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Delimitation of kala-azar risk areas in the district of Vaishali in Bihar (India) using a geo-environmental approach

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Remote sensing and geographical information technologies were used to discriminate areas of high and low risk for contracting kala-azar or visceral leishmaniasis. Satellite data were digitally processed to generate maps of land cover and spectral indices, such as the normalised difference vegetation index and wetness index. To map estimated vector abundance and indoor climate data, local polynomial interpolations were used based on the weightage values. Attribute layers were prepared based on illiteracy and the unemployed proportion of the population and associated with village boundaries. Pearson's correlation coefficient was used to estimate the relationship between environmental variables and disease incidence across the study area. The cell values for each input raster in the analysis were assigned values from the evaluation scale. Simple weighting/ratings based on the degree of favourable conditions for kala-azar transmission were used for all the variables, leading to geo-environmental risk model. Variables such as, land use/land cover, vegetation conditions, surface dampness, the indoor climate, illiteracy rates and the size of the unemployed population were considered for inclusion in the geo-environmental kala-azar risk model. The risk model was stratified into areas of "risk" and "non-risk" for the disease, based on calculation of risk indices. The described approach constitutes a promising tool for microlevel kala-azar surveillance and aids in directing control efforts.

Key words: kala-azar - GIS - NDVI - wetness index - geo-environmental risk model

Visceral leishmaniasis (VL), or kala-azar, is a major public health problem in India that has historically occurred primarily in northeast region of the country (Sanyal 1985, WHO 1990, Joshi et al. 2008). The annual estimates for the incidence and prevalence of kala-azar cases worldwide are 0.5 million and 2.5 million, respectively, among which 90% of the confirmed cases occur in India (Bora 1999, Joshi et al. 2008). In 1977, a sample survey conducted in North Bihar (i.e., Vaishali, Muzaffarpur, Sitamarhi and Samastipur) estimated the number of kala-azar cases to at approximately 70,000, associated with 4,500, deaths whereas in 1991, the number of kala-azar cases reached 250,000, with 75,000 deaths (Thakur 2000). All 11 public health centres (PHCs) in the district of Vaishali were reported to be affected with kala-azar. Mahua was reported to be the most affected and Raghopur was the least affected PHC. In 1990, a total of 9,922 cases and 214 deaths were reported in the district. However, more than 20,000 cases have been reported from the district of Vaishali alone in the last decade and this district accounted for more than 5% of the total kala-azar cases recorded in Bihar. In 2011, 1,905 cases were reported, including 56 deaths between January-September [source: Bihar State Health Society (BSHS), India]. The annual incidence rate of kala-azar cases is greater than 2.61%, whereas the cause-specific rate is 32.78% per 10,000 individual in this district. The sandfly vector (*Phlebotomus argentipes*) of this disease is nocturnal and anthropophilic, exhibiting a short flight range. It reproduces best at temperatures of 25-30ºC and a relative humidity (RH) of 80-85% (Singh et al. 2008, Picado et al. 2010). The requirement of such specific environmental conditions for vector propagation make application of remote sensing (RS) and geographic information systems (GIS) ideal for the study of VL (Hay et al. 1996, Thompson et al. 1999, Sudhakar et al. 2006, Bhunia et al. 2010b).

The primary objective of the present paper is to develop a geo-environmental risk map for kala-azar using environmental and demographic parameter, based on primary and secondary data with the aid of RS and GIS technologies. The identified risk areas can then be marked as priority zones requiring immediate attention from health agencies as well as community participation to address the risk of kala-azar. High resolution satellite data provided important detailed information about landscape features, such as land use, vegetation conditions and soil moisture that were not detected from the low resolution imagery used in the study, which recorded habitat seasonality and the climate. Other recent studies have used different approaches to study the risk of kala-azar in different parts of the world (Thomson et al. 1999, Sudhakar et al. 2006, Bhunia et al. 2010a). However, these studies were based on low resolution satellite data. Additionally in previous studies, the authors have not incorporated the indoor climate, soil moisture or socio-economic factors in kala-azar risk maps for India (Sudhakar et al. 2006, Bhunia et al. 2010b). To our

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knowledge, this combination of geo-environmental parameters is novel. Thus, a combination of RS and GIS technologies will aid the Indian and international the authorities in planning and implementing successful preventive programs for mitigating kala-azar infections.

In this study, the parameters associated with kala-azar endemic areas were considered for inclusion in a geo-environmental risk model for predicting probable endemic areas for kala-azar. Our next step will be to validate this model in both endemic and non-endemic areas.

MATERIALS AND METHODS

Study area - The district of Vaishali (Fig. 1) was selected as the study area due to the high incidence of reported cases of kala-azar during the last decade. The district lies between the latitudes 23º38'N 25º26'N and longitudes 88º05'E 90º11'E, encompassing an area of 2.036 sq km. It is characterised by three distinct seasons; summer (March-June), winter (November-February) and the rainy season (July- October). January is the coldest month (< 5ºC) and April-May are the warmest months (44ºC). The rainy season lasts until the end of September, often extending to the middle of October. The average annual rainfall is 1.154 mm. The district has a population of 2,146,065 based on 2001 census data and is bounded by the Ganges River and its southern side and by the Gandak River on its western side. The region's geomorphology takes the form of a flat plain, including perennial, as well as seasonally flowing watercourses. The principle soil orders throughout the region are Entisols and Inceptisols.

The district of Vaishali is naturally unforested and exhibits with moist deciduous vegetation. The lands around its villages are irrigated and contain cultivated fruit trees, cereals, bamboo, reeds and grasses. The primary indigenous trees in the region are *Musa acuminata* (known locally as *banana*), *Mangifera indica* (mango), *Litchi chinensis* (litchi), *Ficus religiosa* (bo trees), *Borassus flabellifer* (Palmyra palm) and *Phoenix dactylifera* (date palm) and the region is mostly cultivated with maize, paddies and vegetables as well as some riverine vegetation consisting of fruit orchards, other trees and bushes.

Entomological data collection - Ground data were from March-June 2007 to acquire the ground data. Data on the vector (*P. argentipes*) for the summer season (March-June) were collected from 121 locations (villages) that were randomly selected out of 1,572 villages (Fig. 1) within the district using Communicable Disease Centre light traps. For each location, 10 houses were randomly selected, which were separated by a minimum distance of 100 m. The light traps were placed indoors at all 10 (inside both human dwellings and cattle sheds) to collect sandflies and climate data. The numbers of *P. argentipes* captured by the traps were used to calculate the average number of sandflies captured/trap-night for each location, as a measure of the abundance of the local vector population. All species were mounted on microslides using Canada balsam as the mounting medium (Remaudière 1992). Species identifications (Lewis 1978) were also performed. Villages where vector abundance is nil were not included in this study.

Epidemiological data - Data on the incidence of VL data for the year 2007 were obtained from the District Health Offices and BSHS. These incidence data aided us in identifying the spatial locations of VL-affected villages within the study area.

Climatological data source - The climatic data included in our analysis consisted of indoor climate measurements [i.e., room temperature (RT) and RH] for the summer season obtained from 140 villages in the district of Vaishali (Fig. 1) using polymeters (BARIGO, Model 305, Germany) at ground level at the time of sandfly collection. For each village, climate data were collected from the 10 houses where the traps were installed and then averaged to represent the local indoor climate of the respective village.

Socio-economic factors - The examined socio-economic factors consisting of the illiteracy rate and the size of the unemployed population (important variables for the human development index) for the years 1990-2001 were obtained from census records [source: Census of India, 2001 (censusindia.gov.in/].

RS data - Data from the Landsat-5 Thematic Mapper (TM) sensor (P/R: 141/042, date of pass: 15 May 2009) with a spatial resolution of 30 m and seven spectral bands with revisit frequency 16 days (98.9 min per orbit) and 8-bit quantisation were used. The examined satellite image registered using Universal Transverse Mercator (UTM) projection system (zone 45N and datum_WGS-84) based on a second-order polynomial algorithm and nearest-neighbour resampling method. The

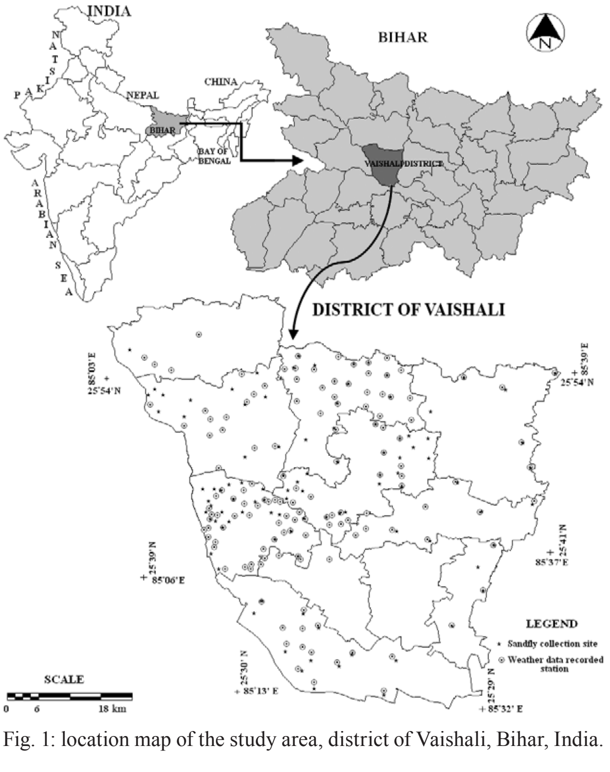

Fig. 1: location map of the study area, district of Vaishali, Bihar, India.

TM products used in this study were the normalised difference vegetation index (NDVI), the tasseled-cap transformation technique [wetness index (WI)] and land use/land cover classification. The NDVI, a standardised vegetation index (Tucker 1979), was calculated for this image and, the minimum, maximum, mean and standard deviation of the NDVI values in the kala-azar affected villages were extracted. Villages, where kala-azar cases are absent, were not included in the model because they were considered to have a low probability of occurrence of the disease. The WI was generated from a tasseled-cap transformed TM image (Qui et al. 1998). The transformation formula for the TM scene is defined in Crist and Cicone (1984) and was implemented in the model in model builder of Erdas Imagine software (version 9.1, Atlanta, Georgia, USA). A land cover map was obtained by performing a supervised maximum-likelihood classification technique (Belward & de Hoyos 1987, Curran et al. 2000). To confirm accurate classification assessments, an error matrix table was derived that expresses the number of sample units (i.e., clusters of pixel) assigned to a particular category relative to the actual location of that category, as verified in the field. Kappa statistics were used to measure agreement or map accuracy (Rosenfield & Fitzpatrick 1986, Congalton 1991).

In our analysis, class density was also calculated for each land use/land cover category. A concentric circle of 500 m radius buffer zone was generated for each of 100 villages around the centred on the centre of the village to estimate the environmental variables. Five hundred meters buffer was selected for the buffer zone size based on the estimated flight range of the sandfly. Class densities for land use/land cover were then extracted from the classified image based on the 500 m buffer zones. The "class density" is the number of times presence of classes within 500 m buffer zone divided by total number of such times considered for calculation (i.e. divided by 100 numbers).

Database creation on GIS platform - The methodology used to develop a geo-environmental based map of kala-azar risk through an index model is illustrated in Fig. 2. In ArcView version 9.3, the data on the sizes of illiterate and unemployed populations were associated with the digitised village boundaries as attribute data and created as separate layers. This was performed using Model Builder (index model) tool in the Spatial Analyst extension. For the vector abundance (sandflies per trap per night) and indoor climatic (temperature and RH) data, local polynomial interpolations using all points only within the defined neighbourhood were performed based on the weightage value (i.e. recorded from the field). Interpolated indoor climate and vector abundance maps were reclassified by dividing each data type into seven categories based on geometric intervals.

The root-mean-square error (RMSE) represents the prediction error, i.e. the difference between predict and actual difference values. For a model, the root-mean-square standardised prediction error should be close to 1 if the predictions are accurate. Another way to look at this is to divide each prediction error by the estimated standard error for the prediction. If the standardized RMSEs are greater than 1, the result is underestimation the variability in predictions; if the standardised RMSEs are less than 1, the result is overestimation the variability in the predictive model.

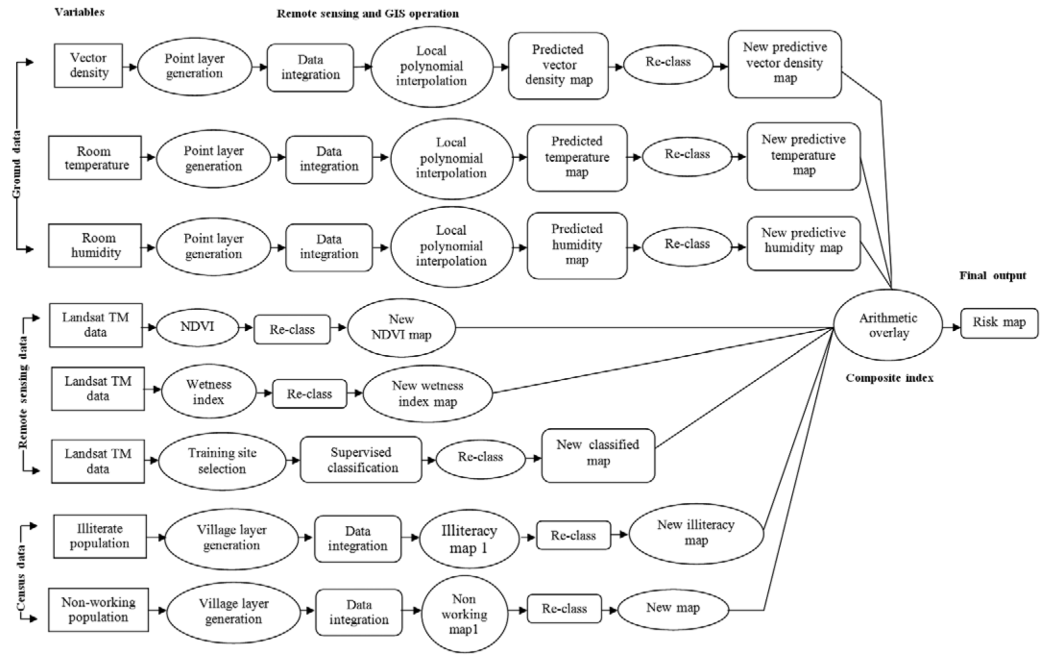

Fig. 2: flow chart map of the study methodology. GIS: geographic information systems; NDVI: normalised difference vegetation index; TM: Landsat-5 Thematic Mapper.

Development of the geo-environmental risk model - Information from several published and peer-reviewed studies on epidemiological aspects and population dynamics of parasites, vectors and human hosts was collected and augmented with a range of other previously reported data (Joshi et al. 2008, Bhunia et al. 2010b). Variables such as the temperature, humidity, vegetation, soil moisture, land use/land cover and the sizes of the illiterate and impoverished populations either individually or in combination are known to be associated with the occurrence of kala-azar. According to the degree of favourable conditions for kala-azar transmission, simple weightings/ratings were calculated for all of the input variables, leading to a geo-environmental risk model. We utilized rating systems based on values from 1-7, where ‘7’ mean very highly suitable, ‘6’ highly suitable, ‘5’ highly to moderately suitable, ‘4’ moderately suitable, ‘3’ as moderately suitable to somewhat unsuitable, ‘2’ less suitable, ‘1’ very less unsuitable and ‘0’ a kala-azar restricted area (no transmission occurred in this area). The model customised the environmental and socio-economic factors encompassing the ranges of different variables associated with the transmission of kala-azar, including indoor temperature (23-32ºC), indoor humidity (55-83%), the NDVI (-0.21-0.38), the WI (-70.56-31.04) and vector abundance (2.35-6.96) in addition to the sizes of the illiterate and unemployed populations to derivine a kala-azar risk index (equation 1). This technique of assigning each value of the input risk variables a score (using the ranges/characteristics provided above) to generate patterns over different geographical areas is usually used to apply a common measurement scale of values to diverse and dissimilar input data to create an integrated analysis (Brooker & Michael 2000, Sabesan et al. 2006). The scores and weights of the eight variables were assigned according to the incidence of VL associated with each category, calculated through overlay analysis and using threshold obtained from the leishmaniasis related literature (Sudhakar et al. 2006, Werneck et al. 2007, Bhunia et al. 2010b, de Almeida et al. 2011) (equation 2). The cell values for each input raster in the analysis are assigned values from the evaluation scale. This makes it possible to perform arithmetic operations on the input rasters, which originally had dissimilar types of values. Each input raster is weighted or assigned a per cent influence based on its importance to the model. The total influence for all rasters equals 100%. The cell values of each input raster are then multiplied by the raster’s weight. The influence of the most important raster is 20%, the second most important is 10%, the third-sixth rasters 15% for each and the seventh and eighth rasters 5% each. The cell values are multiplied by their percentages of influence and then added to create the output raster. In this weighted overlay, sandfly density has a 20% influence, RT a 10% influence, room humidity a 15% influence, land use/land cover a 15% influence, NDVI a 15% influence, WI a 15% influence, population illiteracy a 5% influence and the unemployed population a 5% influence. When the weighted overlay is calculated, the result is a raster of overall suitability is created. Setting the scale value to “restricted” assigns a value to that cell in the output weighted overlay result that is the minimum value of the evaluation scale set, which means that the corresponding area is unacceptable or cannot be used. Restricted areas are excluded from the analysis. In the land-use raster, for example, river/water bodies are restricted for disease transmission. Before running the final model all the layers (e.g., the indoor climate and the attribute layers for illiterate and unemployed populations) were resampled to the 30 m grid of the Landsat-based map using nearest neighbour resampling to match the resolution. The overall relative risks linked to each variable are summarized in Table I. The scores of the risk variables were summed mathematically as follows:

$$\frac{\text{VL risk for an area} = \Sigma^{8}\, Y_i}{i = 1} \qquad (1)$$

where $\sum Y_i = Y_1 + Y_2 + Y_3 + \ldots + Y_8$ are the scores for temperature, humidity, vegetation, soil moisture, land use/land cover and the sizes of the illiterate population and poor living people respectively.

To indicate the VL risk for a locality as a standardised quantity it is represented in terms of a percentage as follows:

$$\text{VL of locality} = \frac{\sum_{i=1}^{8} Y_i \text{ of a locality x } 100}{\text{maximum value of } \sum_{i=1} Y_i \text{ in the study area}} \qquad (2)$$

This approach was used to identify and assess potential risk areas for VL transmission in the district of Vaishali. Each risk index was expressed as an percentage of the highest value possible to compare the risk levels. However, true validation of the model can only be performed by comparing model predictions to the disease incidence report of 2008. The spatial distribution of the case incidence was overlaid on the “risk index value”, layer and the number of cases for each zone was counted manually.

Statistical analysis - Univariate analysis was performed to estimate the significance level of the correlations between the input environmental variables and the disease incidence. The statistical relationships between environmental variables and the incidence of kala-azar cases were assessed based on Pearson correlation coefficients. A simple linear regression analysis was used to investigate the association between the indoor climate and sandfly abundance. The results were considered to be significant if $p < 0.05$.

RESULTS

Entomological data analysis - A total of 389 sandflies belonging to the genera *Phlebotomine* and *Sergentomiya* were collected. Sandfly abundance data were also collected for the summer season (March-June). The highest vector abundance was observed in Sukhwarpur village (9.70), whereas the, lowest was found in Banthu village (0.25). Among the three sandfly species observed at the study sites 61.89% were *P. argentipes* (male:female ratio 1:1.01), while 36.25% were *Sergentomiya* (male:female ratio 1:1.92) and 1.86% were *Phlebotomus papatasi* (male:female ratio 1:0.70). However, in the present study,

TABLE I

Building a raster-based index model by calculating index values and summing up weighted cell values for kala-azar transmission

Data type	Input theme	Operator	Multiplier	Label	Weightage
Ground data	Sandfly density (summer month)	+	20	< 2.86	1
				2.86-3.37	2
				3.37-3.89	3
				3.89-4.91	4
				4.91-5.42	5
				5.42-6.45	6
				> 6.45	7
	Room temperature	+	10	< 24.91	1
				24.91-25.50	2
				25.50-26.10	3
				26.10 -27.29	4
				27.29-27.89	5
				27.89-28.48	6
				28.48-29.08	7
				29.08-29.68	3
				> 29.68	2
	Room humidity	+	15	< 66.58	1
				66.58-67.55	2
				67.55-69.85	3
				69.85-71.41	4
				71.41-73.43	5
				73.34-75.27	6
				> 75.27	7
	Land use/land cover	+	15	River/water body	Restricted
				Sand	1
				Vegetation	2
				Marshy land	4
				Crop land	6
				Agricultural fallow	5
				Dry/moist fallow	3
				Plantation with settlement	7
	Normalised difference vegetation index	+	15	-0.21-0.00	Restricted
				0.01-0.10	5
				0.101-0.15	7
				0.151-0.20	6
				0.201-0.25	3
				0.251-0.30	4
				> 0.301	2
Remote sensing data	Wetness index	+	15	< -3.09	1
				-3.09-2.06	2
				2.06-6.83	3
				6.83-10.79	4
				10.79-13.97	6
				13.97-16.75	7
				16.75-19.92	5
				19.92-23.50	3
				23.50-31.04	2
	Population illiteracy	+	5	No data	0
				Very low	1
				Low	2
				Less moderate	3
				Moderate	4
				High	5
				Very high	6
Census data	Non-working population	+	5	No data	0
				Very low	1
				Low	2
				Less moderate	3
				Moderate	4
				High	5
				Very high	6

only the *P. argentipes* density was considered the development of the model (Fig. 3A). The regression function for local polynomial interpolation was 0.219* x +3.347 with a RMSE of 0.008 units per trap/per night. The entire region was classified into seven categories. The highest values indicate higher risk of disease transmission.

Environmental parameters - A land use map was generated from the Landsat TM (30 m resolution) satellite data to obtain detailed information about land use and their percent coverages within the study site (Fig. 3D, Table II). For the initial classification, the following land use classes were considered: (1) river, (2) other water body, (3) sand, (4) vegetation, (5) marshy land, (6) crop land, (7) agricultural fallow, (8) dry fallow, (9) moist fallow land and (10) plantation with settlement. An accuracy assessment of the result, showed an overall classification accuracy of 89.89%. The values of kappa ranges showed almost perfect agreement (> 0.82) between these land cover classes for the study site. Based on class density a weightage score was assigned to each land cover class, as shown in Table I, II. The highest class density was found for the "plantation with settlement", class which was given the highest score, while the lowest score was given to the "sand" class. In this analysis, the river/water body classes were removed from the model as restricted because transmission could not occur in these areas.

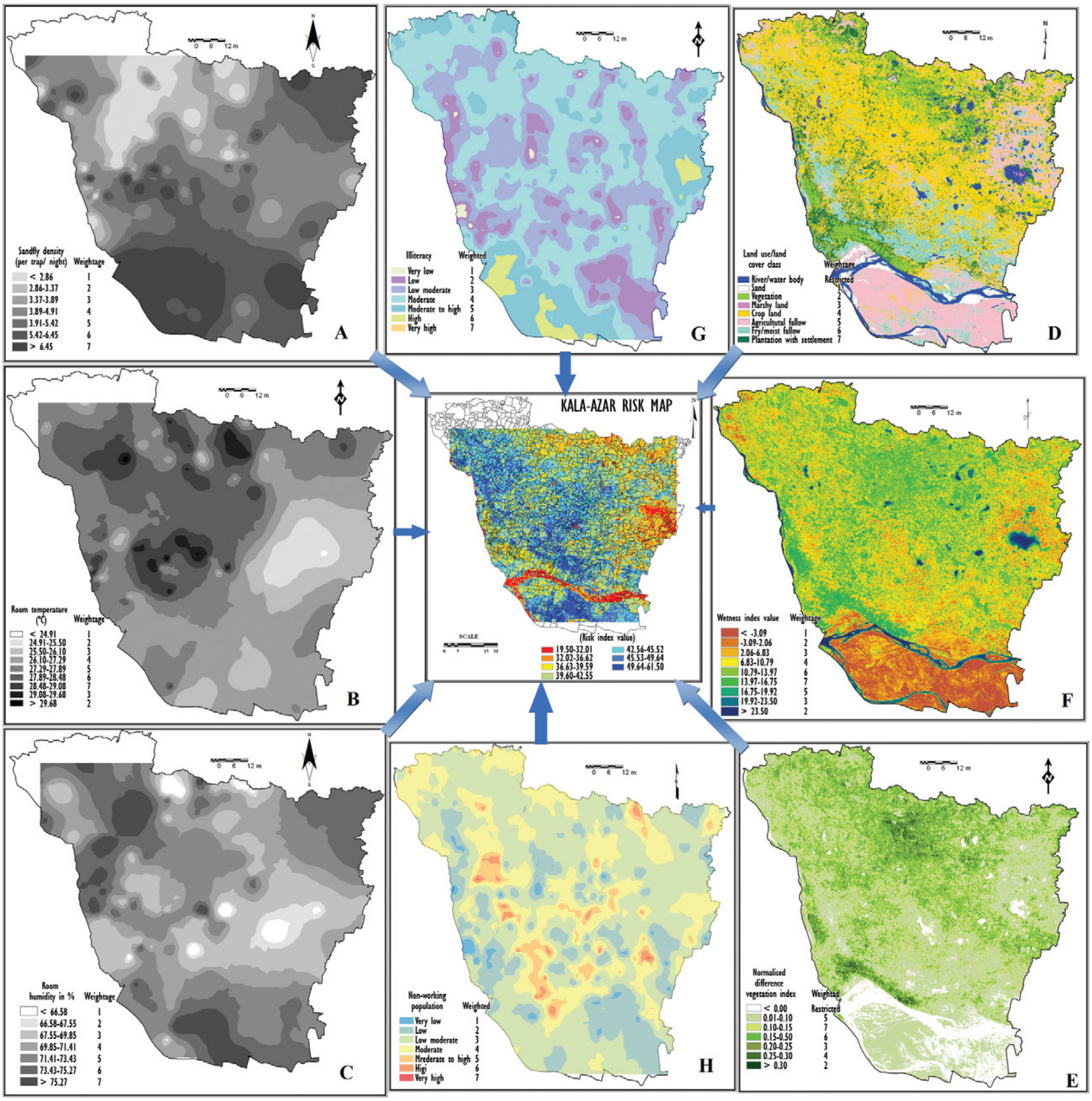

Fig. 3: geo-environmental risk map for visceral leishmaniasis (kala-azar) of the district of Vaishali, Bihar, India.

An NDVI map was generated to differentiate between vegetated and non-vegetated areas (Fig. 3E). The highest NDVI values (0.38) were observed around the study area during the dry season, whereas the minimum NDVI value was -0.21. Based on the density of vegetation, the areas were divided into seven categories related to the percent coverage within the study area, as follows (1) -0.21-0.00 (48.03%), (2) 0.01-0.10 (8.96%), (3) 0.101-0.15 (11.68%), (4) 0.151-0.20 (11.26%), (5) 0.201-0.25 (8.84%), (6) 0.251-0.30 (7.22%) and (7) > 0.301 (3.11%). Classes 1 and 2 represent absent or very low-density vegetation and the vegetation density increases from class 3-7. Pearson correlation co-efficient showed a strong negative relationship ($r = -0.69$; $p < 0.05$) between the case incidence and maximum NDVI values. A significant relationship ($r = 0.48$; $p < 0.05$) was also detected between the average NDVI and the incidence of cases. However, in this analysis, the minimum NDVI had no significant effect ($r = 0.10$; $p > 0.05$). When we overlaid all of the reported cases on the NDVI map, the result showed that most of the cases occurred in non-vegetated areas (Table III).

A WI map was prepared to estimate the dampness of the surface of the study area (Fig. 3F). The WI values for the study area varied from -70.56-31.04. The WI values were divided into nine categories (Table IV). The minimum value of WI indicates the driest areas (i.e., sandy areas), whereas the maximum value indicates wetness (i.e., a water body/river) within the study site. The WI map was then overlaid with the records of kala-azar case incidence for the locality. The distribution of areas with a high recorded incidence of cases is concordant with the distribution of WI values in the range of 13.97-16.75. Average WI values were strongly associated with the occurrence of disease ($r = 0.78$; $p < 0.05$), whereas no such significant relationship was established with the minimum and maximum WI value ($r = 0.28$; $p < 0.094$ - $r = 0.19$; $p < 0.18$, respectively). The results also illustrated that the percentage of cases decreases with the proximity to higher WI values as well as greater distance from the lower WI values.

Climatic parameters - The RT in the summer months within the study sites ranged from 23.97-31.50ºC (Fig. 3B). The summer RT values were categorised into nine classes (Table I). The largest number of potential vectors was observed within the RT range of 28.48-29.08ºC. A simple linear regression analysis showed a moderately significant relationship ($R^2 = 0.47$; $p < 0.05$) between RT and sandfly abundance. The regression function for RT by local polynomial interpolation was $-0.032^* x + 29.586$, with an RMSE of 0.836. However, when the monthly number of VL cases and the temperatures recorded during the study period were compared, a weak, but significant negative correlation, was detected ($r = -0.29$; $p < 0.003$).

TABLE II

Characteristics of various land use/land cover categories and their area covered in kala-azar endemic region

Land use/land cover classes	Description	Area covered (%)	Classes density[a] (%)
River	The perennial river (Ganga and its tributary, Gandak).	2.08	0.69
Sand	Naturally occurring unconsolidated granular material on the river bed.	2.11	1.32
Vegetation	Area characterized with weed/grass and sparsely distributed hard stem plant.	23.82	7.15
Bare surfaces/dry fallow	Area characterized with no green vegetation, unusable and unsaturated land.	10.21	7.23
Marshy land	Land covered with shallow bodies of water consisting of grass, weeds etc.	7.60	13.91
Surface water body	Natural inland water bodies characterized by chaurs, paleo-levees, mender belts, cut-off loops, pond etc.	1.37	2.88
Plantation with settlement	Residential land as well as land used for transportation. Areas dominated by planted trees (small size fruit garden e.g., banana, mango, lichhi etc.) surrounding the houses.	5.28	22.78
Crop land	Characterized by seasonal crops, intensively managed for production of paddy, wheat and vegetables.	16.50	18.86
Agricultural fallow	Areas consisting of shurbland, pasture, not used for cultivation during last four-five years.	20.45	14.99
Moist fallow	Land saturated with water; periphery of the river bank and permanent water body.	10.58	10.19

a: within 500 m buffer zone.

The indoor RH in the summer months at the study sites varied from 61.09-80.60% (Fig. 3C). The room humidity values for the summer months were divided into seven categories (Table I). The results of a simple linear regression analysis showed that RH has significant influence ($R^2 = 0.68$; $p < 0.05$) on vector abundance for the study sites. The results also illustrated that adult *P. argentipes* prefer room humidity values exceeding 75.27%. The regression function for local polynomial interpolation of room humidity was 0.024* x +69.417 with an RMSE of 0.207. RH showed a significant and positive relationship with VL ($r = 0.48$; $p < 0.020$).

Socio-economic characteristics - Data on the illiteracy rates and unemployed populations in the villages of the district of Vaishali were obtained from census records from the Government of India for 2001. The illiteracy rate in the district ranges from 6.25-98.59 (Fig. 3G). A simple correlation drawn between the illiteracy rate in each village and the incidence of kala-azar cases showed a positive relationship ($r = 0.43$; $p < 0.03$). The unemployed population percentage of the population varied from 33.05-87.67 in the district of Vaishali (Fig. 3H). A positive significant relationship ($r = 0.32$; $p < 0.009$) was found between the incidence of disease and the unemployed percentage of the population. Both of the above variables were categorised into six classes (Table I). Villages with higher illiteracy rates (greater than 60%) and unemployed percentage of the populations (more than 70%) were marked as having a high risk of disease transmission.

Geo-environmental risk model - Depending on the obtained risk values (Fig. 3), the geo-environmental kala-azar risk map (Fig. 3) was stratified into different spatial entities, ranging from “high risk” (blue) to “low risk” (red) units. The values for the calculated risk index varied from 19.5-61.5% (the observed maximum, this is the maximum value for the kala-azar risk index map) according to the localities. It is quite clear that this approach works well, as the resulting map of high, moderate, low or no risk of VL corresponded with the actual disease incidence records from BSHS. Importantly, all of the high prevalence points were predicted to be high-risk areas (risk index values > 45.53). All of the intermediate incidence points fell into the high or moderate risk-areas (risk index values 39.60-45.52) and risk index values < 32.02 were predicted as low-risk area. At sites presenting risk index values < 32.02, no cases were found, although some cases migrated from nearby endemic villages. After several discussions with the migratory patients, it was established that actually belonged to the areas of district of Vaishali where index value was greater than 32.20.

The observed cases of kala-azar for the year 2008 were extracted from detailed records from BSHS and then overlaid on the generated geo-environmental kala-azar risk map (Table V). The statistical accuracy of the findings was encouraging, as the correlation between the sampled results (results from District Health Offices and BSHS) and the predictions from the kala-azar risk map was greater than 76%.

TABLE III

Normalised difference vegetation index (NDVI) derived from a Landsat-5 TM data of district of Vaishali, Bihar, India

NDVI	Mean ± SD	Observed kala-azar cases (%)
-0.21-0.00	-0.11 ± 0.06	4.46
0.01-0.10	0.05 ± 0.03	14.64
0.101-0.15	0.13 ± 0.02	26.60
0.151-0.20	0.18 ± 0.01	20.15
0.201-0.25	0.23 ± 0.01	12.59
0.251-0.30	0.28 ± 0.01	14.11
> 0.301	0.34 ± 0.02	7.45

SD: standard deviation.

TABLE IV

Wetness index (WI) derived from a Landsat-5 Thematic Mapper image of the district of Vaishali, Bihar, India

WI	Mean ± SD	Observed kala-azar cases (%)
< -3.09	-36.84 ± 19.47	0.77
-3.09-2.06	-0.52 ± 1.48	2.97
2.06-6.83	4.44 ± 1.38	6.44
6.83-10.79	8.80 ± 1.14	13.16
10.79-13.97	12.38 ± 0.92	21.09
13.97-16.75	15.36 ± 0.80	29.11
16.75-19.92	18.33 ± 0.92	12.86
19.92-23.50	21.71 ± 1.03	10.05
23.50-31.04	27.27 ± 2.18	3.55

SD: standard deviation.

TABLE V

Village wise case incidence verification of kala-azar risk model in the district of Vaishali, Bihar, India

Risk index	Cases observed (%)
19.50-32.01	0.50
32.02-36.62	6.06
36.63-39.59	17.52
39.60-42.55	18.19
42.56-45.52	19.85
45.53-49.64	15.95
49.65-61.50	21.93

DISCUSSION

Risk models have been developed by different researchers and scientists to investigate disease transmission (Werneck et al. 2007, de Almeida et al. 2011). Cross et al. (1996) developed a model of the *P. papatasi* distribution in southwest Asia based on weather and the NDVI, although this model was not validated. No study has been conducted in India to delineate kala-azar "risk" and "non-risk" areas at a microlevel. In a previous reported by our group (Bhunia et al. 2010a), we presented kala-azar risk map at a macro-level based on meteorological variables and land use/land cover data. Here, we considered the village level and socio-economic data for risk prediction. It should be noted that the field data included in the analysis were collected over a short period of time that included no extreme weather events and the number of sandflies collected is known to depend heavily on local circumstances, including the weather, while sandfly trapping results show considerable variation seasonally and between years (Dinesh et al. 2001, Singh & Singh 2009, Picado et al. 2010). Sandfly abundance shows seasonal variation, being higher during the pre-monsoon and post-monsoon periods and lower in the winter months. However, this study was performed during the pre-monsoon season and the model was developed only for the study period, whereas it has been reported that the incidence of kala-azar cases is higher in the summer months than in the other months (Sanyal et al. 1979). Additionally, the entire district addressed in this study has been highly affected by kala-azar over the last 30 years and we considered only the villages reporting a high incidence of kala-azar in 2007. Nevertheless, the transmission of kala-azar is continuing unabatedly (simmering transmission) in the entire area. Therefore, the results of our analysis of environmental variables may be considered in the kala-azar risk model. Consequently, a quantitative longitudinal study is currently being conducted by our team.

Kala-azar transmission, similar to that of other vector-borne infectious diseases, is dependent on microlevel environmental (physiographic and climatic) and demographic variables. Physiographic factors primarily contribute to vector abundance, whereas climatic factors influence extrinsic incubation (of parasites) directly, as well as via vector survival. In the present study, the results showed that the indoor abundance of *P. argentipes* was positively associated with RT (28.48-29.08ºC) and RH (> 75.27%). Epidemics can occur when vector abundance increases due to environmentally favourable conditions or the presence of alternative host species that amplify vector abundance. In contrast, in areas of high vector density, the vector-to-host ratio is high and the disease may become endemic at a high level of prevalence. It has also been also reported that higher sandfly densities suggest a higher probability of successful transmission, subject to the presence of an infective host and positive eco-environmental conditions (Campbell-Lendrum et al. 2001, Sudhakar et al. 2006, Sánchez-García et al. 2010).

It is evident from the findings of the present study that environmental factors are major contributors to the distribution pattern of kala-azar incidence as previously stated by others (Victora et al. 1997, Bucheton et al. 2002, Kishore et al. 2006, Sudhakar et al. 2006, Kesari et al. 2011). Our results showed that environmental factors can be used to model and identify potential risk areas susceptible to kala-azar. This study also confirms that the general abundance of *P. argentipes* in the district is positively associated with RT and RH. It has been shown that climatic variability in general affects sandfly abundance *vis-à-vis* disease occurrence. Previous reports (Ranjan et al. 2005, Sharma & Singh 2008, Bhunia et al. 2010a, Kesari et al. 2011) also support these findings, as these researchers found that adult *P. argentipes* in India "preferred" relatively moderate to high temperatures and very high humidity.

Kala-azar is linked to the environment through the ecology of the vectors. As the conditions under which the vectors can thrive are limited, human VL appears to be a disease that is very sensitive to landscape characteristics (Sudhakar et al. 2006, Kesari et al. 2011). Landscape features, including land cover, land use and their patterns influence the availability of suitable habitat and thus, the abundance of vectors. The primary goal of the present study was to determine whether the environmental conditions that are observable via Landsat 5-TM imagery were correlated with the local abundance of *P. argentipes* and thus with the level of transmission of *Leishmania donovani* and consequent incidence of VL. In this study, plantations associated with settlements, crop land, agricultural fallow, marshy land and moist land were considered at greater risk due to their large areal extent in endemic villages based on the class density within 500 m buffer zones around the centres of endemic villages. The mapping of land cover characteristics with considerable emphasis on variables associated with crop land, agricultural fallow areas, marshy land and moist land using RS satellite data were performed meticulously. The distance of these variables from settlements calculated through GIS analysis with discrete criteria and proximity analysis helped in prioritising the village that are prone to high sandfly populations. The present observations indicated that crop land, agricultural fallow lands with human settlements were indicators of risk-prone areas for kala-azar disease. These variables may support large adult populations of sandflies by providing food and suitable moisture condition.

However, it was found that the sandfly genic potential of an area arising due to its specific environmental composition regarding parameters, such as the proximity to water bodies, crop lands and agricultural fallow land can be assessed for macro-stratification of vectors *vis-à-vis* disease abundance/non-abundance (Kesari et al. 2011). The investigated villages are in close proximity to crop and agricultural fallow lands, yielding grass or weeds, which provide moisture to the soil in adjoining domestic biotopes. The area is generally clay type soils and the land is used for paddies, wheat crops and vegetables each year, which provides moisture to the soil in adjoining domestic biotopes. Moreover, sandflies were found in caves, animal burrows and termite hills, which are well-protected from wind and exhibit high humidity and diffuse light conditions (Kendrick 1986). The capacity of the soil in agricultural or crop lands to maintain a relatively constant humidity is of decisive importance for establishing the correct microclimate burrows for the successful habitation of not only of sandflies, but also their vertebrate hosts. A recent investigation (Elyes et al. 2007) showed that the development of irrigation for agriculture in central Tunisia may offer create a new suitable ecological niche for sandfly species and, subsequently, the emergence of a new fear of leishmaniasis.

It is also obvious from the NDVI results that low density vegetation (minimum NDVI) was associated with a high incidence rate of VL according to the level of vegetation cover in village neighbourhoods (Thompson et al. 2004, Bavia et al. 2005, Bhunia et al. 2010b), which should reflect the densities of trees because most grasses in the area are highly seasonal, flourishing only after rains. However, sandflies require a sugar meal, obtained from plant material (El Said et al. 1986, Schlein & Jacobson 1999). Therefore, sandflies would be expected to be less abundant in areas with little or only soft stem vegetation. These results are in agreement with the findings of Cross et al. (1996) and are also supported by Bavia et al. (2005), who investigated the influence of the minimum NDVI on the distributions of *P. papatasi* and *Lutzomyia longipalpis* in southeast Asia and Brazil, respectively. In the non-endemic villages, the NDVI values do not vary considerably over space.

The presence of surface dampness and high moisture has proven to be a potential environmental factor in the successful breeding of sandflies and the possibility of kala-azar transmission in tropical countries (Kadaro et al. 1993, López et al. 1996, Feliciangeli 2004, Sharma & Singh 2008). Unlike most other biting flies, the immature stages of sandfly species avoid open water (WHO 1991). The role of water bodies in the demarcation of kala-azar endemicity may be due to their effect on the prevailing moisture regime through flooding during the rainy season. Living in proximity to water bodies has been found to be a risk factor for kala-azar (D'Oliveira et al. 1997, Saha et al. 2009). In the present study, kala-azar cases were more likely to occur in regions showing a certain range of WI value, providing evidence that a certain threshold value of WI represents areas that are likely to include *P. argentipes* breeding habitats (i.e., areas far from the permanent water bodies). Alternatively, the region, close proximity to water bodies (e.g., rivers, low lying land, marshy land, chaurs) may detrimental to larval developmental stages (Mukhopadhyayet al. 1990, Bhunia et al. 2011) and/or may be have been cleared of organic remnants that compromise the resting or breeding places of sandfly larvae (ISPAN 1993). Prior investigation have demonstrated that proximity to water bodies may increase kala-azar risk, suggesting the importance of identifying wet areas showing the potential for vector breeding (Boaz 2006, Sudhakar et al. 2006, Kesari et al. 2011). Thus it appears like that proximity to an area of the certain value showing a certain range of WI values represents a more accurate predictor of sandfly breeding than proximity to swamps and/or other permanent water bodies in general.

The influence of human factors, such as literacy levels, economic status and occupations on the occurrence of VL at the microlevel has been described by others (Ranjan et al. 2005, Joshi et al. 2008, Adhikari et al. 2010). Previous studies in south Asia demonstrated an association between kala-azar and poverty (Bern et al. 2000, Thakur 2000). In this study, areas with low income levels and high illiteracy rates are considered high-risk areas for kala-azar transmission. Information, education and communication (IEC) or behavioural communication change (BCC) is an important component in the control and prevention of the disease. Kala-azar is a disease affecting the poorest among the poor. The affected population belongs to the lowest socio-economic group, whose *per capita* income is very low (Adhikari & Maskay 2003, Sharma et al. 2006). Thee individuals do not have knowledge of the symptoms of the disease, such as recurrent, intermittent or remittent fever, often a double rise, loss of appetite, splenomegaly, anaemia, liver enlargement. In the absence of IEC/BCC, they remain without medicinal treatment, which also negatively impacts the community. Furthermore, these populations are unaware of disease prevention techniques, such as spraying insecticides (DDT) in living rooms, sleeping on beds and the use of nets to avoid sandfly bites. A higher unemployed percentage of the population reflects a lower standard of living, which leads to an increased risk of transmission of the disease. These finding can be attributed to the life style and house-building materials of the impoverished which have the effect of increasing the transmission of the disease. Poorly constructed house are easily invaded by sandflies and represent preferred sites for breeding and thus this variable is an important risk factor (Schaefer et al. 1995). The lack of significance of socioeconomic factors may reflect the relative homogeneity of our study population and the fact that at the village level, more proximate factors determine the risk of kala-azar.

In the present study, the individual environmental and socio-economic characteristics were not evaluated as separate entities. Rather, they all considered together in a composite index, as a combination of factors responsible for transmission (Brooker & Michael 2000, Sabesan et al. 2006). Our model does not aim to estimate the prevalence of cases, but only to identify at the microlevel, the areas showing a high risk potential regarding VL transmission. At present, the control strategy of the kala-azar elimination programme is target areas based on their history of kala-azar transmission. However, this strategy is unlikely to yield considerable dividends because VL is well established in this area. The range of the vector *P. argentipes* is shifting and this species becomes quickly established in new areas. Therefore, the present study will very helpful in identifying probable areas of kala-azar transmission and strengthen the kala-azar control strategy. However, it may not be possible to identify positive cases based on the potential "risk" areas (at the microlevel) in this model with any of the existing tools. It may also be concluded that all people living with a risk of infection need not be "infective" because other factors may be involved, such as natural resistance. Nevertheless, a relatively high risk of kala-azar transmission can be reliably expected with increasing values of "risk" as identified by this model.

The kala-azar risk map generated in this study (based on environmental, climatic, entomological and socio-economic factors obtained from RS data and ground surveys) shows promising potential for identifying areas associated a high risk of kala-azar transmission. This kala-azar risk map may be of value to health agencies and local authorities, who may use this value to determine and plan kala-azar surveillance activities as well as to require mitigation programs in the identified areas. Information from the kala-azar risk map can be further used as a management guide to continuously monitor the status of the kala-azar intensity level.

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