A comparative evaluation of endemic and non-endemic region of visceral leishmaniasis (Kala-azar) in India with ground survey and space technology

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In visceral leishmaniasis, phlebotomine vectors are targets for control measures. Understanding the ecosystem of the vectors is a prerequisite for creating these control measures. This study endeavours to delineate the suitable locations of Phlebotomus argentipes *with relation to environmental characteristics between endemic and non-endemic districts in India. A cross-sectional survey was conducted on 25 villages in each district. Environmental data were obtained through remote sensing images and vector density was measured using a CDC light trap. Simple linear regression analysis was used to measure the association between climatic parameters and vector density. Using factor analysis, the relationship between land cover classes and* P. argentipes *density among the villages in both districts was investigated. The results of the regression analysis indicated that indoor temperature and relative humidity are the best predictors for* P. argentipes *distribution. Factor analysis confirmed breeding preferences for* P. argentipes *by landscape element. Minimum Normalised Difference Vegetation Index, marshy land and orchard/settlement produced high loading in an endemic region, whereas water bodies and dense forest were preferred in non-endemic sites. Soil properties between the two districts were studied and indicated that soil pH and moisture content is higher in endemic sites compared to non-endemic sites. The present study should be utilised to make critical decisions for vector surveillance and controlling Kala-azar disease vectors.*

Key words: *Phlebotomus argentipes* - Kala-azar - CDC light trap - RS image - NDVI - landscape

Visceral leishmaniasis (VL) is a tremendous public health issue caused by the intracellular protozoan *Leishmania* parasite, which is transmitted by phlebotomine sandflies. The World Health Organization (WHO 2008) recently reported on the diversity of this disease. This report stated that 2.4 million new cases of leishmaniasis occur each year and 367 million people are at risk in 88 countries, with 12 million people currently infected. The annual incidence of VL is estimated at 500,000 cases per year worldwide with 40,000-80,000 deaths a year (Hussein 2004). At present in India, Bihar (31 out of 38 districts), West Bengal (10 out of 24 districts), Uttar Pradesh (4 districts), that border Bihar, and a small portion of the Jharkhand district are endemic at varying levels. In the Indian sub-continent, VL, also known as Kala-azar, is caused by *Leishmania donovani* transmitted by *Phlebotomus argentipes* (Swaminath et al. 1942). These insects prefer warm climates and are generally abundant during monsoon season (Sharma & Singh 2008). The Vaishali district in Bihar is the most affected by this disease, with more than 2,000 of cases derived from this district over the last five years. The fluctuation in the number of VL reported cases in the last 30 years in Vaishali can be linked to ineffective control strategies for *P. argentipes*. Additionally, due to a lack of knowledge of the ecology of *P. argentipes*, control strategies are not being conducted properly because *P. argentipes* is constantly changing foci and establishing new areas of disease. Hence, understanding the ecology of the sandfly is of the upmost requirement for successfully implementing a control strategy.

Presently, there is a dearth of scientific information defining the ecosystem of sandflies. Analysing the distribution of leishmaniasis (i.e., its identification, standardisation, and location) along with monitoring environmental variables with conventional ground surveys is time consuming, laborious and expensive. With increasing accessibility to new technologies, such as remote sensing (RS), it is possible to monitor land-use features on the earth's surface over various time intervals to develop methods for rapid stratification of highly susceptive areas and to design remedial measures (Bakker et al. 2000, De La Rocque et al. 2004, Mushinzimana et al. 2006, Bhunia et al. 2010a). Another recent development is the use of satellite data to acquire increasing amounts of geographical data available in conjunction with geographic information systems (GISs) to assist with interpretation of ecosystems and environmental parameters related to sandfly-genic conditions (Sharma & Srivastava 1997, Bergquist 2001, Moore 2008, Bhunia et al. 2011). With increasingly

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widespread and combined implementation of RS and GIS technology, professionals have been provided with efficient and accurate tools for mapping, maintaining and managing information on landscape features to further our understanding of the epidemiology for the purpose of controlling of VL in India (Palit et al. 2001, Sudhakar et al. 2006, Bhunia et al. 2010b, 2011).

To delineate the suitable habitats of the VL vector, *P. argentipes* density in relation to environmental characteristics between different ecosystems was assessed in endemic (Bihar) and non-endemic (Jharkhand) Indian states.

MATERIALS AND METHODS

Study area - Cross-sectional descriptive studies were carried out in two different ecological regions of two states of India. Vaishali (25º43'N 26º00'N; 85º04'E 85º38'E), which had the highest prevalence of Kala-azar cases, was selected as the representative endemic site. The Lohardaga district, Jharkhand (23º30'N 23º40'N; 84º40'E 84º50'E) with almost no Kala-azar cases over the past decades, was selected as the representative non-endemic site (Fig. 1). Data on sandfly distribution, frequency and biology (Kesari et al. 2010), as well as on the sampling areas of Lohardaga and Vaishali (Kumar et al. 2009), have been reported. Similar to other regions in the upper Gangetic plain, the climate of Vaishali is humid and tropical. The summers are hot (maximum temperature ~45ºC), whereas the winters are cold (minimum temperature ~6ºC). The region is a relatively flat plain with no relief. The principal soil type found throughout the district is alluvial and alkaline (pH 7.0-8.3). The total estimated annual rainfall in this area is 2,500-5,500 mm. The humidity ranges from 50% (winter) to 95% (rainy and summer seasons).

The non-endemic district (Lohardaga) is a hilly plain with abundant relief in the form of scattered hills and seasonally flowing watercourses. The principal soil found throughout this area is laterite and acidic (pH -5.7 to -6.1). The climate of this area is dry and sub-tropical with an estimated annual rainfall of 900-1,100 mm.

Twenty-five villages in each district were selected randomly from different cardinal locations (East, West, North, South and centre) for two reasons: (i) to garner knowledge about the vector distribution for the study sites and (ii) to understand the influence of local ecological parameters on vector distribution.

RS and collateral data - RS data for this study included data from the Indian RS 1D (IRS-1D) satellites, which maintain near polar, sun-synchronous orbits. Images were acquired nominally at 10.30 am local time on a descending path. The orbit altitude was 817 km and provided 25 days of repetition (358-orbit cycles). It has three payloads: panchromatic, Linear Imaging Self Scanning-III (LISS-III) and Wide Field Sensor. LISS-III scenes with Date of Pass (DoP) for Vaishali were collected on 11 November 1999 and 15 March 2000, with Path/Row 105/53, 105/54. The DoP for Lohardaga was on 3 January 2000 and 23 March 2000 with Path/Row 104/55, 105/55. Resolution for visible and near infrared region (21.2-23.5 m) and resolution in shortwave infrared region (63.6-70.5 m) were used in the present study. The entire scenes were registered to a Universal Transverse Mercator co-ordinate system (zone 45N and datum WGS-84) based on second order polynomial algorithm and nearest neighbour re-sampling method. Mosaics were created from two satellite scenes covering the said districts for each site and the study area was cropped based on their administrative boundary from the mosaic image.

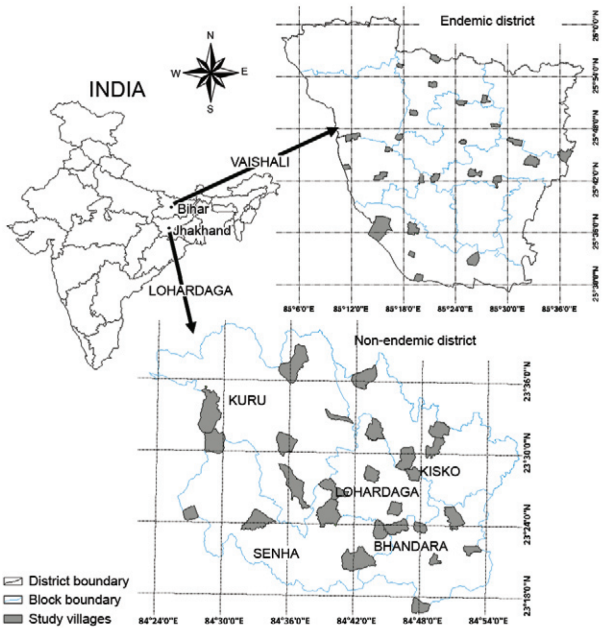

Fig. 1: location map of the study area.

The block boundary map of both districts was scanned and registered with the satellite data. The village boundary layer for each site was also digitised and vector layers were generated.

Ground data collection - Ground data were recorded from October 2007-July 2009 in the endemic area and from February 2008-October 2009 in the non-endemic site. Twenty-five villages from each district were randomly selected from different cardinal locations (East, West, North, South and centre) to perform this research work. In each village from the endemic and non-endemic sites, 10 houses were randomly selected and were sampled both indoors (living rooms) and/or outdoors (e.g., cattle sheds) to collect sandflies using a CDC light trap (Table I). Sandfly collection was made between dusk and dawn (06.00 pm-06.00 am). The female sandflies collected were confined for egg laying in a Hilton's pot. Dead sandflies were preserved in 70% alcohol and transported to the laboratory. Sandfly number, type, gender and vector species were also recorded for each site. All species were mounted on microslides using Canada balsam as mounting media (Remaudière 1992). Species identification (Lewis 1978) was also performed.

Data on relative humidity and temperature indoors were recorded from the relevant sites of vector collection by installing a polymer and were compared with weather parameters to evaluate favourable environmental conditions for vector habitation.

TABLE I

Abundance of *Phlebotomus argentipes* in endemic and non-endemic villages

	Endemic site				Non-endemic site		
Villages	Male	Female	Total	Villages	Male	Female	Total
Akiladabad Diara	0	0	0	Hesal	6	3	9
Daulatpur Chandi	5	8	13	Nandinagra	3	0	3
Bishunpur Basant	9	14	23	Nigni	5	9	14
Randaha	11	5	16	Bhunjaniya	0	0	0
Bazidpur	6	8	14	Manho	6	4	10
Harpur Gangaram	18	10	28	Putrar	3	3	6
Bishunpur Balbhadar	4	3	7	Deodariya	5	7	12
Hussainipur	0	0	0	Hesri	7	4	11
Damodarpur	6	9	15	Hutap	0	0	0
Paharpur	9	13	22	Kekrang	7	4	11
Dhudhua	3	7	10	Porha	0	0	0
Gangapur Lachhmi	12	8	20	Pajhri	6	4	10
Kajri Bhath	7	5	12	Hati	0	0	0
Soharthi	4	3	7	Kanjo	9	3	12
Pirapur	3	9	12	Khanda	5	8	13
Bhanpur Barewa	9	14	23	Dhawra	0	0	0
Mahammadpur Daria	4	6	10	Kharta	10	8	18
Ismailpur	12	13	25	Lawagain	0	0	0
Bakhri Dua	10	5	15	Kuru	1	3	4
Rasulpur	0	0	0	Doba	5	3	8
Sukhwarpur	12	16	28	Bari	3	2	5
Karmupur	7	8	15	Jhaljamira	0	0	0
Mallikpur	5	12	17	Badla	1	3	4
Pirmahammadpur	7	6	13	Nindi	4	3	7
Rampur Karai	4	6	10	Uru	2	7	9

Land use/land covers (LULC) habitat characterisation - LULC maps were produced to identify different classes of land from IRS-1D LISS-III imagery. A supervised classification technique with the Maximum Likelihood algorithm was used to assign the pixels into 10 land cover classes based on the following parameters: (i) their spectral reflectance characteristics, (ii) the author's *a priori* knowledge of the study area, which was guided by research objectives, and (iii) an expected certain degree of accuracy in image classification. To perform accurate classification assessments, an error matrix table was derived that represents a square array of numbers laid out in rows and columns, which expresses the number of sample units (e.g., clusters of pixel) assigned to a particular category relative to the actual category as verified in the field. Kappa statistics were used to measure agreement or map accuracy (Rosenfield & Fitzpatrick 1986, Congalton 1991). All image-processing operations were processed using ERDAS IMAGINE 9.2 software (Leica Geosystems 2008).

Estimation of Normalised Difference Vegetation Index (NDVI) - NDVI is an important vegetation index because it monitors seasonal and inter-annual changes in vegetation growth and activity (Lillesand et al. 2004). An NDVI image was generated from the LISS-III bands [Red $_{\text{Channel 3}}$ and Near infrared (NIR) $_{\text{Channel 4}}$] of IRS-1D (Channel$_{4\ (NIR)}$ – Channel$_{3\ (Red)}$)/(Channel$_{4\ (NIR)}$ + Channel$_{3\ (Red)}$). Channel$_3$ is the reflectance in the visible wavelengths (0.62-0.68 µm) and Channel$_4$ is the reflectance in the reflective infrared wavelengths (0.77-0.86 µm). The index normalises the difference between the bands, so that values range between -1 and +1. Minimum, maximum, mean and standard deviation (SD) of NDVI for each grid square for each village were calculated for each site.

Soil samples - Individual soil samples were collected from five sites in each village from the endemic and non-endemic areas during the ground survey. At each collection type, the soil was dug up to a depth of about 5-6 cm. Each soil sample (at least 200 g was collected in a plastic pot, covered with fine muslin cloth and transported to the laboratory where it was maintained at room temperature (27ºC ± 2) for 45-60 days. Chemical properties were examined to identify favourable breeding characteristics in the soil. The chemical properties of the soil [silicon dioxide (SiO_2) aluminium oxide (Al_2O_3), iron oxide (Fe_2O_3), calcium (Ca), magnesium (Mg), sodium oxide (Na_2O), potassium oxide (K_2O), phosphorous pentoxide (P_2O_5), and titanium dioxide (TiO_2) levels and pH] were employed to assess the breeding ecology of vector *P. argentipes*. Moisture content was determined by the ratio of the weight of water in the soil to the weight of the dry soil.

Statistical analysis - Statistical analysis was performed with SPSS 10.0 software (SPSS Inc, Chicago, IL, USA). Multivariate linear regression analysis was performed to observe the effect of climatic parameters, such as indoor temperature, relative humidity and type of area (endemic/non-endemic) on sandfly density (flies per trap/per night). After processing land cover maps (calculating areas occupied by land cover types) and NDVI computation, data were analysed with the density of *P. argentipes* in SPSS software. Factor analysis (FA) was used to investigate if there was any relationship between land cover classes and the density of *P. argentipes* among different villages in the endemic and non-endemic study sites. FA allows for data reduction and is used to identify underlying variables or factors that explain the pattern of correlations within a set of observed variables. In this study, we were only considering five important variables of LULC classes for both sites due to the small sample size. If we considered the total variables of LULC classes, it might not be possible to obtain significant results. FA generates a table in which the rows are observed raw indicator variables and the columns are the factors or latent variables, which explain the variance in these variables. The cells in this table are factor loadings. The meaning of the factors was deduced from observing which variables were most heavily loaded on which factors. A principal component analysis (PCA) method was used to conduct factor analysis. Eigenvalues are the variances of the factors. PCA can be used for data reduction. Each factor will tend to have either positive or negative loadings of a large or small amount for any particular variable.

RESULTS

Sandfly capture and distribution - From October 2007-July 2009, a total of 355 *P. argentipes* were collected from 25 villages in an endemic site (Vaishali: 47.04% male, 52.96% female). Similarly from villages in the non-endemic site (Lohardaga: 53.01% male, 46.99% female), a total of 166 *P. argentipes* were collected from 25 villages. Sandfly density (flies per trap/per night) in the endemic site varied from 4.56-8.07, whereas in the non-endemic site varied from 2.24-4.89.

Climatic variables and vector abundance - *In situ* indoor temperature and relative humidity for the endemic and non-endemic site were recorded at the time of sandfly collection and considered for risk analysis. Multivariate linear regression analysis was performed to determine the predictor variable for sandfly density. Results showed a significant effect of climatic variables, such as indoor temperature, humidity and type of area (endemic/non-endemic) on vector density (Table II). In this analysis, the non-endemic area was considered as a referent category.

The final model was highly significant (63.27, p value < 0.0001). This finding indicates that these three variables in concert are significant predictors of sandfly density. The adjusted $R^2 = 0.79$, indicates that nearly 79% of the variance of sandfly density could be attributed to these three predictor variables.

Spatial relationship between sandfly abundance and vegetation health (NDVI) - Concerning vegetation health, the NDVI value ranged from -0.24 to +0.44 in the endemic site, while in the non-endemic site, it varied from -0.31 to +0.58. Negative or minimum values for NDVI (0 or < 0) were characterised by non-vegetated land (barren land, water bodies, urban land), while positive or maximum values demarcated vegetated land (e.g., crop land, dense forest). For each village, descriptive statistics for NDVI (minimum, maximum, mean and SD) were calculated (Table III). In general, the maximum NDVI value corresponded with a higher vegetation activity level. Additionally in rice paddies, NDVI values were low at planting time, rose as rice plants grew and dropped again when crops were harvested. The NDVI values were also higher in plantation lands than in agricultural lands. The highest NDVI value corresponds to the zone of minimum suitability. Maximum suitability is recorded in the zones where the vegetation shows the minimum NDVI values (< 0.21).

Spatial distribution of vector P. argentipes in relation to LULC characteristics - LULC characteristics are not similar between endemic and non-endemic sites (Figs 2, 3). Analysis of LULC characteristics within the test villages demonstrated that a majority of the area was covered by agricultural fallow, sparse vegetation, moist fallow land and marshy land in the endemic region (Table IV). In the non-endemic region, a majority of the area was covered with agricultural fallow, dry fallow, crop land and open forest land (Table IV). An accuracy

TABLE II

Significant predictor variables of sandfly density

Predictor variables	Coefficients (βs) (95% CI)	SE(βs)	T statistic	p value
Intercept	-79.75 (-94.40- -65.10)	7.27	-10.96	0.000
Inside room temperature	1.21 (0.68-1.74)	0.26	4.61	0.000
Inside room humidity	0.74 (0.54-0.93)	0.10	7.54	0.000
Area	5.84 (4.13-7.54)	0.85	6.90	0.000

CI: confidence interval; SE: standard error.

TABLE III

Calculation of value of Normalized Difference Vegetation Index (NDVI) endemic and non-endemic site

Region	Mininimum NDVI	Maximum NDVI	Mean NDVI	SD
Endemic site	-0.274	0.441	0.089	0.208
Non-endemic site	-0.313	0.581	0.144	0.263

SD: Standard deviation.

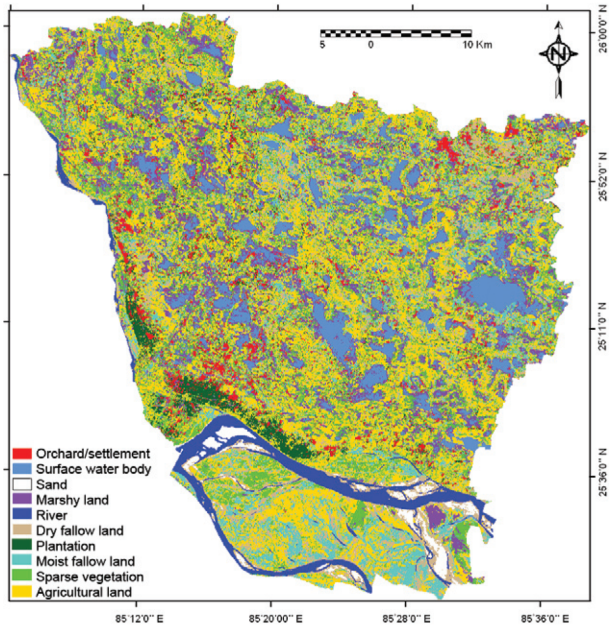

Fig. 2: land use/land cover map of district of Vaishali, India (endemic site).

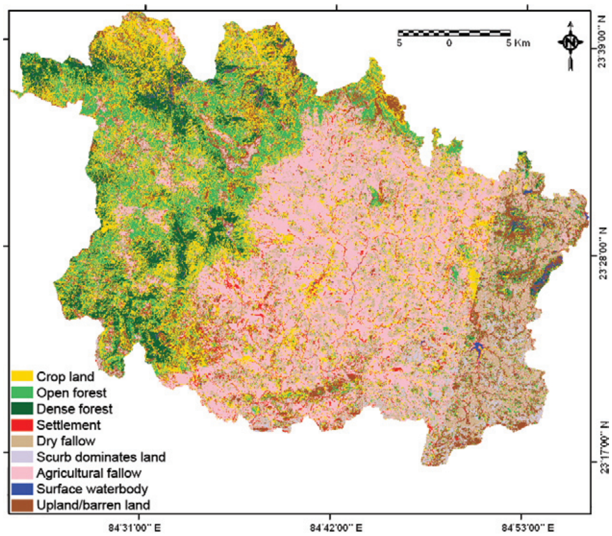

Fig. 3: land use/land cover map of district of Lohardaga, India (non-endemic site).

TABLE IV

Land use/land cover classes of 25 villages and their average percent of area covered in endemic and non-endemic area

Serial number	Land use/ land cover class	Area covered (%)
	District of Vaishali (endemic site)	
1	Orchard/settlement	3.42
2	Surface water body	12.16
3	Sand	0.60
4	Marshy land	10.94
5	River	3.77
6	Dry fallow	4.76
7	Plantation	3.04
8	Moist fallow	11.32
9	Sparse vegetation	16.50
10	Agricultural fallow	33.50

overall classification accuracy: 88%; overall Kappa statistics: 0.855.

Serial number	Land use/ land cover class	Area covered (%)
	District of Lohadaga (non-endemic site)	
1	Crop land	20.36
2	Upland/barren land	8.50
3	Open forest	12.54
4	Dense forest	3.11
5	Water body	0.27
6	Settlement	3.52
7	Dry fallow	11.55
8	Scrub dominated land	2.77
9	Agricultural fallow	37.38

Overall classification accuracy: 86%
Overall Kappa statistics: 0.8105

overall classification accuracy: 88%; overall Kappa statistics: 0.855.

assessment of the results, using additionally known land cover sites as test areas (5 test polygon per class), showed an overall classification accuracy of greater than 85%. Kappa statistics were collected for each land cover category in each study from the public health centres (PHCs) to measure the agreement between the land cover categories. The values of the kappa ranges suggested almost perfect agreement (0.76-0.82) between these land cover classes of an endemic and non-endemic site.

To form our FA analysis dataset, we considered the following variables for the endemic and non-endemic sites: *P. argentipes* density, minimum NDVI, agricultural fallow, water body surface and orchard/settlement. Marshy land and dense forest were also considered for endemic and non-endemic sites, respectively. We conducted FA separately on two different datasets. For both

TABLE V

Factor loading (pattern matrix), extraction method - principal component analysis

Variables	Endemic area		Variables	Non-endemic area	
	Factor 1	Factor 2		Factor 1	Factor 2
Presence and/or absence of *Phlebotomus argentipes*	0.7688	-0.1902	Presence and/or absence of *Phlebotomus argentipes*	0.4580	0.4894
Minimum NDVI	0.6641	-0.3520	Settlement	0.0620	0.8793
Agricultural fallow	-0.5243	-0.3937	Water body	0.5271	-0.6831
Surface water body	-0.2219	0.8478	Dense forest	0.9028	-0.1344
Marshy land	0.6816	0.4151	Agricultural fallow	-0.8227	-0.2896
Orchard/settlement	0.6910	0.1141	Minimum NDVI	0.1533	-0.2315

sign indicates load of variable either positive or negative on factor with their higher or lesser amount. NDVI: Normalised Difference Vegetation Index.

TABLE VI

Test lanes and sample number including chemical properties and water retaining capacity of the soil

Area	Test lanes	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	Na_2O	K_2O	P_2O_5	TiO_2	pH	Water molecules
Endemic	1	41.10	10.76	9.62	8.12	4.61	8.76	2.11	0.02	0.01	7.50	12.45
	2	42.43	11.76	8.96	6.20	3.76	9.24	2.14	0.04	0.00	7.80	15.01
	3	41.90	14.75	8.34	4.76	2.43	8.76	1.76	0.06	0.01	8.10	11.73
	4	41.04	12.66	10.42	5.62	2.42	12.76	2.43	0.08	0.03	8.20	10.12
	5	41.12	14.72	8.34	4.76	2.43	8.76	1.76	0.05	0.01	7.60	11.73
	Average	41.52	12.93	9.14	5.89	3.13	9.66	2.04	0.05	0.01	7.84	12.21
	SD	0.62	1.78	0.89	1.39	1.01	1.75	0.28	0.02	0.01	0.30	1.78
Non-endemic	1	61.22	9.23	12.57	1.23	0.56	2.41	1.83	2.72	1.66	6.18	3.72
	2	62.32	9.43	12.87	1.32	0.58	2.45	1.83	2.76	1.68	6.18	3.80
	3	62.20	9.13	12.77	1.28	0.58	2.30	1.80	2.66	1.56	6.10	3.70
	4	62.00	9.02	12.67	1.22	0.55	2.38	1.81	2.68	1.46	6.05	3.77
	5	61.88	9.40	12.81	1.30	0.57	2.44	1.79	2.58	1.62	6.08	3.75
	Average	61.92	9.24	12.74	1.27	0.57	2.40	1.81	2.68	1.60	6.12	3.75
	SD	0.43	0.17	0.12	0.04	0.01	0.06	0.02	0.07	0.09	0.06	0.04

Al_2O_3: aluminium oxide; CaO: calcium oxide; Fe_2O_3: iron oxide; K_2O: potassium oxide; MgO: magnesium oxide; Na_2O: sodium oxide; P_2O_5: phosphorous pentoxide; SD: standard deviation; SiO_2: silicon dioxide; TiO_2: titanium dioxide.

datasets, we extracted two factors using PCA: group, according to scatter plot and eigenvalue greater than 1.

We found with variables in an endemic site that the density of *P. argentipes* has a positive load on minimum NDVI, marshy land and orchard/settlement, whereas agricultural fallow and water body surface have a negative load on the first factor in an endemic site (Table V). For the second factor, it was found that water body surface has a high load, while marshy land and orchard/settlement have a lesser load. The density of *P. argentipes*, minimum NDVI and agricultural fallow have a negative load.

In the non-endemic site, results demonstrated that the density of *P. argentipes* has a positive load on settlement, water body, dense forest and minimum NDVI, whereas agricultural fallow has a negative load on the first factor. It was observed that *P. argentipes* density and settlement have a positive load on the second factor, whereas water body, dense forest, minimum NDVI and agricultural fallow have a negative load.

Soil properties of the endemic and non-endemic sites - Estimation results from the chemical analysis of soil in the endemic and non-endemic foci were highly variable. Results indicate that Al_2O_3, Na_2O, K_2O, Ca and Mg are higher in the endemic site, whereas SiO_2, P_2O_5 and TiO_2 are higher in the non-endemic site (Table VI). The pH of the soil in breeding locations was alkaline in nature (range 7.50-8.50, mean 7.84, SD 0.30) in the endemic site; however, in the non-endemic site, the pH was slightly acidic (range 6.05-6.18, mean 6.12 SD 0.06). The moisture content in the endemic site was much higher (12.21%) than in the non-endemic site, which symbolised suitable conditions for vector habitation.

DISCUSSION

Climate data (indoor temperature and relative humidity) derived from ground survey by *in situ* measurements were used to determine predictor climatic variables that affect sandfly density. Temperature and relative humidity were shown to play important roles in the transmission by influencing vector survival, parasite development rate and sandfly feeding behaviour (WHO 2000, Benkova & Volf 2007, Dinesh et al. 2008). Our study indicates that indoor relative humidity and temperature are highly associated with increased risk for *P. argentipes* breeding and leishmaniasis infection. Previous work reported that prediction of disease occurrence is based on climatic and/or environmental requirements of sandfly (Cringoli et al. 2005, Rinaldi et al. 2006). However, our results showed significant associations between vector abundance, indoor temperature and relative humidity, which might be a relevant predictor for disease prevalence. One study (Kumar et al. 2009) supports that the presence of cracks and crevices, due to high levels of relative humidity and temperature, generates essential breeding conditions for *P. argentipes*. These findings are also supported by another study (Ready 2008), which demonstrated spatiotemporal modelling of the distributions of the leishmaniasis and their sandfly vectors in relation to climate change in Europe.

A knowledge, attitude and practices study was recently conducted in endemic and non-endemic areas for the purpose of socio-economic and house structure assessment (Kesari et al. 2010). During this study, it was found that houses with mud plastered walls, thatched roofs and mixed dwellings were suitable areas for the breeding and propagation of *P. argentipes*. It was also observed that cattle sheds, mixed dwellings and interconnections between the houses strongly were associated with the presence/absence of vector.

Analysis of LULC variables using space technology in Kala-azar endemic and non-endemic regions indicated varying composition and depicted unique environments, which influence the availability of suitable habitat for the vector, as well as its abundance. The physiographical characteristics of the surface environment derived from the land cover data play an important role in the dynamics of leishmaniasis transmission (Defries & Townshend 1999). Different land cover classification approaches vary in their potential for discernment and consequently in their utility for meeting specific needs (e.g., the identification of all examples of a particular habitat) (Rogers 1991, Patza et al. 2000, Franklin & Wulder 2002). FA analysis results corroborated breeding preferences of *P. argentipes* by landscape elements. Minimum NDVI (areas with grass/weed cover or crop land), marshy land [high sediment water bodies containing grass/weeds, mass green colour in the standard false colour composite (FCC) image] (Sudhakar et al. 2006) and orchard/settlement (soft stem plants, e.g., banana, bamboo, sugarcane associated with hamlets) produced high loading when analysed with *P. argentipes* density in an endemic region. Water bodies, which are a blue colour in the standard FCC image (Lillesand & Kiefer 1999) and dense forest, which are a deep red colour in the standard FCC image (Lillesand & Kiefer 1999), generate a high load in the non-endemic site. Marshy land maintains dampness near its surface and increases humidity in the surrounding environment, which might be important for sandfly oviposition and breeding. Marshy areas might promote opportunistic growth of commonly found secondary vegetation (such as bamboo and banana) that provides the sandfly with a sugar meal within the study site. Similarly, human settlement has been associated with vector density because humans are feed on by their preferred host and also act as a reservoir. These results are consistent with previous studies where water bodies and vegetation influence sandfly density thus affecting leishmaniasis incidence (Kalluri et al. 2006, Sudhakar et al. 2006). Contrary to our hypothesis, we observed a strong negative relationship with agricultural fallow, which was potentially due to this analysis being based on dry season data.

Satellite-derived proxy for vegetation status derived from NDVI values showed that the majority of sandflies are present in the less dense vegetation zone. This relationship is potentially due to the fact that areas surrounded by peridomestic soft stem plants or less vegetation might help the male sandflies feed (i.e., take their sugar meal) (Schlein & Jacobson 1999). The minimum NDVI value positively correlated with sandfly density. Therefore, the NDVI values are extremely valuable and effective in analysing the conditions of Kala-azar occurrence (Cross et al. 1996, Bavia et al. 2005). Non-endemic areas have shown that proximity to the dense forest is an important determinant of the risk of Kala-azar transmission and can increase vector densities. Forest areas maintain the sub-surface soil moisture and influence the local climate, which might aid suitable sandfly propagation. The distance of the dense forest from hamlets, approximated using GIS analysis with distance criteria and proximity analysis, helped in prioritising the villages prone to high sandfly populations. In areas where the predominant land use is forest, the temperature is generally much cooler because of the abundance of shade provided by trees. Contrarily, humidity is greater because of the increased ability of the vegetation and soil to retain moisture. In this situation, there is a high probability that permanent breeding sites will be present and this condition would suggest the possibility of high Kala-azar transmission. The close proximity of the forest to human settlements might also enable trees to provide refuge for digesting sandflies and thereby protect the vector from insecticides.

The distribution and abundance of sandflies depends on moist soil that is rich in organic and nitrogenous matter (Napier & Smith 1926, Adler & Theodor 1957). The importance of physicochemical properties of the soil governing the breeding and distribution of sandflies has been emphasised (Sivagnaname & Amalraj 1997); however, in India, pH and the presence of inorganic constituents in the soil were found as characteristics of the breeding sites of sandflies (Kesari et al. 1991, Singh et al. 2008). Substrate soil analysis from endemic (Vaishali) and non-endemic (Lohardaga) regions showed that calcium oxide, magnesium oxide, Al_2O_3, Fe_2O_3, Na_2O and K_2O levels, pH and moisture content were higher in the non-endemic site, whereas the percentage of TiO_2 SiO_2 and P_2O_5 was lower in the endemic site (Table V). In general, high clay content in the soil and an average pH of 9.0 were identified as significant features of the areas where leishmaniasis was prevalent in endemic in India and Sudan (Hoogstraal & Heyneman 1969, Singh et al. 2008). Our data support these previous studies. In sum, the chemical properties and water retaining capacity of the soil in endemic sites mitigates the abundance of vector *P. argentipes*.

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