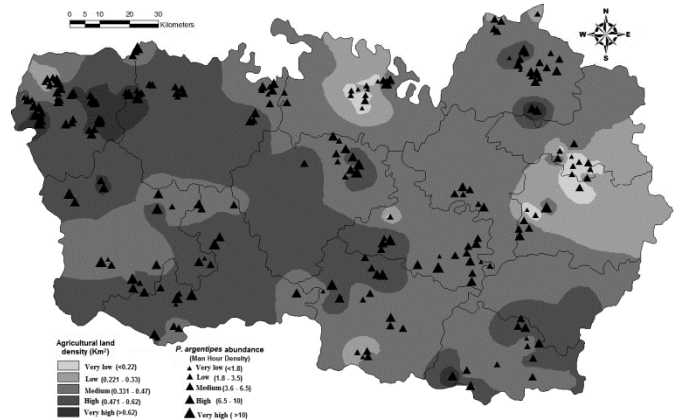
argentipes. Because the large scale of agricultural land use in the villages is spread out in a well irrigation network for seasonal cropping, moisture may be retained in the sub-soil, and high humidity exists in its surrounding areas. Water management in the irrigation schemes, the preference of crop and crop varieties, cropping patterns, and chemical inputs may influence vectorial capacity. Our results also showed that the abundance of *P. argentipes* was found most often in houses fewer than 10 metres from the agricultural and crop land. As the distance from agricultural land increased, the abundance of *P. argentipes* density decreased. Possible reasons for this were: the crop phagostimulant chemical may have attracted male sand fly species; the optimal soil moisture and high relative humidity may have been favored as a breeding site; or, the area was a resting place of the species. Our results also showed that the female population was slightly higher nearer the agricultural land compared with the male population. This could be because the *P. argentipes* is relevant to endophilic flies, which breed inside the living rooms or cattle sheds [26,27]; conversely, male sand flies go outside to penetrate and imbibe plant sap [28].

Knowledge of the seasonal activities of the sand fly and the link to the surrounding agricultural environment of the region may aid in determining the breeding status of sand flies. This study could serve as a good pilot study for future research, and for integrated vector control management, which may have an impact on the epidemiology of VL, especially in the Indian sub-continent. Further research is required to understand the role of agricultural environment (*i.e.* seasonal cropping patterns, outdoor collection of vectors, etc.) in relation to *P. argentipes* habitat suitability.

Acknowledgements

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