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SPECIAL THEME: INFECTIOUS DISEASE

Zoonotic cutaneous leishmaniasis in central Tunisia: spatio–temporal dynamics

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Background Zoonotic cutaneous leishmaniasis (ZCL) is endemic in many rural areas of the Southern and Eastern Mediterranean region where different transmission patterns of the disease have been described. This study was carried out in a region located in Central Tunisia and aimed to investigate the spatio–temporal dynamics of the disease from 1999 to 2004.

Methods Incident ZCL cases were defined by clinical diagnosis, confirmed by a positive skin test and/or parasitological examination. Annual ZCL rates were calculated for 94 regional sectors that comprise the study region of Sidi-Bouzid. Spatial and temporal homogeneity were initially investigated by chi-squared tests. Next, spatial scan statistics were used to identify spatial, temporal and spatio–temporal clusters that display abnormally high incidence rates. A hierarchical Bayesian Poisson regression model with spatial effects was fitted to signify explanatory socio-geographic factors related to spatial rate variability. Temporal ZCL dynamics for the 94 sectors were described via a linear mixed model.

Results A total of 15 897 ZCL cases were reported in the 6-year study period, with an annual incidence rate of 669.7/100 000. An outbreak of the disease was detected in 2004 (1114/100 000). Spatial clustering is evident for the whole time period. The most likely cluster according to the spatial scan statistic, contains seven sectors with abnormally high incidence rates and ~5% of the total population. ZCL rates per sector are mostly related to the urban/rural index; sectoral population density and the number of inhabitants per household do not appear to contribute much to the explanation of rate variability. The dynamics of the disease within the study period are satisfactorily described by quadratic curves that differ for urban and rural areas.

Conclusions ZCL rates vary across space and time; rural/urban areas and environmental factors may explain part of this variation. In the study region, the Sidi Saâd dam—constructed in the early eighties and identified by previous studies as a major reason for the first outbreak of the disease—seems to be still related to increased ZCL rates. The most likely spatial cluster of high incidence rates contains regions located close to the dam. Our findings of increased incidences in urban areas support the hypothesis of increased incidences in peri-urban environments due to changes in sandfly/rodent living habits over recent years.

Keywords zoonotic cutaneous leishmaniasis, spatial clustering, spatio–temporal dynamics

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Zoonotic cutaneous leishmaniasis caused by *Leishmania major* and *L. tropica* is found in many countries of the Southern and Eastern Mediterranean region. It is an important health problem, with clinical manifestations varying from simple cutaneous to mucocutaneous and disseminated lesions. *Phlebotomus papatazi* (sandfly) is the proven vector of *L. major*,

and rodents *Psammomys obesus* and *Meriones* spp. serve as animal reservoir hosts. Some countries of the region have endemic foci of zoonotic and anthroponotic cutaneous leishmaniasis, which could cause epidemics among non-immune populations if they are involved in the transmission cycle. There are also some new foci where leishmaniasis has never before been recorded. Ben Salah *et al*.[1] presented several reasons behind the increased incidence of leishmaniasis in the region. The majority of them depend on human activities such as environmental modifications, resettlement of non-immune populations or development of agro-industrial projects, military activities, urbanization and so on.[2] Environmental modification, such as construction of dams, can change the temperature and humidity of the soil and vegetation, which may result in changes in the composition and density of sandfly species as well as changes in populations of rodent species. The formation of new settlements with non-immune populations facilitates the outbreak of leishmaniasis. For example, the outbreak of ZCL in the central and southern governorates of Tunisia in 1982–83 occurred following the construction of the Sidi Saad Dam.[3] Control of reservoir hosts is an important component of the control strategy against zoonotic forms of leishmaniases. Some new approaches to control the transmission of ZCL through environmental modifications have been studied in Jordan and Tunisia with support of the EMRO/CTD/TDR Small Grants project. The destruction of *Psammomys obesus* burrows by deep ploughing, removal of chenopods and planting of trees in a 2–3 km zone surrounding human settlements has resulted in a significant reduction of the incidence of cutaneous leishmaniasis among the local human population.[4]

In this article, we present results that emerged from a ZCL surveillance system carried out in the Sidi-Bouzid region, Central Tunisia. This study aims to estimate ZCL incidence rates from 1999 to 2004, to identify spatio–temporal patterns and correlate them to socio-geographic factors. Some studies of visceral leishmaniasis in Sudan that develop ideas similar to ours (although using different variables and statistical techniques) are presented in Thomson *et al*.[5] and Elnaiem *et al*.[6] Assunção *et al*.[7,8] and Werneck *et al*.[9] explored the space–time dynamics of leishmaniasis in Brazil using a methodological framework similar to the one displayed in this article.

Material and methods

Study area

Sidi-Bouzid is an endemic region for the disease. It is an area of 7500 km^2 located in central Tunisia and lying within the boundaries of 34°30′ and 35°29′ S and 8°55′ and 10°04′ W. The region is subdivided according to the Tunisian Census Bureau into 94 sectors (Figure 1). Its population according to the 2005 census is 395 506.

Subjects

Incident ZCL cases were patients attending for the first time a primary health centre (PHC) in Sidi-Bouzid during 1999–2004,

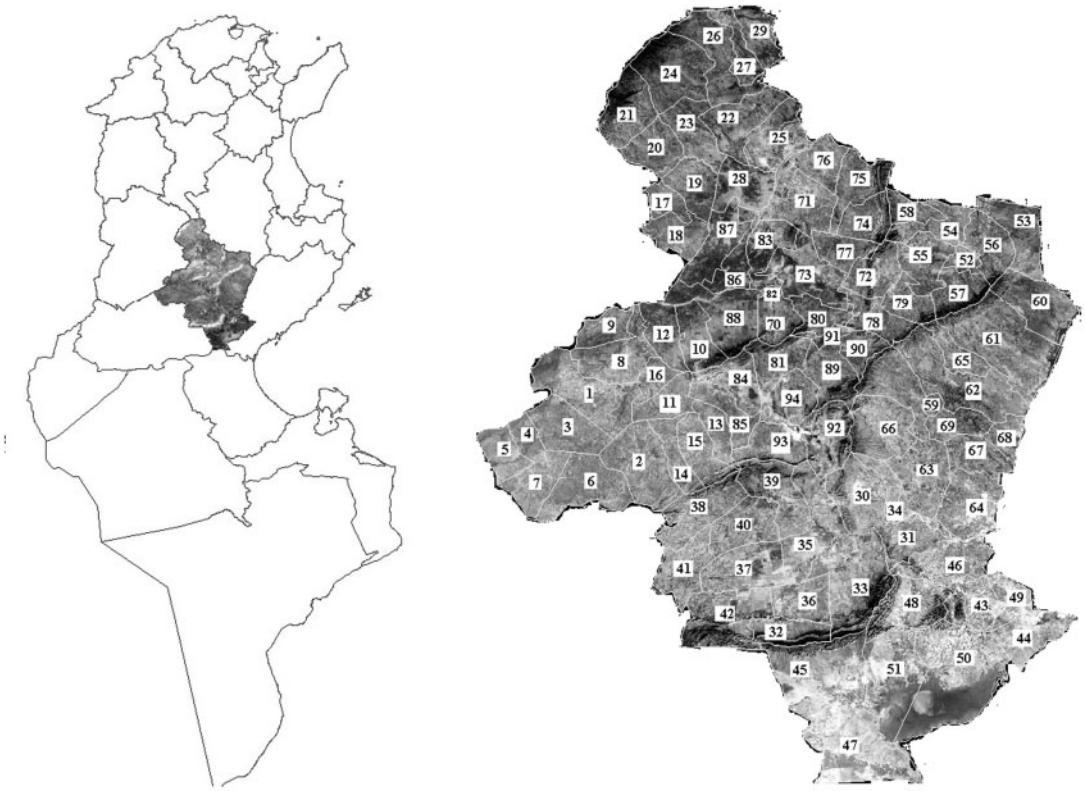

Figure 1 Left: Tunisian regions and the study area (highlighted). Right: A LandSat image of the study region with sectoral boundaries/Ids superimposed. Red tones correspond to vegetation, white-grey tones correspond to soil, green tones correspond to sparse vegetation and blue tones correspond to urban areas

and reported to a specific passive surveillance system for leishmaniasis. Patients with one or more active skin lesions, clearly demarcated, were diagnosed as cutaneous leishmaniasis. Most of ZCL diagnoses were based on clinical (multiple chronic lesions mostly located in uncovered parts of the body, mainly painless and nodo-ulcerative) and epidemiological criteria (multiple patients in the dwelling or the compound, lesions emerging between September and April among individuals living in the study area during the transmission season). Non-typical lesions were confirmed by parasitological examination using direct smear and/or culture. PHC provided basic demographic data (age and sex) for the patients.

Population data

Demographic data for the study area were obtained by the national Tunisian Statistical Agency. The 2005 census data for Sidi-Bouzid were used as an approximation for the 1999–2004 urban/rural populations and the number of urban/rural households for the 94 sectors of the study.

Temporal homogeneity

Annual homogeneity of ZCL rates at the sectoral level was tested by a chi-squared test which compares the total number of observed ZCL cases to those expected under the hypothesis of constant rates through time. Temporal clusters were identified using the scan statistic proposed by Kulldorff.[10]

Spatial clustering

Initially we tested for spatial homogeneity in aggregate ZCL rates and then proceeded to separate tests for each year. Again the chi-squared test was used in order to compare the number of observed ZCL cases per sector with those expected under the hypothesis of constant rate through space. To identify spatial clusters we applied a spatial scan statistic with an underlying Poisson model[11] to the aggregate cases for the 6 years of the study. That is we assume the number of ZCL cases to be Poisson distributed across sectors and test the null hypothesis that the risk of ZCL is the same in all sectors. The adopted methodology imposes a circular window on the map and allows the centre to move over the area so that at any given position, the window includes different sets of neighbouring sectors. For practical reasons, the centre of the window is positioned only at the 94 sector centroids; at each position the radius of the circular window is varied continuously from zero up to a maximum radius so that the window never includes >50% of the total population. In total, the method creates a very large number of distinct circular windows, each with a different set of neighbouring sectors within it, and each a possible candidate for containing a cluster of ZCL cases. For each window the method tests the null hypothesis against the alternative hypothesis that there is an elevated (or reduced) risk within, compared with outside, the window. For cluster identification, two criteria were posed: no geographical overlap between clusters and no cluster centring in another cluster. The spatial scan statistic has been used in a large number of epidemiological studies.[12–14] Application of the spatial scan statistic is highly facilitated by the SatScan software,[15] which is freely available (SaTScan™ is a trademark of Martin Kulldorff. The SaTScan™ software was developed under the joint auspices of (i) Martin Kulldorff, (ii) the National Cancer Institute and (iii) Farzad Mostashari at the New York City Department of Health and Mental Hygiene) from www.satscan.org.

Spatio–temporal clustering

A space–time scan statistic proposed by Kulldorff[16] was employed to identify sectoral clusters in space and time. The statistic is defined by a cylindrical window with a circular geographic base and with height corresponding to time. The base is centred on one of several possible centroids located throughout the study region, with the radius varying continuously in size. The height reflects any possible time interval of less than or equal to half the total study period, as well as the study period as a whole. The window is then moved in space and time so that for each possible geographic location and size, it also visits each possible time interval. In effect, we obtain an infinite number of overlapping cylinders of different size and shape, jointly covering the entire study region. Cases are assumed to be Poisson distributed with constant risk over space and time under the null hypothesis, and with different risk inside and outside at least one of the cylinders, under the alternative hypothesis. For each cylinder the number of disease cases inside and outside the cylinder are noted, together with the expected number of cases reflecting the population at risk. On the basis of these numbers, the likelihood is calculated for each cylinder. The cylinder with the maximum likelihood is denoted the most likely cluster. For cluster identification, we used the two aforementioned criteria: no geographical overlapping between clusters and no cluster centroids in other cluster.

Bayesian Poisson regression

To analyse spatial variability in ZCL rates, a hierarchical Bayesian Poisson Regression model [The main advantage of a hierarchical modelling procedure is that it partitions a complex model into a hierarchical structure. Such models are known to perform well for high-dimensional models (i.e. many parameters) as in our case][17] was fitted to the aggregate ZCL counts per sector. In this log-linear model the difference between the logarithm of observed cases minus the logarithm of expected cases (under the hypothesis of spatial homogeneity) was expressed as a linear function of three variables: urban/rural index, population density and inhabitants per household. Under the assumed model the observed cases per sector are Poisson distributed. The model smoothes raw ZCL counts by fitting random effects that allow for spatial correlation using the intrinsic conditional autoregressive (CAR) prior.[18] These random effects represent the effect of latent (unobserved) risk factors. There is a second set of random effects in the model for which we assume an exchangeable normal prior. The random effect for each area is thus the sum of a spatially structured component and an unstructured component. This is termed a convolution prior.[19]

Linear mixed model

Visual inspection of the temporal dynamics of the disease for the 94 sectors over the six-year study period indicates that a

sector's profile can be approximated by a quadratic function of time expressed in years. We can then fit such a function to each sector's data separately, resulting in estimates for sector-specific polynomial coefficients. Further, analysis of covariance can then be used to investigate what factors influence a sector's evolution over time. Instead of implementing a two-stage procedure, more efficient estimation for sector specific parameters can be obtained using a linear mixed (effects) model.[20] The fixed effects are regression parameters, which are assumed to be the same for all subjects, while the random effects are subject-specific regression coefficients. In the application we allow for different intercepts and slopes for urban and rural areas.

Results

A total of 15 897 ZCL cases were reported in Sidi-Bouzid from 1999 to 2004 with an average crude incidence rate of 669.7 annual cases/100 000 inhabitants. About 63.4% of the cases were from rural areas (83 sectors), which account for 71% of the total population (395 506 inhabitants). Age and sex distribution as well as clinical features of the disease are depicted at Table 1. Apparently, the disease is mostly characterized by multiple lesions.

The temporal evolution of ZCL is depicted at Figures 2 and 3. Figure 3 indicates a tendency of high incidence rates to occur at the eastern part of the region as time evolves. There is an

Table 1 Demographic and clinical features of ZCL cases in Sidi-Bouzid from 1999 to 2005 (annual percentages in parentheses)

Year	1999	2000	2001	2002	2003	2004	Total
Age							
<1 Year	66 (2%)	56 (2%)	34 (2%)	30 (2%)	101 (4%)	215 (5%)	502 (3%)
1–5 Years	518 (19%)	409 (15%)	252 (13%)	195 (11%)	317 (14%)	789 (18%)	2480 (16%)
6–14 Years	1027 (38%)	991 (38%)	592 (30%)	417 (24%)	635 (26%)	1075 (24%)	4737 (30%)
15 and more	1105 (41%)	1203 (45%)	1086 (55%)	1098 (63%)	1359 (56%)	2327 (53%)	8178 (51%)
Annual totals	2716 (100%)	2659 (100%)	1964 (100%)	1740 (100%)	2412 (100%)	4406 (100%)	15 897 (100%)
Gender							
Male	1247 (46%)	1235 (46%)	854 (43%)	738 (42%)	1086 (45%)	1941 (44%)	7101 (45%)
Female	1469 (54%)	1424 (54%)	1110 (57%)	1002 (58%)	1326 (55%)	2465 (56%)	8796 (55%)
Annual totals	2716 (100%)	2659 (100%)	1964 (100%)	1740 (100%)	2412 (100%)	4406 (100%)	15 897 (100%)
Clinical descriptions							
1 Lesion	1074 (40%)	1422 (53%)	923 (47%)	819 (47%)	1172 (49%)	2135 (48%)	7545 (47%)
2 to −5 Lesions	1381 (51%)	1052 (40%)	853 (43%)	802 (46%)	1017 (42%)	1875 (43%)	6980 (44%)
>−6 Lesions	261 (9%)	185 (7%)	188 (9%)	119 (7%)	223 (9%)	396 (9%)	1372 (9%)
Annual totals	2716 (100%)	2659 (100%)	1964 (100%)	1740 (100%)	2412 (100%)	4406 (100%)	15 897 (100%)

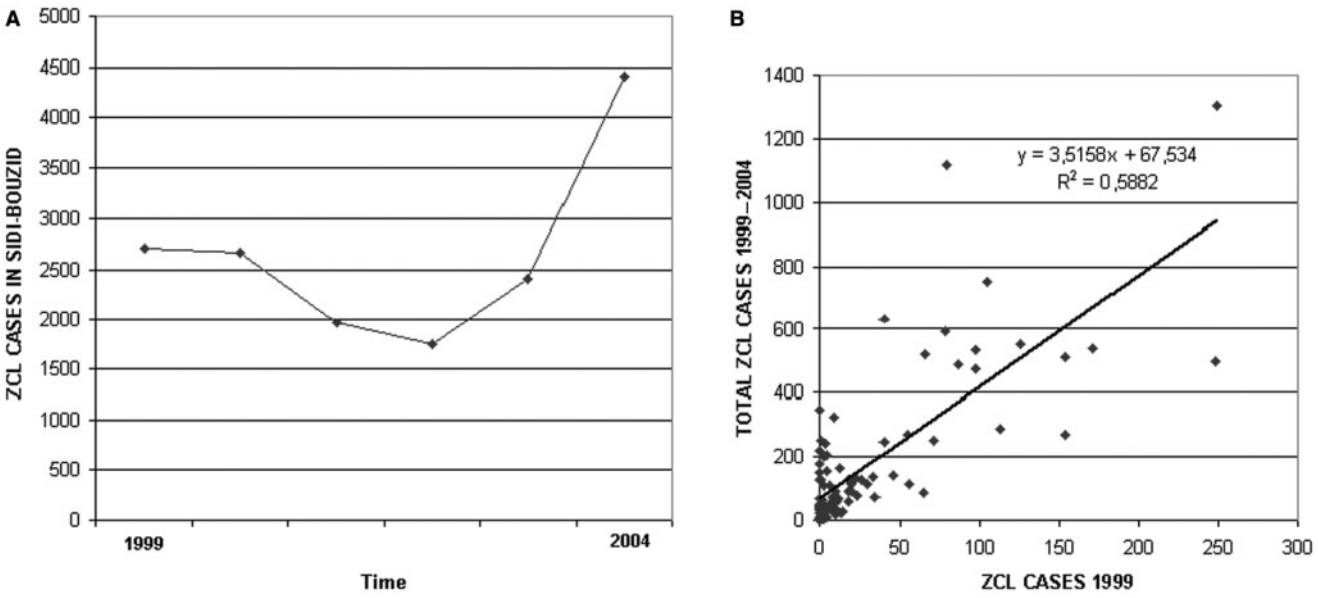

Figure 2 Left: ZCL cases in Sidi-Bouzid from 1999 to 2004. Right: Linear association between cases observed during the first year of the study and total cases from 1999 to 2004 for the 94 sectors

obvious outbreak of the disease in 2004. The chi-squared test does not support the hypothesis of temporal homogeneity ($\chi^2 = 1675.8$, df = 5, $P < 0.0001$) and Kulldorff's scan statistic reports a significant temporal cluster of high rates occurring in 2004 (observed cases are 1.66 times the expected under the hypothesis of temporal homogeneity with a log-likelihood ratio of 602 and a *P*-value <0.001). As indicated by the graph at the right part of Figure 2 there is a strong association between ZCL cases per sector during the first year of the study and total ZCL cases during the 6-year study-period (Pearson's *r* statistic equals 0.77 for the correlation between sectoral ZCL cases in 1999 and total cases during the study period. The graph is log transformed as most sectors cluster in low numbers). Thus, a sector with high incidence rates at the beginning of the study period is quite likely to display high rates for the whole time period.

ZCL rates varied across different regions, during each year and over the whole study region. This was determined by chi-squared tests on spatial homogeneity; their corresponding values are: 4974.8 for 1999, 4392 for 2000, 7048.5 for 2001, 3490.2 for 2002, 9817.5 for 2003, 4248.8 for 2004 and 4176.3 for the aggregate cases. These values provide strong evidence against the null hypothesis since the critical value for a chi-squared distribution with 93 degrees of freedom at a 0.01 confidence level is 128.

Figure 4 presents spatial clusters of high/low incidence rates for the sum of ZCL cases for each sector from 1999 to 2004. The underlying probability model for the 94 sectors is Poisson distributed and clusters were derived under two criteria: no geographical overlap between clusters and no cluster centres in another cluster. (To save space, the figure that corresponds to the second criterion is not displayed). Both methods suggested a spatial cluster of high incidence rates located at the northeastern part of the region. Observed cases in this cluster are four times more than the expected ones under the hypothesis of spatial homogeneity; the cluster contains seven sectors: Sidi Lefi, Sidi Khlif, El Amra, El Hania, El Makarem, Fayedh and Ouled Haffouz. Apparently, this cluster is neighbouring to the Sidi-Saad dam where the first outbreak of the disease was identified in the early eighties; the dam is located outside of the study region, very close to the borders of the spatial cluster. The population of the set of regions that comprise the cluster is 18 589; except Ouled Haffouz all other sectors are rural. When we allow for overlapping between clusters (but no cluster centroids in other clusters) three more clusters of high incidence rates, located at the central part of the region, are identified; they contain 20 regions with observed cases being at least two times the expected ones. Spatial clusters of low incidence rates are present at the northern, western and eastern parts of the region. When we allow for regions to overlap, practically all sectors of the northwestern part of the study-region belong to at least one cluster of low incidence rates.

Examination of space–time clustering reveals very similar results regarding the spatial distribution of incidence rates; in this case though, we also have a time frame that denotes the period for which each cluster deviates significantly (Figure 5). For example, the secondary cluster of high incidence rates at the centre of the region is prominent from 1999 to 2003, whereas for the most likely cluster the corresponding period starts in 2000 and ends in 2004. No evidence of spatial or spatio–temporal clustering exists for the southern/southwestern part of the region.

The rate of Leishmania infection depended on whether populations were in urban or rural location. This was determined by a Hierarchical Bayesian Poisson regression

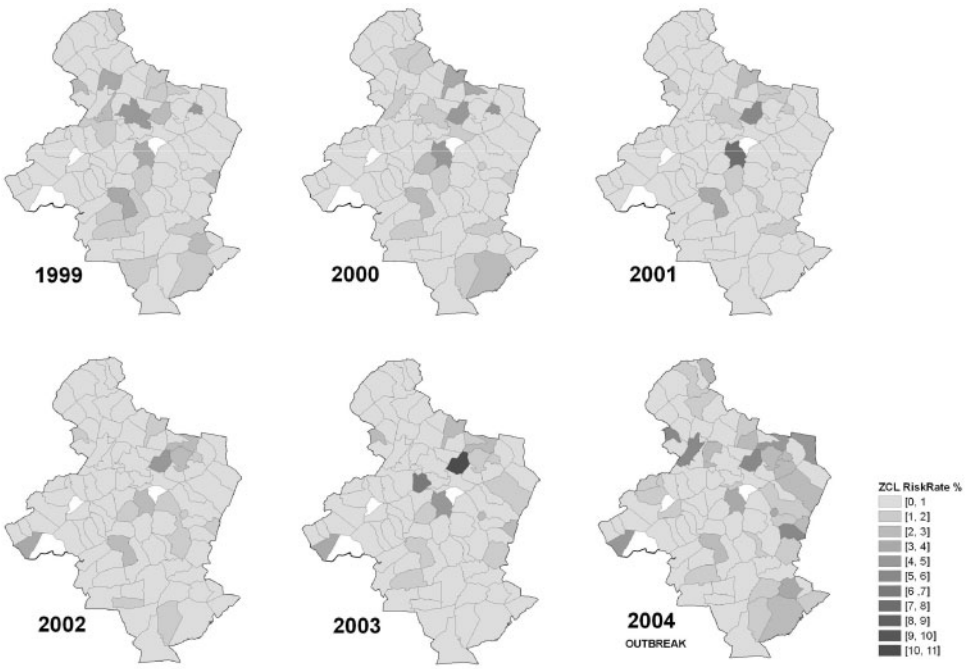

Figure 3 ZCL rates in the sectors Sidi-Bouzid from 1999 to 2004 in terms of percentage of cases in the population of each sector

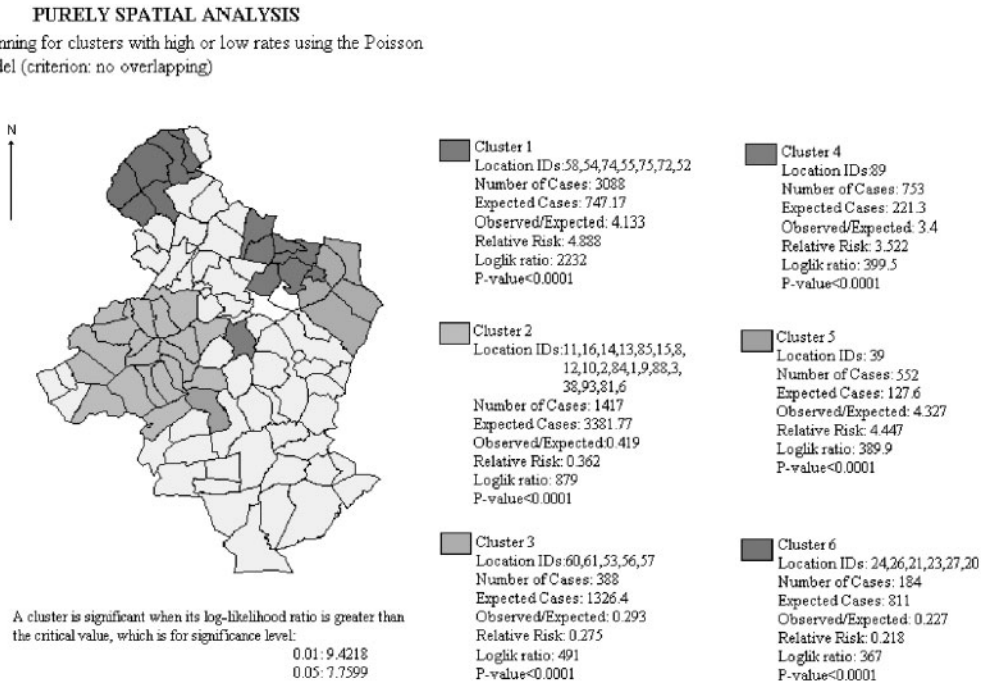

Figure 4 Spatial clusters derived from the spatial scan statistic under the Poisson model and the criterion of no geographical overlapping between clusters

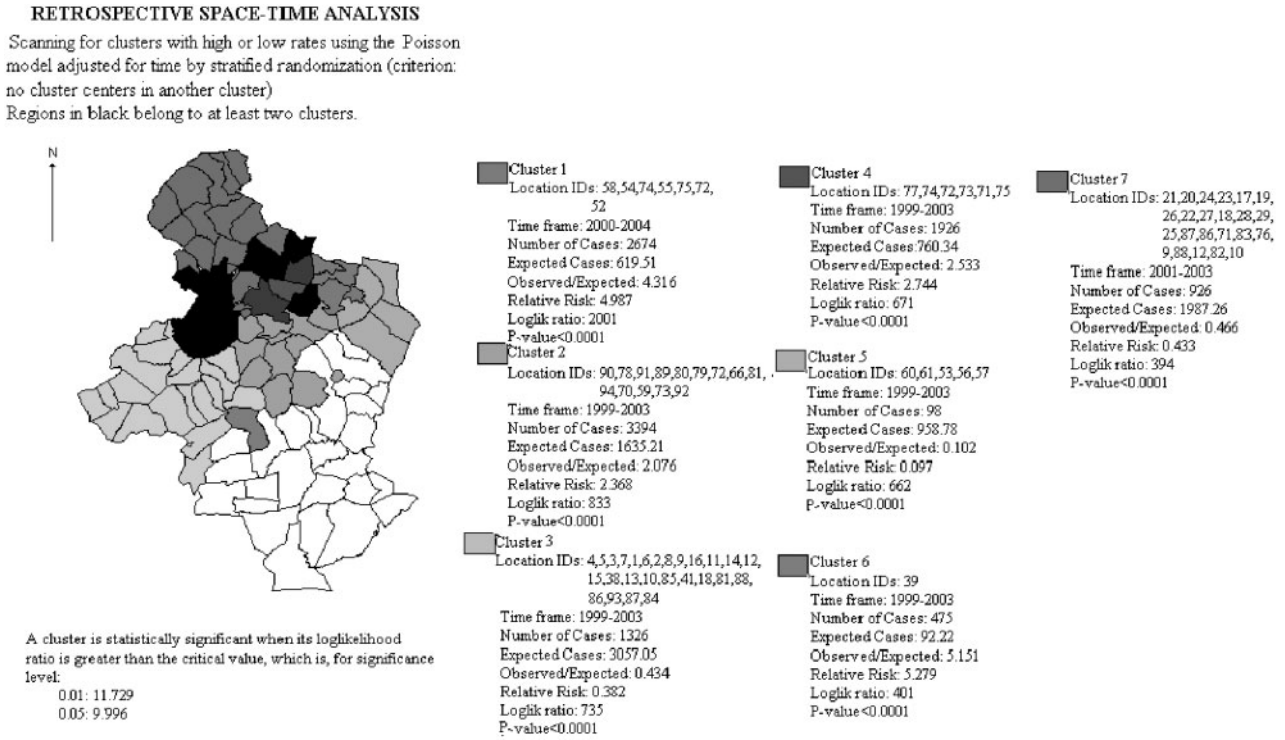

Figure 5 Clusters in space and time derived from the scan statistic under the Poisson model and the criterion that no cluster centres in another cluster (cluster overlapping is allowed-regions in black belong in at least two clusters)

model that revealed strong dependence of the observed rates per sector to the urban/rural index. The negative sign of the corresponding coefficient ($\alpha 2$ in Table 2) manifests lower rates in rural areas; this observation may seem awkward at first but it is consistent with previous findings that report high incidence rates in peri-urban areas.[2] It is also consistent with our findings from a large set of geo-referenced ZCL cases reported during 2004 in the northern part of the study region (data not shown).

Table 2 Summary statistics for the posterior distributions of the parameters of the Bayesian Poisson regression model

Node	Mean	SD	MC error	2.5%	Median	97.5%	Start	Sample
α0	1.62	0.7357	0.02816	0.149	1.615	3.127	70 000	150 003
α1	−0.0171	0.0263	9.599E-4	−0.0763	−0.0148	0.0281	70 000	150 003
α2	−1.421	0.5052	0.0188	−2.447	−1.397	−0.5376	70 000	150 003
α3	−0.1911	0.1382	0.0053	−0.4517	−0.1936	0.1026	70 000	150 003
Tau	1008.0	1423.0	34.51	1.045	469.6	5074.0	70 000	150 003
Tau.h	0.791	0.3724	0.009	0.5433	0.7599	1.102	70 000	150 003
Deviance	677.4	14.1	0.053	651.7	676.7	707.0	70 000	150 003
Deviance*	689.8	13.74	0.042	665.9	685.7	725.1	70 000	150 003

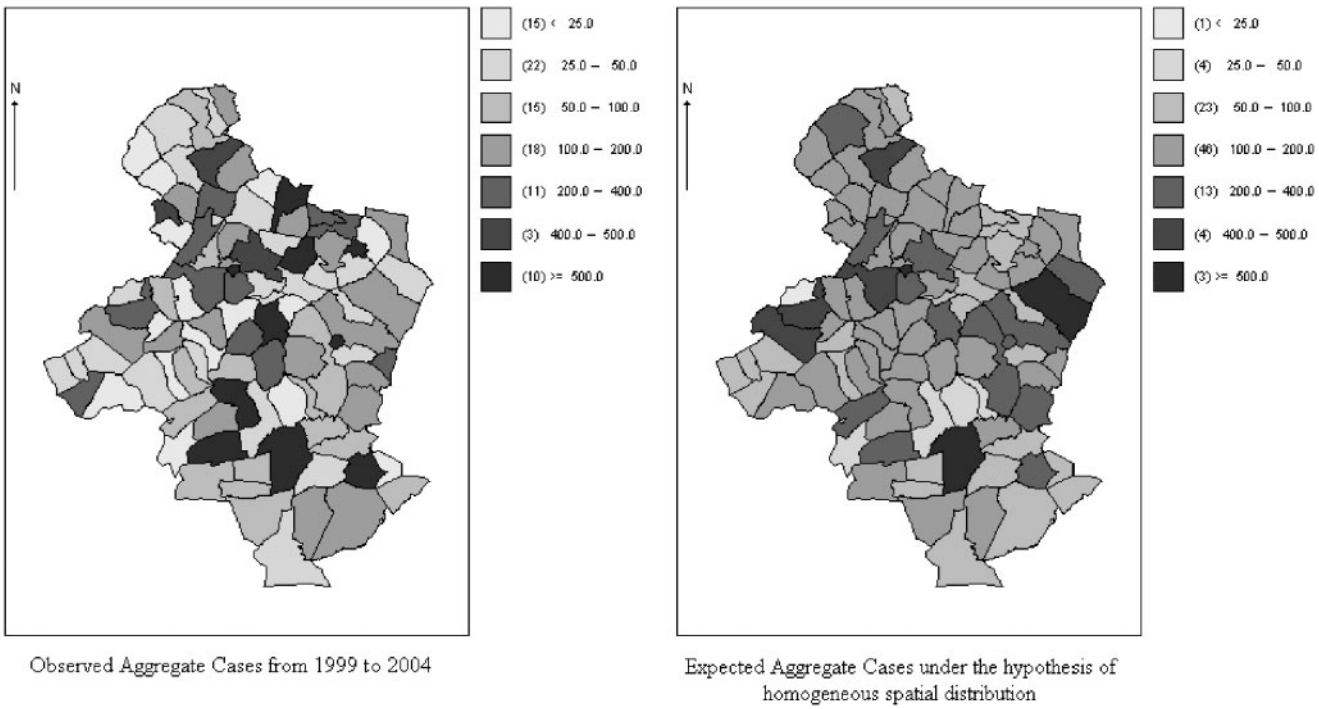

Figure 6 Left: Observed aggregate cases for the study period. Right: Expected aggregate cases under the hypothesis of homogeneous spatial ZCL distribution. Note: Parentheses contain the number of regions that fall in each class

Population density appears to be a weak explanatory factor since its posterior distribution is centred at zero (α1 at Table 2), whereas the coefficient that corresponds to inhabitants per household (α3) tends to take negative values contrary to what one would expect. Observed aggregate ZCL cases, expected cases under the hypothesis of homogeneous spatial distribution of ZCL, model fit and estimated relative risk, are depicted at Figures 6 and 7.

The three explanatory variables of the model are not strongly correlated (Pearson's r is 0.45 for the correlation between population density and inhabitants per household indicating more inhabitants per household in rural areas. Pearson's r is <0.15 for the correlation between inhabitants per household and the urban/rural index and the correlation between population density and the urban/rural index). Tau and Tau.h in Table 2 denote the precision (inverse of variance) for the spatial random effects and the exchangeable normal random effects respectively. Apparently, spatial random effects are much less dispersed around zero. Unexplained log-relative risk of disease appears to be higher in the most likely spatial cluster derived from the spatial scan statistic (to save space the distribution of spatial effects and random error in the study area is not depicted). Moreover, high values are also present at the southern part of the region in which vegetation is very sparse (Figure 1). The last two rows in Table 2 depict the deviance of the model with and without an exchangeable normal prior for the random effects, respectively. That is in one model we assume convolution and in the other only spatial CAR priors for the random effects.

Observed values indicate superiority (in terms of short-term prediction) for the model with the convolution priors. Sampling from the posterior distribution for the parameters of interest was accomplished via Markov Chain Monte Carlo and

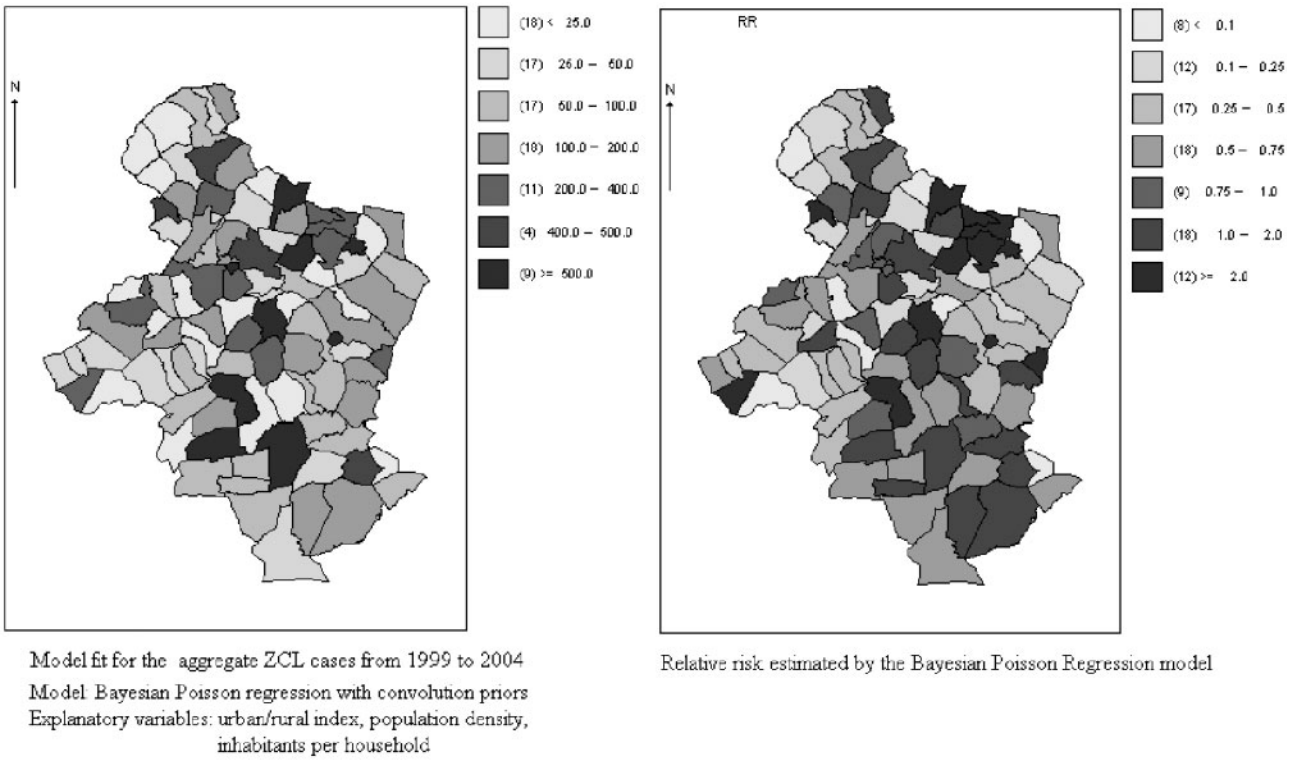

Figure 7 Left: Predicted cases from the Bayesian Poisson regression model. Right: Relative risk per sector. Note: Parentheses contain the number of regions that fall in each class

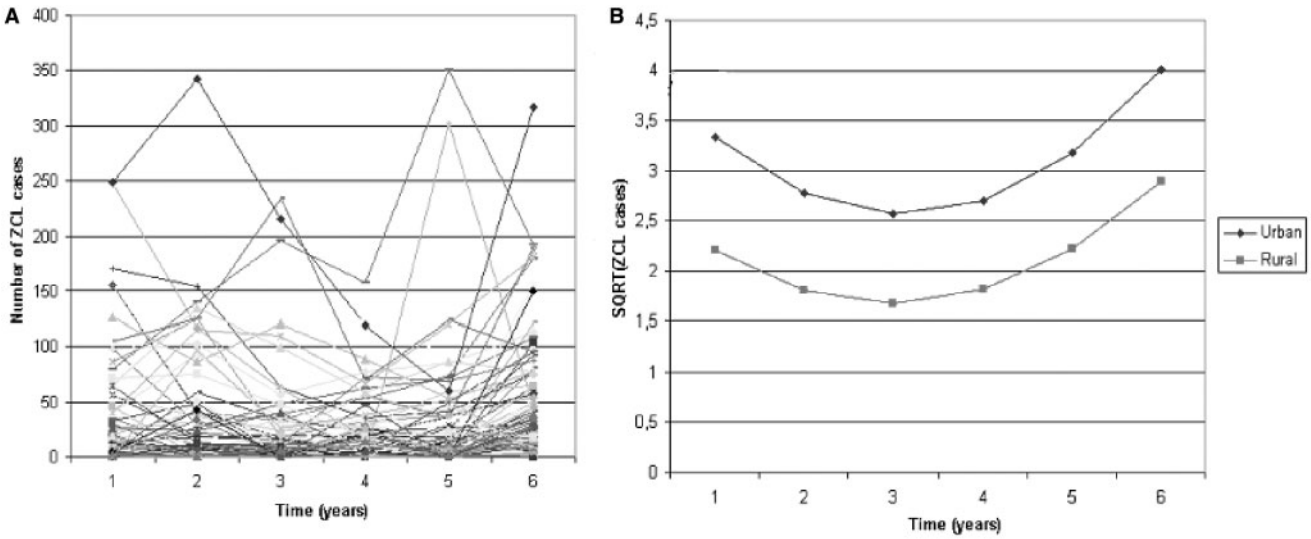

Figure 8 Left: Temporal evolution of raw sectoral ZCL counts. Right: Estimated average dynamics for urban/rural sectors

Gibbs sampling. We run three chains and 70 000 iterations (under over-relaxation) for each one till convergence was reached; convergence was checked via the Gelman-Rubin criterion[21] and visual inspection of the (overlapping) chains. The basic rule of thumb[22] that MC error should be <5% of the standard deviation for each parameter of interest was also satisfied (Table 2). After convergence was ensured, 50 000 more iterations per chain were run and posterior inference was based in these final iterations.

Urban and rural areas display different average profiles as far as the temporal ZCL dynamics are concerned (Figure 8). This feature is uncovered from the final stage of the analysis where

we examine sectoral heterogeneity in the evolution of ZCL rates via a linear mixed model (LMM). For this model, we worked with the square roots of the sectoral rates since this transformation brings data closer to the normality hypothesis in which the adopted methodology is based. The LMM model is a combination of fixed and random effects. Fixed effects are constituted by different coefficients for the (quadratic) evolution of the transformed rates over the study period. Regarding random effects, we allow for randomly varying intercepts and slopes; the best fitting covariance matrix is an unstructured one, indicating increased heterogeneity not only in the starting levels of the rates but also in the way the dynamics evolve through time. A simple structure was chosen for the residual covariance matrix after comparing likelihood based information criteria for alternative (mainly autoregressive in time) specifications.

Discussion

Over 70% of compulsory notified diseases in Tunisia are accounted for by zoonotic cutaneous leishmaniasis (ZCL), viral hepatitis and tuberculosis. Therefore, ZCL is a major public health problem for the country. Since 1982, an epidemic emerged in central Tunisia and expanded to the whole central and southern parts of the country (15/23 governorates are considered as endemic since 2002). The epidemics are cyclic and annually, two to three thousand cases are reported. The epidemic curve displays peaks and inter-epidemic periodicity of 5–6 years. Key factors driving spatio–temporal dynamics of the disease are presently unknown. These might include dynamics of rodent populations, dispersal of vectors, climate changes, vegetation and soil type and establishment of dense human settlements in areas where a sylvatic transmission of leishmaniasis is high (rodent-vector-rodent cycle). Currently, prediction of epidemic peaks and geographic spread is at an infant stage; thus, prevention programmes are difficult to design and implement. Evaluation of the importance of such parameters will be critical for understanding the epidemiological features of ZCL and to predict future evolution.

As evident from past experience in the study region, the development of agricultural and water resources projects might enhance ZCL transmission and introduce the parasite to new areas through environmental changes in several ways. In 1982, the Sidi Saâd dam was constructed in northern Sidi Bouzid and the previously flooded shoals surrounding the dam, dried. Consequently, chenopod plants (which prefer soil of higher salinity) and are the exclusive food for *P. obesus*, the main reservoir of ZCL, expanded in these areas. The dramatic increase in rodent population was associated with the emergence of ZCL epidemic in man. Furthermore, drilling of hundreds of wells in other areas might have increased humidity and enhanced the vector density (*P. papatasi*) exposing humans to higher transmission. All these factors may have contributed to the explosion of a ZCL epidemic in 1992 in the town of Sidi Bouzid. Health authorities implemented a control programme based on ploughing fields of chenopods around the town where *P. obesus* was very dense. This intervention that was planned and evaluated by Pasteur Institute, led to a significant reduction of the incidence among humans with a prevention fraction of disease exceeding 90%. Consequently, in 2000, the Tunisian National Control of Parasitic Diseases Program (PDP) adopted a new multi-disciplinary strategy to intervene in ZCL transmission. It introduced ecological surveillance of areas at risk for ZCL, before the occurrence of the epidemics. Ecological surveillance consists of surveying for the emergence of rodent colonies, such as *P. obesus*, previously shown to harbour and increase transmission of ZCL. Indeed, previous work in the Pasteur Institute showed that ~90% of *P. obesus* in the governorate of Sidi Bouzid showed evidence of anti-leishmanial antibodies at the end of the transmission cycle. Therefore, in all governorates where ZCL is endemic, PDP instituted rodent surveillance of a radius of 2 km around villages with >5000 inhabitants. Despite this significant effort, and the analysis of transmission dynamics of the disease in other regions, control strategies remain unsatisfactory, as indicated by the number of annual cases. A global and prospective vision regarding the assessment of exposure of communities to ZCL in case of rural development is lacking. Therefore, the ability to integrate demographic, epidemiological and ecological factors is critically needed.

This study depicted that sectors neighbouring to the Sidi Saâd dam still display increased ZCL rates. Indeed, two alternative techniques that identify spatial clusters and two alternative space–time clustering methods indicated that the most likely cluster of high incidence rates is formed by seven sectors located close to the dam. Our findings coincide with previous ones reporting significant heterogeneity in the dynamics of ZCL incidence rates; ZCL occurred in outbreaks, clustering in space and time. Explanatory variables like people living per household and population density did not have strong predictive power at the sectoral level in contrast to the urban/rural index. To our knowledge this is the first study that examines the relationship of such variables with leishmaniasis. Our findings of increased incidences in urban areas support the hypothesis of increased incidences in peri-urban environments due to changes in sandfly/rodent living habits over the last years. We plan to explore this hypothesis further in a forthcoming publication, by analysing a data set of geo-referenced ZCL cases, collected in the study area during 2004.

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Conflict of interest: None declared.

KEY MESSAGES

- The Sidi Saâd dam—constructed in the early eighties and identified by previous studies as a major reason for the first outbreak of ZCL in central Tunisia—seems to be still related to increased incidence rates.
- The rate of Leishmania infection depended on whether populations were in urban or rural location. Increased incidences occur in urban areas.
- Average ZCL dynamics during the study period (1999–2004) can be described by quadratic curves. An outbreak occurred during 2004.

References

[1] Ben Salah A, Louzir H, Chlif S *et al*. The predictive validity of naturally acquired delayed-type hypersensitivity to leishmanin in resistance to leishmania major-associated cutaneous leishmaniasis. *J Infect Dis* 2005;**192:**1981–87.

[2] Neouimine NI. Leishmaniasis in the eastern Mediterranean region. *Eastern Mediterr Health J* 1996;**2:**94–101.

[3] Ben Rachid MS, Ben-Ismail R. Current situation in regard to leishmaniasis in Tunisia. *Research on Control Strategies for the Leishmaniases. Proceedings of an International Workshop held in Ottawa*, June 1–4, 1987, Canada: IDRC-CIID, Manuscript report 184e.

[4] Kambawi S. Environmental manipulation in the control of a zoonotic cutaneous leishmaniasis focus. *Archives d'Institut Pasteur Tunis* 1993;**70:**383–90.

[5] Thomson M, Elnaiem DA, Ashford RW, Conner S. Towards a kala-azar risk map for Sudan: mapping the potential distribution of Phlebotomus orientalis using digital data on environmental variables. *Trop Med Int Health* 1999;**4:**105–13.

[6] Elnaiem DA, Schorscher J, Bendall A *et al*. Risk mapping of visceral leishmaniasis: the role of local variation in rainfall and altitude on the presence and incidence of kala-azar in eastern Sudan. *Am J Trop Med Hyg* 2003;**68:**10–17.

[7] Assunção RM, Reis IA, Oliveira CL. Diffusion and prediction of leishmaniasis in a large metropolitan area in Brazil with a Bayesian space-time model. *Stat Med* 2001;**20:**2319–35.

[8] Machado-Coelho GLL, Assunção RM, Mayrink W, Caiaffa WT. American cutaneous leishmaniasis in southeast Brazil: space-time clustering. *Int J Epidemiol* 1999;**28:**982–89.

[9] Werneck GL, Costa CHN, Walker AM, David JR, Wand M, Maguire JH. Multilevel modeling of the incidence of visceral leishmaniasis in Teresina, Brazil. *Epidemiol Infect* 2006;**135:**1–7.

[10] Kulldorff M. Spatial scan statistics: models calculations and applications. In: Balaktrishnan N. and Glaz J. (eds). *Recent Advances on Scan Stastics and Applications*. Boston, USA: Birkhauser, 1999.

[11] Kulldorff M. A spatial scan statistic. *Communications in Statistics: Theory and Methods* 1997;**26:**1481–96.

[12] Chaput EK, Meek JI, Heimer R. Spatial analysis of human granulocytic ehrlichiosis near Lyme, Connecticut. *Emerg Infect Dis* 2002;**8:**943–48.

[13] Bakker MI, Hatta M, Kwenang A *et al*. Population survey to determine risk factors for Mycobacterium leprae transmission and infection. *Int J Epidemiol* 2004;**33:**1329–36.

[14] Jennings JM, Curriero FC, Celentano D, Ellen JM. Geographic identification of high gonorrhea transmission areas in Baltimore, Maryland. *Am J Epidemiol* 2005;**161:**73–80.

[15] Kulldorff M. and Information Management Services, Inc. SaTScan™ v6.0: software for the spatial and space-time scan statistics. http://www.satscan.org/, 2005.

[16] Kulldorff M, Heffernan R, Hartman J, Assunção RM, Mostashari F. A space-time permutation scan statistic for the early detection of disease outbreaks. *PLoS Med* 2005;**2:**216–24.

[17] Dunson DB. Commentary: practical advantages of Bayesian analysis of epidemiologic data. *Am J Epidemiol* 2001;**153:**1222–26.

[18] Besag J, York J, Mollie A. Bayesian image restoration, with two applications in spatial statistics. *Ann I Stat Math* 1991;**43:**1–59.

[19] Mollie A. Bayesian mapping of disease. In: Gilks WR, Richardson S, Spiegelhalter DJ (eds). *Markov Chain Monte Carlo in Practice*. New York: Chapman & Hall, 1996;359–79.

[20] Verbeke G, Molenberghs G. *Linear Mixed Models in Practice: A SAS Oriented Approach*. Springer, 1996.

[21] Gelman A, Rubin DB. Inference from iterative simulation using multiple sequences. *Stat Sci* 1992;**7:**457–511.

[22] Spiegelhalter D, Thomas A, Best N, Lunn D. WINBUGS user manual version 2.0, 2004.