Geospatial Health 5(2), 2011, pp. 205-215

Incidence of visceral leishmaniasis in the Vaishali district of Bihar, India: spatial patterns and role of inland water bodies

Gouri Sankar Bhunia[1], Shreekant Kesari[1], Nandini Chatterjee[2], Dilip Kumar Pal[3], Vijay Kumar[1], Alok Ranjan[1], Pradeep Das[1]

[1]Department of Vector Biology and Control, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Agamkuan, Patna, Bihar, India; [2]Department of Geography, Presidency College, Kolkata, West Bengal, India; [3]Department of Surveying and Land Studies, PNG University of Technology, Lae, Papua New Guinea

Abstract. The role of the distribution of inland water bodies with respect to the transmission of visceral leishmaniasis (VL) and its dominant vector, *Phlebotomous argentipes*, has been studied at the regional scale in Bihar, eastern India. The Landsat TM sensor multispectral scanning radiometer, with a spatial resolution of 30 m in the visible, reflective-infrared and short-wave-infrared (SWIR) bands, was used to identify water bodies using the normalized differential pond index (NDPI) calculated as follows: (Green – SWIR I)/(Green + SWIR I). Nearest neighbour and grid square statistics were used to delineate spatial patterns and distribution of the sandfly vector and the disease it transmits. The female *P. argentipes* sandfly was found to be associated with the distance from open water and particularly abundant near non-perennial river banks (68.4%; $P < 0.001$), while its association with rivers was focused further away from the water source ($\chi^2 = 26.3$; $P < 0.001$). The results also reveal that the distribution of VL is clustered around non-perennial riverbanks, while the pattern is slightly random around the perennial river banks. The grid square technique illustrate that the spatial distribution of the disease has a much stronger correlation with lower density of open waters surfaces as well as with sandfly densities ($\chi^2 = 26.0$; $P < 0.001$). The results of our study suggest that inland water presence poses a risk for VL by offering suitable breeding sites for *P. argentipes*, a fact that should be taken into account when attempting to control disease transmission.

Keywords: visceral leishmaniasis, inland water body, normalized differential pond index, India.

Introduction

Visceral leishmaniasis (VL), also known as kala-azar, is an important parasitic disease in India where it affects large numbers of people. The disease is caused by the protozoon parasite *Leishmania donovani*, which is transmitted to humans through the bite of the female sandfly *Phlebotomous argentipes*, the established vector in India (Swaminath et al., 1942; Dinesh et al., 2000). About 12 million people worldwide are believed to be infected with the parasite, and 1-2 million new cases are registered each year, whereas 350 million people are at risk (http:// www.who.int/leishmaniasis/en/) (Reddy et al., 2007). According to the national vector borne disease control programme in India (http://planningcommission.nic.in/plans/annualplan/) close to 165 million people are at risk for the disease in four states of eastern and northern India, i.e. Bihar, Jharkhand, Uttar Pradesh and West Bengal. Of these, Bihar alone accounts for 90% of all Indian cases (Bora, 1999).

The *P. argentipes* vector is closely associated with environmental variables (Basimike and Mutinga, 1997; Feliciangeli, 2004; Kishore et al., 2006; Sharma and Singh, 2008; Guernaoui and Boumezzough, 2009) and certain weather parameters (Ghosh et al., 1999; Bhunia et al., 2010). Changed environmental parameters, brought about by construction and development or natural, ecological disturbances, influence its distribution. In spite of considerable advances in our understanding of sandfly biology and disease transmission patterns (Comer and Tesh, 1991; Ashford, 2001), control is still not effective. Since the sandfly breeding-sites have never been satisfactorily investigated, control measures that act specifically against the immature stages of the vector are not a feasible approach on their own (Hanson, 1961; Alexander and Maroli, 2003; Feliciangeli, 2004).

Geographical information system (GIS) is a powerful tool facilitating our understanding of the prevalence of disease and the distribution of risk factors in the spatial domain. The concurrent use of GIS and statistical tech-

---

Corresponding author:
Pradeep Das
Department of Vector Biology and Control
Rajendra Memorial Research Institute of Medical Sciences (ICMR)
Agamkuan, Patna 800007, Bihar, India
Tel. +91 0612 263 5570; Fax +91 0612 263 4379
E-mail: drpradeep.das@gmail.com

niques assist the determination of risk factors and the delimitation of risk areas, thus contributing to optimization of resources and better strategies for controlling the disease (Beck et al., 1997, 2000; Bavia et al., 2001; Thompson et al., 2002). Remote sensing has been used as an adjunct in various epidemiologic studies (Hay et al., 1996) but has so far not been fully utilised with respect to kala-azar. Nevertheless, ecological indicators have been exposed by this approach (Victora et al., 1997; Beck et al., 2000; Coasta et al., 2003; Sudhakar et al., 2006), revealing the role of environmental markers such as vegetation vigor, soil characteristics and humidity, among other factors, (Kadaro et al., 1993; Thomson et al., 1999; Elnaiem et al., 2002; Singh et al., 2008). As suggested by Andrade Filho et al. (2001), the sandfly's ability of rapid adaption to manmade environments may contribute to increased transmission of the disease. As vector-related changes are reinforced by the progressive deterioration of the natural sandfly habitats in northern India, the role of the environment in *Leishmania* transmission there warrants further study.

Inland water bodies are widespread in India's north-east and the provision of humidity from them may facilitate sandfly breeding. The plains and banks along the Ganges River are not only strongly associated with high numbers of kala-azar cases, but these areas are also characterised by permanent, as well as non-permanent, water bodies. To our knowledge, no study has focused on the potential role of natural water bodies, such as ponds and rivers, for *P. argentipes* breeding. In order to delineate the potential hydrological relationship between the vector and kala-azar transmission, the associations between inland water bodies, sandfly prevalence and *Leishmania* infections were investigated.

Materials and methods

The study sites

Hajipur (22.669 km^2) and Lalganj (13.722 km^2), two highly VL-affected areas in the Vaishali district of Bihar, with populations of 203,729 and 349,694, respectively (2001 census), were selected for the study (Fig. 1). These sub-districts, located next to each other on the floodplains of the Ganges River to the south and its tributary Gandak to the west, are confined within latitudes 25°39'00.18'' and 25°56'16.32'' north and longitudes 85°06'02.54'' and 85°20'17.61'' east. The land is criss-crossed by perennial, as well as seasonally flowing, watercourses and contains ancient levees ("chaurs"), i.e. relict palaeo channels transformed into meander belts, ox-bow lakes and cut-off loops, which are subject to frequent avulsions and flooding. The ground throughout the region consists of entisols (recently formed surface soils in unconsolidated parent material) and inceptisols (soils devoid of clay or organic matter). These soils are well supplied with lime and characterised by pH of 7-8 and low base-exchange capacity.

The climate is humid and tropical with an estimated average annual rainfall of 1,200-1,400 mm. The mean minimum temperature is 26.5 °C in the summer, 18.8 °C in the rainy season, and 9.4 °C during the winter with corresponding maximum temperatures of 36.3 °C, 33.5 °C and 21.7 °C, respectively (Indian Meteorological Department, Patna, Bihar, India).

Clinical data

The data used in the study were collected from two public health centres (PHCs), one for each study area. The kala-azar incidence rate is approximately 0.5 per 1,000 people in 2007, resulting in 335 cases for Hajipur and 381 for Lalganj (District Malaria Office, Bihar State Health Society). The diagnosis was based on the presence of *Leishmania* parasites in spleen/bone marrow aspirations according to tests using rk39 kits (Boelaert et al., 2000; Srivastava et al., 2011) provided by the national vector borne disease control programme, Government of India.

Sandfly collection and identification

All sandflies were collected in 320 US Centers for Disease Control (CDC) light traps (Emami and Yazdi, 2008; Dinesh et al., 2009), placed in 75 villages in both study areas biweekly between April and October, 2008. The traps were placed at different distances from the rivers in randomly selected sites (living rooms and/or cattle sheds) after sunset (18:00 hours) and collected before sunrise at 06:00 hours). The captured sandflies, stored until examined in 70% ethanol in test tubes labeled with area, village and the number of sandflies caught, were calculated as the number of specimens per trap per night per house. The specimens were mounted on micro slides with Canada balsam according to Remaudière (1992) and examined by stereo-microscopy, identifying species and sex by genital morphology as described by Lewis (1978).

Since *P. argentipes* is the recognised vector in India and the river banks are the preferential human settlement areas, we focused on this species and the perennial and non-perennial rivers.

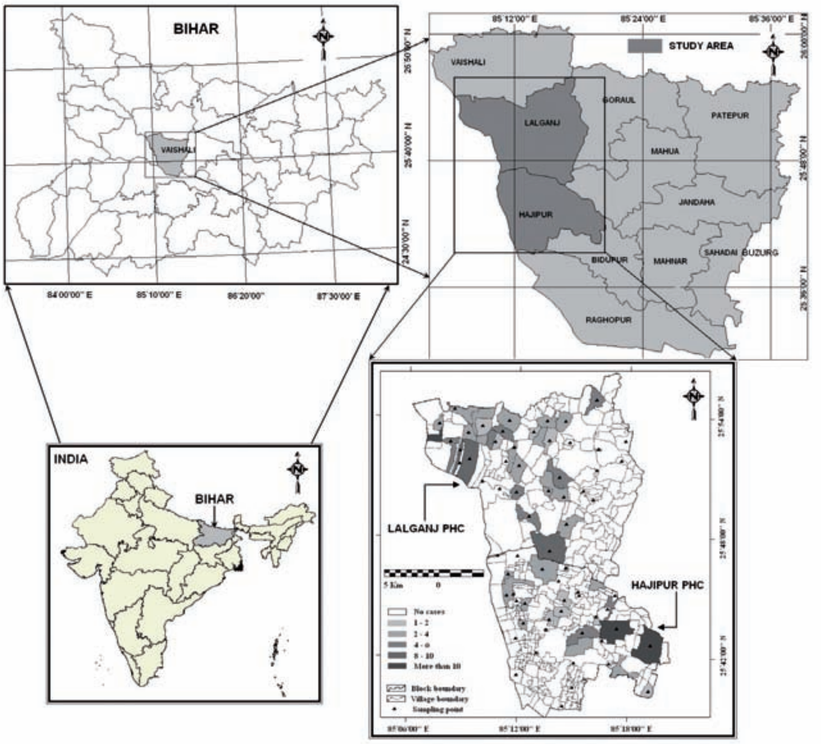

Fig. 1. The study areas related to the general geography of the Indian subcontinent.

Remotely sensed data and image processing

A 30 m-resolution image (P/R: 141/042, DoP: 30/10/2006) originated by the Landsat-5 Thematic Mapper (TM) (http://landsat.gsfc.nasa.gov/about/tm.html) was obtained from the Global Land Cover facility (http://www.landcover.org). The imagery consisted of visible, reflective-infrared, shortwave-infrared (SWIR), and thermal-infrared bands, and processing was done with ERDAS Imagine version 9.2 (http://erdas.com). The index used was obtained from the geo-coded Landsat TM data by the normalized differential pond index (NDPI) transformation tool (Lacaux et al., 2007) combining the short-wave infrared band-I (channel 5) and the green band (channel 2). The NDPI differs from the SWIR index, which is based upon the short-wave and near-infrared (NIR) absorption by water (Yi et al., 2007; Soti et al., 2009) and was preferred since it discriminates between actual water bodies and humid soil and vegetation allowing specific detection of open water surfaces such as rivers and ponds (Fig. 2). The NDPI combines the digital counts for the SWIR-I (1.55-1.75 µm) and the green bands (0.525-0.605 µm) as follows:

$$\text{NDPI} = \frac{(\text{Green}_{\text{Channel 2}} - \text{SWIR I}_{\text{Channel 5}})}{(\text{Green}_{\text{Channel 2}} + \text{SWIR I}_{\text{Channel 5}})} \tag{1}$$

The threshold values (which vary in different regions, different temporal regimes, and also for different images) were chosen from the histogram of the NDPI image and the resulting map compared with actual locations in the study area. As the many land cover categories obtained were deemed to be too complex for analysis, a simplified reclassification process available in ArcView version 9.3.1 (ESRI; Redlands, CA, USA) was used to show only the water bodies.

Since images of the land surface during the rainy season were unavailable due to overcast weather conditions, post-flooded lands typical for the post-monsoon season had to be used to extract information about the inland water bodies. The water body density was derived by the Radial Basic Function interpolator technique (Buhmann, 2003).

Spatial statistical analysis

A point layer including all villages affected by kala-azar in the study areas was generated and a second order nearest neighbour analysis undertaken to measure the distribution of disease incidence data locations according to whether they are clustered, random or regular. The ratio (R) between the average observed nearest neighbour distance ($\overline{D}_{obs}$) and the expected value of the nearest neighbour distance in a random pattern is:

$$R = \frac{\overline{D}_{obs}}{0.5\sqrt{A/n}} \tag{2}$$

where n is the number of points and A the area (in ha). The ratio R varies from 0 (completely clustered) to 1 (random) to 2.149 (completely dispersed). In this analysis, the distribution was considered dispersed if the the mean of the observed nearest neighbour distance was found to be greater than that of a random pattern (R >1), while clustering was indicated when the the mean of the observed nearest neighbour distance was found to be less than that of a random pattern (R <1). To decide how probable it is that the observed pattern occurred by chance, a significance test was employed. The test statistic is:

$$Z_r = \frac{\overline{D}_{obs} - \overline{D}_{ram}}{SE_r} \tag{3}$$

where is the expected mean nearest-neighbour distance in a random pattern and the standard error of the mean nearest neighbour distance, i.e.:

$$SE_r = \frac{0{,}26136}{\sqrt{\frac{n^2}{A}}} \tag{4}$$

where n and A are the same as defined for equation (2).

If $Z_r > 1.96$ or $Z_r < -1.96$ (probability <95%), we concluded that the calculated difference between the observed pattern and the random pattern was statistically significant. Alternatively, if $-1.96 < Z_r < 1.96$ (probability <95%), we concluded that the observed pattern was not significantly different from a random pattern even if the point appeared visually clustered or dispersed.

The relation between the density of water bodies and the case incidence vis-à-vis vector density were analysed by using a grid system with a 2 km^2 square size. A subset layer was first produced from the NDPI image by the clipping/sub-setting method (Campbell et al., 2005; Alaguraja et al., 2010) to assess the ratio of water surfaces to the total area for each square. Next, a point layer was generated and the density entered as an attribute. Based on these values, a predictive water body surface area was generated using the radial basic functions (RBF) interpolator (Beatson et al., 2000; Heryudono and Driscoll, 2010). The spatial distributions of the reported kala-azar cases and the sandfly densities were finally added onto the map and the correlations determined.

For the statistical analysis, Stata version 10.0 (http://www.stata.com/), was employed as devised by Hamilton (2009). Poisson regression analysis, allowing model-dependent variables that describe count data (Cameron and Trivedi, 1998; Kleinbaum et al., 1998), was applied to predict the sandfly density between the measured distances from the river banks also taking the river types into account. The analysis permitted measuring the relationship between disease incidence and sandfly density as well as the determination of the kala-azar incidence rate regard to the relative amount of water surface (water body density) and sandfly density per 2 km^2 grid area. The statistical significance was defined as P <0.05.

Results

Water body distribution

The 30 m resolution image from the Landsat-5 TM instrument (Fig. 2a) display NDPI values in the study areas ranging from 0.07 to 18.92. The brightest parts of the image (NDPI >3.93) indicate the perennial rivers and other water bodies. The reclassified image (Fig. 2b) provides better contrast showing all water surfaces, though now in grey. The relative distribution of open water surfaces (i.e. the water body density) is shown in Figures 3 and 4.

Sandfly collection

The traps produced a total of 754 adult sandflies from 226 of the sampling sites corresponding to at least one sandfly in 48 out of the 75 villages investigated. All specimens were identified as either *Phlebotomous* (male/female ratio = 1:1.51) or *Sergentomyia* (male/female ratio = 1:1.08). *P. argentipes* was the most frequently captured species, comprising 516 (68.4%) of all specimens captured. Calculation of the mean frequency confirmed the abundance of *P. argentipes* (male/female ratio = 1:1.50) throughout the area. With 229 (30.4%) specimens, *Sergentomyia* spp. were common at all sampling sites, while only nine *P. papatasi* (male/female = 2:1) specimens (1.2%) were found (Table 1).

River type and vector density

The total number of *P. argentipes* captured near the rivers (the main focus of this investigation) was 212

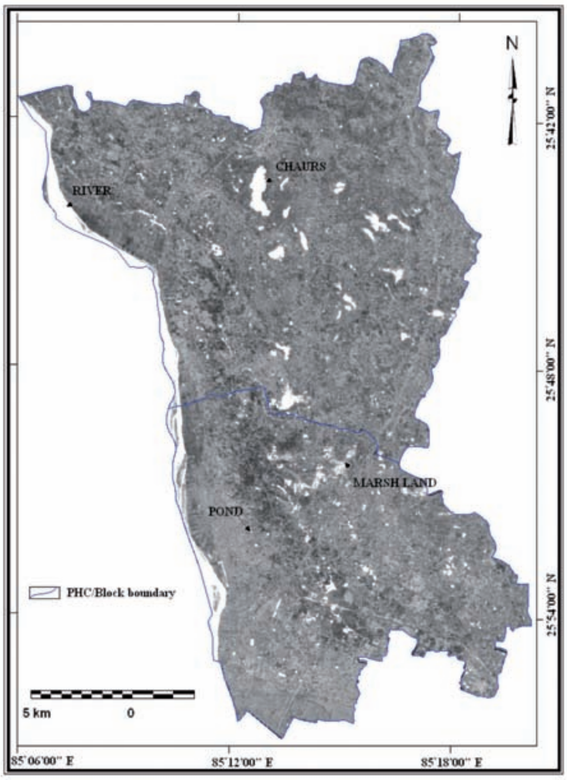

Fig. 2a. The NDPI image of the study areas, derived from Landsat TM data. The brightest areas (almost white) indicate surface water bodies.

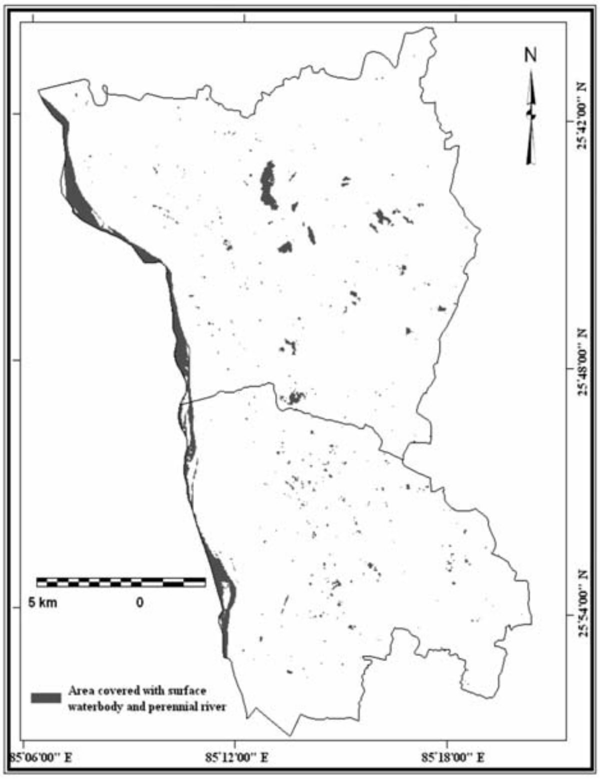

Fig. 2b. Reclassification of the NDPI image of study area indicating only the inland surface water bodies (in grey).

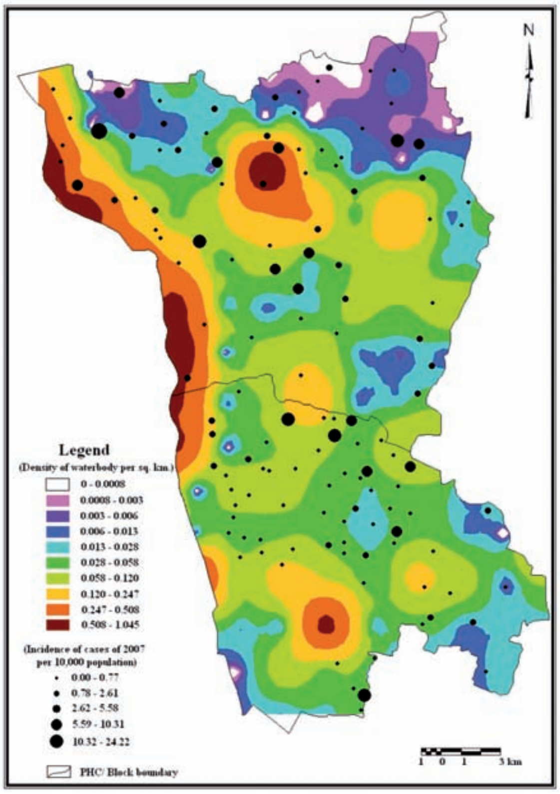

Fig. 3. Relation between the density of water body and disease incidence rate. The density surface of water body derived by the radial basic function interpolator technique. The disease incidence rate is represented through graduated symbol of the study area.

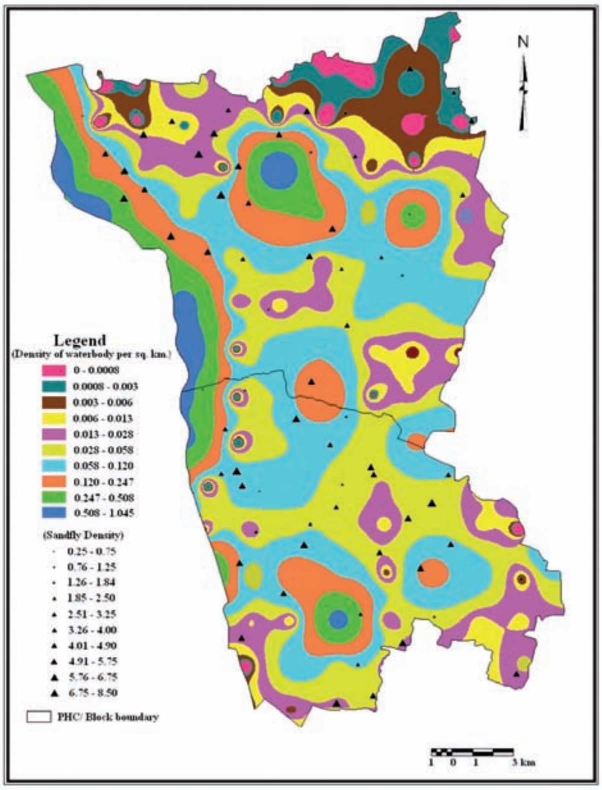

Fig. 4. Relation between the density of water body and sandfly density. The density surface of waterbody derived from radial basic function interpolator technique. Sandfly density represented through graduated symbol of the study area.

Table 1. Vector distribution and relation to the river types (perennial or non-perennial).

River type	Distance to river bank (m)	No. of traps per site	No. of positive sites	Sites with *P. argentipes*	*P. argentipes**		*P. paptasi**		*Sergentomiya**		*P. argentipes* density**
					Male	Famale	Male	Female	Male	Female	
Perennial	<250	25	18	3	8	5	1	0	4	7	4.33
Non-perennial	<250	25	21	8	27	39	1	1	6	8	8.25
Perennial	250-500	25	20	10	27	17	0	1	7	11	4.40
Non-perennial	250-500	25	22	18	17	49	0	0	9	19	3.67
Perennial	500-750	25	21	7	21	20	0	0	26	25	5.86
Non-perennial	500-750	25	19	11	16	46	0	1	11	12	5.64
Perennial	750-1,000	25	20	7	27	27	1	0	12	9	7.71
Non-perennial	750-1,000	25	17	15	19	39	0	0	19	18	3.87
Perennial	1,000-1,250	20	19	6	12	21	0	0	0	0	5.50
Non-perennial	1,000-1,250	20	16	14	5	16	0	1	10	3	1.50
Perennial	1,250-1,500	20	5	5	3	9	0	2	3	5	2.40
Non-perennial	1,250-1,500	20	7	5	5	9	0	0	0	0	2.80
Perennial	>1,500	20	12	4	11	4	0	0	3	2	3.75
Non-perennial	>1,500	20	9	7	8	9	0	0	0	0	2.43
Total	NA	320	226	120	206	310	3	6	110	119	NA

*Number of sandfly specimens; **measured as the total number of *P. argentipes* (male + female) trapped, divided by number of sites positive for *P. argentipes* (each trap used only once).

(41.1%) along the perennial and 304 (58.9%) along the non-perennial river banks. The difference between the river types was even larger when only the female sandflies were considered, i.e. 66.8% along the perennial and 33.2% along the non-perennial river banks, (Table 1, columns 6, 7).

The vector density in the 250 m wide buffer zones along the river banks (Table 1, column 5), were directly proportional to the distance from the perennial river banks, showing a positive association (r = 0.69), whereas a negative correlation (r = -0.87) was generated (P <0.001) with respect to the non-perennial rivers. The Poisson regression analysis showed a significant interaction between the distance from the river and sandfly density (likelihood ratio (χ^2) = 26.3; P <0.001) and indicated a significant relation between non-perennial rivers and vector density (likelihood ratio (χ^2) = 27.7; P <0.001), suggesting that increasing the distance from the non-perennial river banks would lead to decreased sandfly densities (Table 2). However, the analysis did not find a significant interaction between the perennial

Table 2. Poisson regression analysis for predicting sand fly vector density with regard to distance from the river.

Variable	Coefficients	Standard error	|Z|	P-value
Constant	1.696	0.184	9.20	<0.001
Distance from perennial river	-0.141	0.179	-0.79	0.431
Constant	1.969	0.122	16.05	<0.001
Distance from non-perennial river	-0.492	0.104	-4.72	<0.001
Constant	1.906	0.983	1939	<0.001
Distance from both river types*	-0.409	0.086	4.73	<0.001

*Perennial river is the referent category here

Table 3. Distribution of *P. argentipes* density in the kala azar-affected villages of Hajipur and Lalganj as reported to the PHCs of the Vaishali district.

Village investigated	*P. argentipes* density	No. of VL cases	Village investigated	*P. argentipes* density	No. of VL cases
Tajpur	4.25	3	Karanpura	8.25	5
Khaji	1.75	1	Ismailpur	2.00	3
Pirapur	6.50	2	Kushdeo	5.00	4
Jazira	5.75	1	Etwarpur Nizamat	3.75	1
Bishunpur Basant	7.50	5	Ekara	8.75	4
Shiurampur	4.75	1	Etwarpur Jagir	14.75	5
Anarra	9.25	7	Purkhaulu	2.75	1
Keshopur	3.00	2	Pukhaulu	2.50	5
Kharauna	7.25	4	Harauli Fatehpur	2.00	1
Atullahpur	1.50	2	Shahbazpur	4.75	5
Chak Chameli	4.75	2	Mathurapur	2.75	1
Khanjahan Chak	3.25	5	Patti	6.00	4
Basant Jahanabad	4.75	2	Yusufpur	1.5	1
Asdharpur	0.75	1	Rampur	4.00	3
Gadai Sarae	1.75	5	Samaspura	3.00	3
Manua Alah Baksh	8.00	1	Pahetia	2.00	1
Senduari Gobind	8.50	5	Chak Salae	4.00	4
Jalalpur Anurudh	6.50	1	Piuraina	1.25	2
Salempur	4.25	1	Bhakurahal Sital	4.75	5
Kutubpur	7.75	4	Rikhar	0.25	2
Chak Rasul	2.75	2	Banthu		

river type and vector density (likelihood ratio (χ^2) = 0.63; P = 0.429).

Vector density and disease prevalence

We found a highly significant correlation between sandfly density and the presence of disease (likelihood ratio (χ^2) = 8.8; P = 0.002). The correlation analysis was only performed for villages from where cases had been reported (Tables 3 and 4).

River type and kala-azar density

The results of the nearest neighbour analysis showed that the kala-azar cases were strongly clustered along the non-perennial river banks (R scale = 0.094, SE_r = 0.020, Z_r = 21.04), whereas a slightly random pattern was found along the perennial river banks (R scale = 0.341, SE_r = 0.156, Z_r = 6.63). In addition, a significant relationship was found between distance from the river and case incidence when the distribution over the buffer zones was taken into account. As can be seen in Table 5, most of the endemic villages were located between 250 m and 1 km away from the perennial river banks. However, although inhabitants residing at the distance of 500 m to 750 m from the river were safer, they were still at high risk.

Effect of water body density on disease incidence vis-à-vis sandfly density

In the grid square analysis, the rate of kala-azar incidence was shown to be correlated with vector densities as well as the densities of waterbodies. As evident from Table 6 and Figures 3 and 4, the results illustrate that there is significant relationship between the incidence of disease and vector density vis-à-vis water body density (likelihood ratio (χ^2) = 26.0; P <0.001). The results show that when the water body density per 2 km^2 grid area increases, the sandfly density vis-à-vis incidence of disease decreases.

Table 4. Poisson regression analysis between disease incidence and sandfly density.

Variable	Coefficients	Standard error	|Z|	P-value
Constant	0.617	0.177	3.49	<0.001
Vector density	0.865	0.278	3.12	0.002

Table 5. Kala azar case distribution with regard to distance from the river.

Distance from the river (m)	Case distribution (%)	Standard deviation	Kurtosis	Skewness
<250	24.15	4.32	0.45	-1.07
251-500	29.47	6.42	1.70	-1.07
501-750	33.33	3.95	0.76	0.58
751-1,000	13.04	2.50	1.50	0.20

Discussion

The sandfly prefers damp surfaces for its eggs (Aiello, 2002; Sharma and Singh, 2008) and the larvae of *P. argentipes* require relative humidity exceeding 70% (Napier, 1926; Bern et al., 2000; Sharma and Singh, 2008). Since humidity sustains the development and survival of the established sandfly vector of kala-azar, the water body density in Bihar is probably one of the most important land covers influencing the distribution of this disease. This variable, strongly influenced by rainfall and the seasonal water flow in the Ganges River and its tributaries, might therefore be one of the root causes for sustaining kala-azar in the study area. This reasoning is supported by the finding that vector density vis-à-vis disease prevalence evinced a declining pattern with decreasing distance from areas of high water body density typified by the perennial rivers. While most of the endemic villages are located near the non-perennial rivers, the risk for flooding and destruction of houses near the perennial river banks has forced people to settle at a relatively safe distance (≥250 m) from them, explaining why the prevalence of kala-azar was found to be lower closer to these river banks.

Although inland water bodies, in our opinion, must all be considered strong indicators for sandfly presence, we only investigated the river effect in detail. The most plausible explanation for finding the highest numbers of the vector near the non-perennial river banks (<250 m), while further away from the perennial river banks (750-1,250 m), is that heavy rainfall common to this region increases the risk for flooding of the latter. The relatively long lag time until the non-perennial river beds fill up is less detrimental to the habitats, allowing most eggs to survive and the development of the larvae to continue. The situation with regard to the perennial rivers is the opposite (Mukhopadhyay et al., 1990; ISPAN, 1993).

The non-perennial river banks are not only the first choice for human settlement, but they are also preferred by the sandflies. These confounding factors promote clustering along these rivers as opposed to the perennial river banks. A positive standard score (Z_r) was found, indicating that the nearest neighbour distance of the observed pattern is higher than what could be expected. The score indicates that the observed pattern of disease distribution has a tendency of clustering nearer the non-perennial river banks but not along the perennial river banks. These areas obviously have a high potential for kala-azar transmission (Tables 2 and 6) but our study also suggests that regions characterised by relatively low water body densities (<0.006/km^2) do not influence the vector vis-à-vis the disease ratio. In this scenario the identification of distribution and spatial extent of the water bodies vis-à-vis the vector would have a higher impact on kala-azar prevalence and sandfly ecology associated with surface water, estimated through Poisson regression between the river types vis-à-vis its density.

It is difficult to establish statistically significant and stationary relationships between surface water and sandfly abundance or kala-azar disease prevalence. For example, unlike most other biting flies, the immature stages of sandflies species avoid open water (http://whqlibdoc.who.int/hq/1991/WHO_CWS_91.3_3_eng.pdf). Thus, the presence of streams and other water bodies and streams influence vector abundance and its distribution in two ways: firstly, the increased surface humidity associated with water body and river bank enhances the generation of suitable breeding areas and host-seeking behaviour; secondly, density and the type of surface water available to the sandfly

Table 6. Poisson regression analysis for predicting the rate of Kala azar incidence with the density of water bodies and *P. argentipes* within the 2 km^2 squares of the study site.

Variable	Coefficients	Standard error	|Z|	P-value
Constant	0.675	0.169	3.98	<0.001
Water body	-1.710	0.547	-3.13	0.002
Sandfly density	0.087	0.036	2.38	0.017

for oviposition can alter the insect abundance. The first effect can lead to increased sandfly abundance by accelerating the reproductive cycle (mating, host-seeking and blood feeding), while the influence of the latter is less certain. The areas surrounded by large amounts of water bodies and perennial river banks experience increasing wetness of the soil near the surface and this can expand saturated low land areas due to flooding during the rainy season, which can diminish the number of larvae. Previous studies have shown that moist and humid habitats are preferred by the sandfly for oviposition, development and survival of immature stages (Feliciangeli, 2004; Kishore et al., 2006; Sharma and Singh, 2008). Our study has demonstrated that low densities of water bodies and presence of non-perennial rivers, might be supportive in making the environment more moist and humid, ultimately promoting the multiplication of the sandfly populations. The inland water bodies are highly influenced by the hydrological framework in this region as the terrain is flat with an average altitude less than 100 m above the mean sea level, which is subject to frequent channel avulsions resulting in sedimentation due to lack of maintenance. Conversely, water availability near saturated grounds, or along perennial river banks, as well as high water body density regions permit periodic flushing during the dry seasons by the reception of lower amounts of rainfall, can also be effective in dislodging sandflies. Such changes in vector numbers may also lead to increased disease transmission.

Our findings strongly support the fact that the higher moisture content of the surrounding areas of non-perennial rivers and lesser density of water bodies play an important role in the maintenance of sandfly density, promoting transmission of the disease. This also implies that the satellite images of inland water bodies may be successfully used in predicting future distributions of the sandfly vector and kala-azar incidence. Hence, an effective kala-azar control strategy without considering the inland water bodies would not be successful.

It should be noted that only *P. argentipes* was considered in this study as it is the proven vector of Indian kala-azar, but it is more than likely that other species also follow the pattern discovered. However, although other species were found, *Sergentomiya* in particular, their role in transmission remains unproven.

Acknowledgements

We thank GLCF Community for free access to satellite data. The work was financillay supported by Indian Council of Medical Research (ICMR), New Delhi under Senior Research Fellowship Grant. We also thank our field assistants and technical staffs, Mr. N. K. Sinha, Mr. S. A. Khan, and Mr. A. K. Mondal of the Vector Biology and Control Division of RMRIMS for their kind cooperation during the field data collection. We also thank Bihar State Health Society and District Malaria Office, Vaishali for providing the disease incidence report. Finally, we are grateful to anonymous reviewers for giving the valuable suggestions for improving the quality of this paper.

References

Aiello SE (Eds), 2002. The Merck veterinary manual. 8th edition. Merck & Co. Whitehouse Station, New Jersey, USA.

Alaguraja P, Durairaju S, Yuvaraj D, Sekar M, Muthuveerran P, Manivel M, Thirunavukkarasu A, 2010. Land use and land cover mapping – Madurai district, Tamilnadu, India using remote sensing and GIS techniques. Int J Civil Struct Eng 1, 91-100.

Alexander B, Maroli M, 2003. Control of Phlebotomine sandflies. Med Vet Entomol 17, 1-18.

Andrade Filho JD, Valente MB, Andrade WA, Brazil RP, Falcao AL, 2001. Flebotomous do Estado de Tocantins, Brasil (Dipteria: Psychodidae). Rev Soc Bras Med Trop 34, 323-329.

Ashford RW, 2001. *Phlebotomus* fevers. In: Service MW (Ed). The Encyclopedia of Arthropod-transmitted Infections. CABI Publishing, Wallingford, England, UK, 377-401.

Basimike M, Mutinga MJ, 1997. Studies on the vectors of leishmaniasis in Kenya: phlebotomine sandflies of Sandai location, Baringo district. East Afr Med J 74, 582-585.

Bavia ME, Malone JB, Hale L, Dantas A, Marroni L, Reis R, 2001. Use of thermal and vegetation index data from earth observing satellites to evaluate the risk of schistosomiasis in Bahia, Brazil. Acta Trop 79, 79-85.

Beatson RK, Light WA, Billings S, 2000. Fast solution of the radial basis function interpolation equations: domain decomposition methods. Siam J Sci Comput 22, 1717-1740.

Beck LR, Lobitz BM, Wood BL, Wood LW, 2000. Remote sensing and human health: new sensors and new opportunities. Emerg Inf Dis 6, 217-226.

Beck LR, Rodriguez MH, Dister SW, Rodriguez AD, Washino RK, Roberts DR, Spanner MA, 1997. Assessment of remote sensing based model for predicting malaria transmission risk in villages of Chipas, Mexico. Am J Trop Med Hyg 56, 99-106.

Bern C, Joshi AB, Jha SN, Das ML, Hightower A, Thakur GD, Bista MB, 2000. Factors associated with visceral leishmaniasis in Nepal: bed-net use is strongly protective. Am J Trop Med Hyg 63, 184-188.

Bhunia GS, Kesari S, Jeyaram A, Kumar V, Das P, 2010. Influence of topography on the endemicity of Kala-azar: a study based on remote sensing and geographical information system. Geospat Health 4, 155-165.

Boelaert M, Criel B, Leeuwenburg J, Van Damme W, Le Ray D, Van der Stuyft P. 2000. Visceral leishmaniasis control: a public health perspective. Trans R Soc Trop Med Hyg 94, 465-471.

Bora D, 1999. Epidemiology of visceral leishmaniasis in India. Nat Med J India 12, 62-68.

Buhmann MD, 2003. Radial Basis Functions. Cambridge University Press. The Pitt Building, Trumpington Street, Cambridge, UK.

Cameron AC, Trivedi PK, 1998. Regression analysis of count data. Econometrics Society Monographs No. 30. Cambridge University Press, Cambridge, UK.

Campbell MV, Fischer RL, Pangburn T, Hardenberg MJ, 2005. Using High Spatial Resolution Digital Imagery. US Army Corps and Engineer. Engineer Research and Development Centre, ERDC TR-05-1.

Coasta LB, Bertúlio MA, Rodrigues H, Lima GBM, Ramos SR, Moraes Filho VC, Ribeiro ALM, Souza CO, Silva AM, Barros IM, 2003. Ocorrência de flebotomíneos vetores da leishmaniose visceral nos municípios do Estado de Mato Grosso. Rev Abrasco 8, 639.

Comer JA, Tesh RB, 1991. Phlebotomine sandflies as vectors of vesiculovirus: a review. Parassitologia 33, 143-150.

Dinesh DS, Bhattacharya SK, Das P, 2009. Peridomestic breeding and resting sites of sandflies (Diptera: Psychodidae) in Bihar, India. Entomol News 120, 496-501.

Dinesh DS, Kar SK, Kishore K, Palit A, Verma N, Gupta AK, Chauhan DS, Singh D, Sharma VD, Katoch VM, 2000. Screening sandflies for the natural infection with *Leishmania donovani*, using a non-radioactive probe based on the total of the parasite. Ann Trop Med Parasitol 94, 447- 451.

Elnaiem DA, Hassan HK, Ward RD, 2002. Associations of *Phlebotomus orientalis* and other sandflies with vegetation types in the eastern Sudan focus of Kala-azar. Parasitology 13, 198-203.

Emami MM, Yazdi M, 2008. Entomological survey of phlebotomine sand flies (Diptera: Psychodidae) in a focus of visceral leishmaniasis in central Iran. J Vector Borne Dis 45, 38-43.

Feliciangeli MD, 2004. Natural breeding places of phlebotomine sandflies. Med Vet Entomol 18, 71-80.

Ghosh K, Mukhopadhyay J, Desai MM, Senroy S, Bhattacharya A, 1999. Population ecology of *Phlebotomus argentipes* (Diptera: Psychodidae) in West Bengal, India. J Med Entomol 36, 588-594.

Guernaoui S, Boumezzough A, 2009. Habitat preferences of Phlebotomine sand flies (Diptera: Psychodidae) in Southwestern Morocco. J Med Entomol 46, 1187-1194.

Hamilton LC, 2009. Statistics with STATA: updated for version 10. 10 Davis Drive, Belmont, CA, USA.

Hanson WJ, 1961. The breeding places of *Phlebotomus* in Panama (Diptera, Psychodidae). Ann Entomol Soc Am 54, 317-322.

Hay SI, Tucker CJ, Rogers DJ, Packer MJ, 1996. Remotely sensed surrogates of meteorological data for the study of the distribution and abundance of arthropod vectors of disease. Ann Trop Med Parasitol 90, 1-19.

Heryudono ARH, Driscoll TA, 2010. Radial basis function interpolation on irregular domain through conformal transplantation. J Sci Comp 44, 286-300.

ISPAN, Irrigation Support Project for Asia and the Near East, 1993. The Kala-azar epidemic in Bangladesh and its relationship to flood control embankments. USAID, Environmental study.

Kadaro AY, Ghalib HW, Ali MS, Eltoum I, Ismail A, Gaafar A, Kemp M, Kordofani AAY, Reed SG, El-Hassan AM, Kharazmi A, Hag-Ali M, Mustafa MD, 1993. Prevalence of cutaneous leishmaniasis along the Nile river north of Khartoum (Sudan) in the aftermath of an epidemic in 1985. Am J Trop Med Hyg 4, 44-49.

Kishore K, Kumar V, Kesari K, Dinesh DS, Kumar AJ, Das P, Bhattacharya SK, 2006. Vector control in leishmaniasis. India J Med Res 123, 467-472.

Kleinbaum DG, Kupper LL, Muller KE, Nizam A, 1998. Applied regression analysis and other multivariable methods - Third Edition. Brooks/Cole Publishing Company, Duxbury Press, Pacic Grove, CA, USA.

Lacaux JP, Tourre YM, Vignolles C, Ndione JA, Lafaye M, 2007. Classification of ponds from high-spatial resolution remote sensing: application to Rift Valley fever epidemics in Senegal. Remote Sens Environ 106, 66-74.

Lewis DJ, 1978. The phlebotomine sandflies (Diptera: Psychodidae) of the Oriental region. Bull Brit Mus 37, 217-343.

Mukhopadhyay AK, Rahman SJ, Chakravarty AK, 1990. Effects of flood control on immature stages of sandflies in flood prone Kala-azar endemic villages of North Bihar, India. WHO/VBC.90: 986.

Napier LE, 1926. An epidemiological consideration of the transmission of Kala-azar in India. India Med Res Memoir 4, 219-265.

Reddy M, Gill SS, Kalkar SR, Wu W, Anderson PJ, Rochon PA, 2007. Oral drug therapy for multiple neglected tropical diseases: a systematic review. JAMA 298, 1911-1924.

Remaudière G, 1992. A simplified method for mounting aphids and other small insects in Canada balsam. Rev Fr Entomol 14, 185-186.

Sharma U, Singh S, 2008. Insect vectors of *Leishmania*: distribution, physiology and their control. J Vector Borne Dis 45, 255-272.

Singh Ram, Lal Shiv, Saxena Vijay K, 2008. Breeding ecology of visceral leishmaniasis vector sandfly in Bihar state of India. Acta Trop 107, 117-120.

Soti V, Tran A, Bailly J-S, Puech C, Lo Seen D, Bégué A, 2009. Assessing optical earth observation systems for mapping and monitoring temporary ponds in arid areas. Int J Appl Earth

Obs Geoinf 11, 344-351.

Srivastava P, Dayama A, Mehrotra S, Sundar S, 2011. Diagnosis of visceral leishmaniasis. Trans R Soc Trop Med Hyg 105, 1-6.

Sudhakar S, Srinivas T, Palit A, Kar SK, Bhattacharya SK, 2006. Mapping of risk prone areas of Kala-azar (visceral leishmaniasis) in parts of Bihar state, India: an RS and GIS approach. J Vector Borne Dis 43, 115-122.

Swaminath CS, Shortt HE, Anderson LAP, 1942. Transmission of Indian Kala-azar to man by bites of *Phlebotomus argentipes* Annandale & Brunetti. India J Med Res 30, 473-477.

Thompson RA, Lima JWO, Maguire JH, Braud DH, School DT, 2002. Climatic and demographic determinants of American visceral leishmaniasis in northeastern Brazil using remote sensing technology for environmental characterization of rain and region influences on leishmaniasis. Am J Trop Med Hyg 67, 648-655.

Thomson MC, Elnaiem DA, Ashford RW, Connor SJ, 1999. Towards a kala azar risk map for Sudan: mapping the potential distribution of *Phlebotomus orientalis* using digital data of environmental variables. Trop Med Int Health 4, 105-113.

Victora CG, Huttly SR, Fuchs SC, Olinto MT, 1997. The role of conceptual frameworks in epidemiological analysis: a hierarchical approach. Int J Epidemiol 26, 224-227.

Yi Y, Yang D, Chen D, Huang J, 2007. Retrieving crop physiological parameters and assessing water deficiency using MODIS data during the winter wheat growing period. Canad