SPATIAL CLUSTERING AND EPIDEMIOLOGICAL ASPECTS OF VISCERAL LEISHMANIASIS IN TWO ENDEMIC VILLAGES, BARINGO DISTRICT, KENYA

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Abstract. Visceral leishmaniasis (VL) seroprevalence in Kenya is unknown because of the lack of a practical and accurate diagnostic test or surveillance system. A novel serological assay was used to estimate the seroprevalence of Leishmania-specific antibodies, and Global Information System and spatial clustering techniques were applied to study the presence of spatial clusters in Parkarin and Loboi villages in Baringo District in 2001. VL seroprevalences were 52.5% in Parkarin and 16.9% in Loboi. Significant associations among seropositivity and house construction, age, and proximity to domestic animal enclosures were found. A significant spatial cluster of VL was found in Loboi. The spatial distribution of cases in the two villages was different with respect to risk factors, such as presence of domestic animals. This study suggests that disease control efforts could be focused on elimination of sand fly habitat, placement of domestic animal enclosures, and targeted use of insecticides.

INTRODUCTION

The leishmaniases are a group of mainly zoonotic infections caused by protozoan parasites of the genus Leishmania. These infections produce a variety of clinical diseases depending on the virulence or tropism of the parasite and differential host immune responses.1 The World Health Organization estimates that with 350 million people at risk worldwide, 12 million people are infected with Leishmania parasites and that as many as 2 million new cases occur each year. Recent reports detail a visceral leishmaniasis (VL) pandemic in the Horn of Africa and parts of India, Nepal, Bangladesh, and Central and South America. A major epidemic reported in the Sudan from 1989 to 1993 was responsible for the deaths of approximately 10% of that country’s population.2

Whereas the definitive diagnosis of VL leishmaniasis requires demonstration of parasites from smears or in vitro cultivation, these methods can be time consuming and involve at least one invasive, and at times risky, procedure such as lymph-node biopsy, splenic or bone marrow aspiration. A variety of serological methods, including indirect immunofluorescence (IFA),3 enzyme-linked immunosorbent assay (ELISA),4,5 and polymerase chain reaction (PCR)6 are associated with a number of problems including cross-reactivity with other pathogens, high cost and/or need for sophisticated equipment. The direct agglutination test (DAT)7 is a sensitive, specific, and simple test, but the main disadvantage is that it can have high background seropositivity in endemic areas.

New diagnostic tests for VL have been a major focus of numerous research groups.8,9 Martin and co-workers reported the use of a soluble antigen prepared from Leishmania donovani as the foundation for an ELISA that could detect specific IgG antibodies in kala azar patients.10 Soluble antigens produced by Leishmania (exo-antigens) are released into the vertebrate host and sand fly vector. The assay was improved and developed into an ELISA that can detect Leishmania-specific IgM and IgG antibodies in patients with visceral and cutaneous leishmaniasis.11 In preliminary studies, the sera from 129 visceral leishmaniasis patients (Brazil, Italy, North Africa, Nepal) with sera from matched controls were tested. The test reported a sensitivity of 98.2%. Testing this ELISA with small subsets of naïve North American normals, Kenyan endemic normals, malaria, African trypanosomiasis, echinococcosis, filariasis, and schistosomiasis case patients yielded a specificity of better than 97%.10–12

Visceral leishmaniasis, caused by Leishmania donovani, is endemic in Baringo District, Kenya. The first case of VL was recorded in 1948 in the District Hospital at Kabarnet.13 Some scientists believe that nomadic Turkana may have introduced the disease into the area from the north. Others speculate that Kenyan soldiers returning from North Africa after World War II were responsible for introduction of the parasite. Phlebotomus martini is the vector of the parasite, and man is the only known reservoir.14 Currently, the disease is widespread throughout Baringo District.

A number of studies have used diagnostics, epidemiologic surveys, and Global Information System (GIS) technology to identify potential risk factors with several tropical diseases.15–17 The current study applied a multidisciplinary approach with integration of new technologies. The objectives in this study were to determine in two villages in a VL-endemic area of the Baringo District of Kenya 1) the current seroprevalence of Leishmania-specific antibodies, 2) the presence of spatial clustering (hot spots) of seropositive cases, and 3) the identification of associations with certain risk factors in those same villages.

MATERIALS AND METHODS

Study area. Baringo District is the only region in Kenya where both VL and cutaneous leishmaniasis (CL) have been found together.18 It covers an area of approximately 10,000 km² and is located north of the Equator in Kenya’s Rift Valley Province. Marigat Township is central to this region and a
site where most VL studies have been conducted in the past. Generally, the district includes a range of landscapes varying between fertile highlands as high as 2,700 m elevation and semiarid lowland at about 900 m elevation. The Tugen hills and the adjacent highlands in the southwest receive 1,200–1,500 mm average yearly rainfall and have daily average temperatures ranging from 10°C to 32°C. In contrast, lowland areas to the west, east, and north of the Tugen Hills receive annual rainfall from 300 to 700 mm and the temperatures vary between 16°C and 42°C. The rainy season is from March to September, with maximum rainfall in May and August. Three main ethnic groups, all classified as agropastoralists, inhabit Baringo District: the Tugen, Pokot, and Njemps. The district is sparsely populated due to harsh physical and climatic conditions. There have been no reports of cutaneous or mucocutaneous leishmaniasis in the two study areas.

**Study sites.** This epidemiologic study was conducted in the villages of Parkarin and Loboi (0°35’N latitude, 36°06’E longitude) in the Rift Valley Province, Baringo District of central Kenya, and approximately 207 kilometres northwest of Nairobi. The population of Parkarin (N = 286 inhabitants) was spread over an area of 3.3 km². The population of Loboi (N = 643 inhabitants) was spread over an area of 2.3 km². Both villages, being only 7 km apart, have the same subtropical climate. Outwardly, the two villages appear to be very similar and both have had a long history of kala azar. The villagers live in family units (homesteads) each consisting of several huts. Within the homestead, householders may live together in one house or in 2–3 houses. Each homestead has domestic animals including cows, goats, sheep, chickens, and dogs. Animal pens and corrals for animals are built adjacent to or near the buildings in each homestead, and in some cases the buildings were within the corral. The economic status of the population of Parkarin is uniformly low. Most villagers live in huts made of sticks and mud, tend to domestic animals, and have no income. Terrain throughout the village is very dry with scattered Acacia trees, thorn bushes, and little ground vegetation. The population of Loboi is a mix of very poor families, day laborers, shopkeepers, and professional staff working at the Lake Bogoria National Park. Loboi has a health post, a number of shops, schools, and public utilities. Parkarin has no health post, therefore, residents must travel 7 km to Marigat when in need of medical assistance. These health posts are staffed by nurses and community health workers who are responsible for patient screening for kala azar, as well as taking blood smears and providing malaria treatment to patients. Although the workers are familiar with kala azar clinically, there are no tools for diagnosing or treating this disease in the endemic area. Another dramatic difference between the two villages is the amount of vegetation. Loboi has more overhead cover from Acacia trees, whereas Parkarin has more open areas with denser scrub and a tree line of Acacia concentrated along the irrigation canal. Loboi also has more termite mounds (N = 188; 87.1 mounds/km²) than Parkarin (N = 113; 33.7 mounds/km²).

**Study population.** This study included 489 (53%) research volunteers from the population of Loboi and Parkarin. It was composed of individuals of both sexes, 5 years of age or older, that volunteered for the study. All participants had been living in the community for at least 3 months and called the community their primary home. This study was approved by the Walter Reed Army Institute of Research (WRAIR) Human Use Review Committee (HURC) and the Kenya Medical Research Institute (KEMRI) Ethical Review Committee. Informed written consent was obtained from all volunteers prior to the employment of any examining, sampling, and questioning measures. Written parental consent was obtained for children under 18 years old. Children under the age of 5 years and temporary visitors to the communities were excluded from the study.

**Survey components.** A community-wide survey consisting of a census, GIS mapping, blood sampling by venipuncture, medical histories, and epidemiologic questionnaires was conducted in the two villages during the period from May to July 2001. The epidemiologic questionnaire captured information concerning house construction; the presence and location of domestic animals; the presence of domestic dogs; the proximity of the resident from typical sand fly breeding sites (termite mounds and animal enclosures); how long the family had lived at the present site; and, the daytime occupation of each resident. Each participant was assigned a unique personal identifier and household address. All participant information was recorded in a database and linked to diagnostics results.

**Medical examination.** A team composed of two clinicians experienced in VL diagnosis, a guide, and several clinical assistants and translators worked systematically through each village. Whereas the clinician conducted the medical examination, an assistant administered the medical history questionnaire. The study clinician recorded the participant’s condition and any clinical abnormalities and/or VL-related symptoms. Degree of splenomegaly was determined by palpitation and graded by use of the Hackett model.¹⁹

**Serology.** Seroprevalence was determined by an ELISA based on an antigen derived from Leishmania donovani promastigotes cultivated in a protein-free, serum-free medium.¹¹,¹² Leishmania donovani (Ld, Strain WR0130E) washed promastigotes were inoculated into 200 mL of a defined, conditioned protein-free medium (XOM) to give a final density of 1 × 10⁶ cells/mL. The parasites were incubated at 25°C for 72 hours in roller bottles. Thenceforth, the spent medium was harvested by centrifugation at 11,000 × g for 20 minutes and the relative protein concentration of the soluble antigens was estimated by measuring the optical density at 280 nm.²⁰

Plate sensitization was effected by coating polystyrene, 96-well microtiter plates (Immulon 4, Dynatech Laboratories, Chantilly, VA) with 100 μL of the Ld antigen solution (5 μg protein per well).

Plates were then blocked with 0.5% casein (Sigma Chemical Co., St. Louis, MO) in PBS for 1 hour at room temperature. The blocking buffer was removed by aspiration and the serum samples (100 μL of 1:1000 dilution) and appropriate controls were added to the microtiter plate and the plate incubated at 26°C for 40 minutes. After washing with 0.05% PBS-Tween-20 (PBS-Tween) buffer four times, goat anti-human IgG (whole molecule) conjugated with horseradish peroxidase (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD) was added at a 1:5,000 dilution in blocking buffer and the plate incubated at 26°C for 30 minutes. The plate was then washed four times with PBS-Tween buffer and 100 μL of TMB substrate (Kirkegaard & Perry Laboratories Inc.) was added to each well. The plate was incubated in the dark, and the optical density (OD) was periodically read at 650-nm wavelength in an ELISA plate reader (Molecular Devices,
Menlo Park, CA) until the OD value of a reference positive control reached 0.75. At this point, 100 µL of a stop solution (1.0 M phosphoric acid) was applied to the plate and the final OD reading taken at 450 nm. A positive reference serum was used in all plates, and only interassay variation of less than 10% was accepted. The lower limit of positivity (cutoff) was determined by the mean of the negative controls subset + 3 standard deviations.21

Global Information System integration. Global Information System (GIS) and spatial clustering statistical techniques were applied to evaluate the presence of high-risk areas and spatial clustering Leishmania seroprevalence. All residences, streets, public buildings, latrines, termite mounds and other features of interest in the villages were mapped using Trimble ProXR GPS receivers to permit the calculations of latitude, longitude, and altitude. During the mapping, each house was assigned a unique household identification number and information about construction type was also recorded. Pathfinder Office software, version 1.1 (Trimble Navigation, Sunnyvale, CA) was used to perform differential correction of all feature locations and to create a locational database for use in GIS analyses. Seroprevalences were calculated by household. For the GIS analysis, ArcView software, version 3.0, (Environmental Systems Research Institute, Inc., Redlands, CA) was used to merge the locational database with the study group data. A surface analysis of cumulative Leishmania risk across the entire village at the household level was performed by using the Inverse Distance Weighted (IDW) interpolator model22 using ArcView Spatial Analyst v.1.0 (ESRI, Inc. Redlands, CA). IDW weights the contribution of each input (control) point by a normalized inverse of the distance from the control point to the interpolated point. IDW assumes that each input point (household) has a local influence that diminishes with distance. It weights the points closer to the processing points more than those farther away. A specified number of points (or all points) within a specified radius are used to determine the output value for each location.

Statistical analysis. Chi-square and Fisher’s exact tests were used to compare differences in proportions. The Student t test or Mann-Whitney U, a nonparametric test, was used to compare differences in continuous variables. To evaluate ELISA results and splenomegaly size on a qualitative scale, the Bartholomew test was applied.23 Because the study population included sets of familial members, the data were presumed to violate the standard logistic regression assumption of independent response probabilities across observations. Potential risk factors and VL seropositivity were evaluated by odds ratios (OR) using random effects logistic regression for each village, where the homesteads were defined as the group variable.23 To describe the amount of aggregation existing in VL seropositivity within homesteads and/or households units, the percentage of explained variance attributed (rho) was estimated after adjusting for age and gender. All reported confidence intervals (CI) are 95%, and all reported P values are two-sided. These statistical analyses were carried out using Stata v. 6.0 (Stata Corporation, College Station, TX).

Spatial analysis. To evaluate the presence of spatial disease clusters and to identify focalized areas of “high risk” for VL, we applied a spatial scan statistic.25 The spatial scan statistic is well suited for geographic disease surveillance. This method takes into account the uneven spatial geographic distribution of cases and population densities; it does not require a priori assumptions about the number, place, or size of locations that may be identified as clusters; it adjusts for multiple testing inherent in the search for multiple clusters; and it searches for either high or low incidence or prevalence areas. The spatial scan statistic works by aggregating together the unique combinations of small-area geographies that have a high probability of being clusters. This statistical test can detect spatial clusters of any size located anywhere in the study area. The spatial scan statistic imposes a geographic circular window to perform purely spatial analysis of varying size on the map surface and allows its center to move so that at any position and size across the study area, the circular window includes different sets of adjacent households. As the circular window is placed at each household, this spatial method creates a large number of distinct geographical circles, with different sets of households areas within them, and each is a possible candidate for containing a spatial cluster of prevalent leishmaniasis cases. For any given geographic position of the household, the radius of the circular window varied continuously from zero to a previously user-defined maximum. Although the choice of maximum cluster window size is somewhat arbitrary, it is important to make the choice of maximum cluster window a priori to avoid the problems of multiple hypothesis testing. In this analysis, we chose 50% of the total study population as the maximum to consider. By choosing 50% as the maximum, we evaluated all sizes from zero percent up to 50% and adjusting for the multiple testing related to each of them. We assumed that the geographic spatial distribution of leishmaniasis cases follow a Bernoulli distribution. The most likely spatial cluster was determined by computing maximum likelihood ratios. The spatial scan statistic uses the Monte Carlo simulation to evaluate the statistical significance of the most likely spatial cluster. In this study, the simulated P value of the statistic was obtained through 9,999 Monte Carlo simulations, where the null hypothesis of no high spatial leishmaniasis clusters was rejected at an α level of 0.05. Spatial risk patterns for VL was expressed as relative risk and was estimated by household as the observed VL seroprevalence/expected VL seroprevalence by using the spatial scan statistic. These spatial analyses were carried out by using the SaTScan v.2.1 software.26

RESULTS

Study area and population. The census conducted at the onset of this study showed that there were a total of 643 villagers in Loboi and 286 villagers in Parkarin living in 235 homesteads: 176 in Loboi and 59 in Parkarin. Population density in Loboi was approximately four times that in Parkarin, with 279.6 and 85.4 persons per km², respectively; 52.5% of the villagers were males, and 62.2% were less than 20 years old with a median age of 15 years. The mean number of persons per homestead in Loboi was 3.7 (standard deviation [SD] = 2.7) and in Parkarin it was 4.8 (SD = 3.8). There was no significant difference in mean age and gender between both villages. In wall construction, there was a clear and significant difference in both villages. Parkarin had more houses made of mud and sticks than Loboi (78.0% versus 31.4%, P < 0.05) (Table 1). Residents of Parkarin had lived an average of 11.5 years (SD = 8.9) in their community, whereas residents of Loboi had lived in theirs an average of 9.8 years (SD = 10.7). People generally resided longer in their houses in
Parkarin (mean 4.5 years; SD = 4.5) than in Loboi (mean 2.8 years; SD = 3.3).

**Sample population.** From the potential study population, 289 villagers in Loboi and 200 in Parkarin volunteered to participate in this study, representing 44.9% and 69.9%, respectively. Of these, 245 (50%) were males (Table 1). The participants lived in 180 homesteads (76.6% of the total 235 homesteads). The population studied in Loboi was not significantly older than that of Parkarin ($P = 0.924$). The average number of people per homestead sampled in Loboi was significantly lower than in Parkarin ($P < 0.001$). There were also significant differences with respect to wall construction, domestic animals, presence of goats, dogs and where animals were kept at night between villages ($P < 0.05$) (Table 2).

**Clinical and serological findings.** Of the 481 (98.4%) individuals examined, a total of 155 (32%) presented with some degree of splenomegaly: 90 (45%) of the 200 participants in Parkarin and 65 (23%) in Loboi. Of these 155 individuals, only 11 (7.1%) presented with other classic signs and symptoms of kala azar such as pallor, weight loss, and fever. However, we were unable to rule out other potential causes of chronic fever such as typhoid fever or malaria.

The overall antibody prevalence by ELISA was 31.5% (154 of 489). The antibody prevalences were significantly different between the two villages, 52.5% and 16.9% in Parkarin and Loboi, respectively ($P < 0.001$). The difference in seroprevalence between females ($29.4\%, 72\text{ of } 245$) and males ($33.6\%, 82\text{ of } 244$) was not statistically different ($P = 0.364$), even when the analysis was performed for each village separately. The mean age of positive subjects (25.6 years) was greater than that of subjects without antileishmanial antibodies (19.6 years). The difference was statistically significant whether the population was analyzed as a whole ($P < 0.001$) or separately for each village. There was a strong linear association of increasing seroprevalence of VL with age groups ($P < 0.001$ by $\chi^2$ for trend) in both villages (Figure 1).

In Loboi, a significant level of aggregation was found only within homesteads units (rho = 27%, 95% CI = 13 to 48, $P < 0.001$) and not within household units. Households or household aggregation did not explain a significant proportion of the variance of VL seropositivity in Parkarin.

**Epidemiologic factors.** From the serological and epidemiologic surveys, we found a number of factors strongly associated with altered risk of leishmaniasis. For each 10-year increase in age, the likelihood of being seropositive was higher.

### Table 1

<table>
<thead>
<tr>
<th>Feature</th>
<th>Population</th>
<th>Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feature</td>
<td>All people No.</td>
<td>Loboi No. (%)</td>
</tr>
<tr>
<td>No. of homesteads</td>
<td>929</td>
<td>643 (69.2)</td>
</tr>
<tr>
<td>No. of homesteads</td>
<td>235</td>
<td>176 (74.9)</td>
</tr>
<tr>
<td>Wall construction (stick and mud)</td>
<td>101</td>
<td>55 (31.4)</td>
</tr>
<tr>
<td>Mean (SD) number of people per homestead</td>
<td>4.0 (3.0)</td>
<td>3.7 (2.7)</td>
</tr>
<tr>
<td>Gender</td>
<td>439</td>
<td>305 (47.7)</td>
</tr>
<tr>
<td>Male</td>
<td>486</td>
<td>334 (52.3)</td>
</tr>
<tr>
<td>Age group (years)</td>
<td>287</td>
<td>201 (33.2)</td>
</tr>
<tr>
<td>&lt; 10</td>
<td>102</td>
<td>63 (31.4)</td>
</tr>
<tr>
<td>10–20</td>
<td>252</td>
<td>167 (27.6)</td>
</tr>
<tr>
<td>21–30</td>
<td>184</td>
<td>144 (23.8)</td>
</tr>
<tr>
<td>31–40</td>
<td>87</td>
<td>63 (10.4)</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>57</td>
<td>30 (5.0)</td>
</tr>
<tr>
<td>Mean (SD) age (years)</td>
<td>18.0 (14.1)</td>
<td>17.9 (13.6)</td>
</tr>
</tbody>
</table>

Note: Denominators total varied slightly due to missing data; SD, standard deviation.

* $P < 0.05$ by chi-square or Fisher’s exact test compared between villages.

### Table 2

<table>
<thead>
<tr>
<th>Feature</th>
<th>Visited No.</th>
<th>Loboi No. (%)</th>
<th>Parkarin No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of homesteads in the village</td>
<td>180</td>
<td>127 (70.6)</td>
<td>53 (29.4)</td>
</tr>
<tr>
<td>Presence of domestic animals</td>
<td>No</td>
<td>71</td>
<td>56 (45.5)</td>
</tr>
<tr>
<td>Yes</td>
<td>105</td>
<td>67 (54.5)</td>
<td>38 (71.7)</td>
</tr>
<tr>
<td>Presence of cattle</td>
<td>No</td>
<td>114</td>
<td>81 (65.9)</td>
</tr>
<tr>
<td>Yes</td>
<td>62</td>
<td>42 (34.1)</td>
<td>20 (37.7)</td>
</tr>
<tr>
<td>Mean (SD) number of cattle</td>
<td>2.5 (4.9)</td>
<td>2.8 (5.6)</td>
<td>1.9 (3.1)</td>
</tr>
<tr>
<td>Presence of sheep</td>
<td>No</td>
<td>156</td>
<td>112 (91.1)</td>
</tr>
<tr>
<td>Yes</td>
<td>20</td>
<td>11 (8.9)</td>
<td>9 (17.0)</td>
</tr>
<tr>
<td>Mean (SD) number of sheep</td>
<td>1.4 (8.3)</td>
<td>1.7 (9.8)</td>
<td>1.2 (2.9)</td>
</tr>
<tr>
<td>Presence of goats</td>
<td>No</td>
<td>99</td>
<td>79 (64.2)</td>
</tr>
<tr>
<td>Yes</td>
<td>77</td>
<td>44 (35.8)</td>
<td>33 (62.3)</td>
</tr>
<tr>
<td>Mean (SD) number of goats</td>
<td>7.6 (12.4)</td>
<td>5.7 (17.3)</td>
<td>12.2 (12.4)*</td>
</tr>
<tr>
<td>Presence of chicken</td>
<td>No</td>
<td>91</td>
<td>66 (53.7)</td>
</tr>
<tr>
<td>Yes</td>
<td>85</td>
<td>57 (46.3)</td>
<td>28 (52.8)</td>
</tr>
<tr>
<td>Mean (SD) number of chicken</td>
<td>3.5 (5.2)</td>
<td>3.6 (5.5)</td>
<td>3.3 (4.5)</td>
</tr>
<tr>
<td>Presence of dogs</td>
<td>No</td>
<td>127</td>
<td>96 (78.7)</td>
</tr>
<tr>
<td>Yes</td>
<td>48</td>
<td>26 (21.3)</td>
<td>22 (41.5)</td>
</tr>
<tr>
<td>Animal shelters</td>
<td>Inside house</td>
<td>23</td>
<td>21 (17.4)</td>
</tr>
<tr>
<td>Adjacent corral</td>
<td>76</td>
<td>44 (36.4)</td>
<td>32 (64.0)</td>
</tr>
<tr>
<td>Away from homestead</td>
<td>2</td>
<td>1 (0.8)</td>
<td>1 (2.0)</td>
</tr>
</tbody>
</table>

Note: Denominators total varied slightly due to missing data; SD, standard deviation.

* $P < 0.05$ by chi-square or Fisher’s exact test compared between villages.
in Loboi (OR = 1.88, 95% CI = 1.32 to 2.69, \( P < 0.0001 \)) than Parkarin (OR = 1.52, 95% CI = 1.16 to 1.99, \( P = 0.002 \)). In univariate logistic regression analysis for Loboi, residents living in households made of sticks and mud, as well as presence of domestic animals, especially cattle, sheep, and goats, were more likely to be seropositive to VL. In the multiple logistic regression analysis adjusted for age and gender, these potential risk factors remained statistically significant associated to VL with the exception of cattle. In Parkarin, presence of domestic animals in households (especially sheep and goats) was negatively associated with a positive ELISA and appears to be protective in the univariate analysis (Table 5). However, only presence of goats remained negatively associated to VL in the multiple logistic regression analysis. Therefore, these data suggest an inverse odd ratio risk relationship in the two villages studied. In both villages gender, proximity to active or inactive termite mounds, and presence of dogs were not significantly associated with VL.

**Spatial analysis.** Based on the serological findings, we generated risk pattern maps by household for Loboi (Figure 2) and Parkarin (Figure 3) that defined areas of high risk. The spatial scan statistic revealed the presence of a spatial cluster of *Leishmania*-specific seropositives in the village of Loboi (Figure 4). This spatial cluster contained 11% (16 of 152) of the total houses sampled in the village and 29% (14 of 49) of the total ELISA cases. Villagers living within this spatial cluster were 3.3 times more likely to have *Leishmania*-specific antibodies than people living in other areas in the village (Table 4; \( P = 0.0003 \)). In addition, this spatial cluster occurred in the northwestern corner of the village in a wooded area in very close proximity to a seasonal river, a kind of setting commonly associated with VL. The rest of the village consisted of more open areas with scattered *Acacia* trees, shrubs, and little ground cover. There were two other spatial clusters that appear graphically (but not statistically) in Loboi, and their settings were nearly identical to that of the primary cluster. There was not a specific high risk pattern and no significant spatial cluster was found (\( P = 0.296 \)). The spatial distribution of seropositives among households appeared to be homogeneous (Figure 3), and this may be due to the high seroprevalence of VL found in this village.

**DISCUSSION**

To our knowledge, this study represents the first geographic epidemiologic analysis in two villages to study the microepidemiology of VL in Africa and probably in other continents. We identified microgeographic areas where there is more likely to be high *Leishmania* exposure and spatial cluster of seroposposites through GIS mapping and spatial statistic techniques.

Baringo District has been the focus of many epidemiologic studies on leishmaniasis. As early as 1983, Jahn and Diesfeld\(^{27}\) used a crude ELISA for detecting *Leishmania* specific antibodies in VL patients seen in the Baringo District Hospital at Kabarnet. With limited success, they applied this ELISA in clinical routine and sero-epidemiologic surveys. Low titers were recorded in healthy individuals from VL foci, but their values were easily distinguished from those of active patients. After that small study, Jahn and co-workers, using the same ELISA, showed that children between 2 and 15 years old were the most affected age group for VL and that male patients predominated slightly at 57%.\(^{28}\) All VL cases came from the semiarid and arid parts of the district below 1500 m, where pastoralism predominated. A positive correlation was reported for active cases and the proximity of homes to seasonal rivers. However, no satisfying explanation was found for the clustering of cases. ELISA values above the cutoff, taken as the borderline nonspecific reaction, were found in about half of the population, suggesting that asymptomatic infections were common.\(^{29}\)

Schaefer and colleagues tested Baringo District inhabitants from 26 clusters, averaging 97 people, with the leishmanin skin test (LST).\(^{30}\) There was an obvious aggregation of LST positivity centered on recent VL cases with approximately 11% of the 2,411 individuals tested having a marked DTH reaction. The level of LST positivity was twice as high in males as females. LST positivity increased with age to a stable level after 15 years of age, reflective of an endemic situation. Schaefer and co-workers later measured *Leishmania*-specific antibodies using the direct agglutination test (DAT) in a defined, endemic rural area of Baringo District composed of 30

**FIGURE 1.** Seroprevalence of *Leishmania*-specific antibody by age group and study sites in Kenya, 2001.
clusters, each averaging 98 people. From 2,934 individuals samples, 78 were DAT seropositive; 54 of those had a history of VL. The seroconversion rate was 9 of 1,000 person-years of observation among 2,332 seronegative individuals retested the following year. During the entire study period, VL was diagnosed in only 10 patients, with an incidence rate of 2.2 of 1,000 person-years of observation. Household contacts of individuals with previously confirmed VL had a higher frequency of DAT positivity than the rest of the population. These results suggested domiciliary transmission.

Using another approach, Schaefer and colleagues used PCR with capillary blood samples dried on filter paper. Assaying 20 parasitologically confirmed VL case-patients, 21 subclinical cases, and 11 healthy controls from a longitudinal study of anthropoontic VL in Baringo District, they were able to detect *Leishmania* DNA 10 months before diagnosis and up to 3 years after treatment that was classified as successful. Alarmingly, these findings showed that subclinical and treated cases may remain potential reservoirs for long periods.

The study results suggest that males and females have similar risks to VL, and seropositive cases are clustered within households. The results of this study are interesting and, at the same time, confounding. We see dramatic differences in seroprevalences between the two villages (16.9% versus 52.5%). GIS mapping and spatial scan statistic showed the presence of a spatial cluster only in the village with the lowest seroprevalence. This spatial cluster found may be related to the wooded environment of the northwestern corner of Loboi that provides a good habitat for sand flies. When merging ELISA data with the results of the epidemiologic survey, we find strong positive associations between seropositivity, age, and poor living conditions. House building materials (mud and sticks) related to lower socioeconomic status were associated with higher risk. However, we find that the presence of domestic animals is only associated with increased risk in Loboi. The figures from Parkarin indicate that there was an inverse relationship between the presence of animals and exposure to *Leishmania*. For both villages, there was a significant association between ELISA seroprevalence and distance to the nearest corral ($P = 0.015$ for Loboi and $P = 0.004$ for Parkarin) (data not shown).

We surmise that this result may be due to the fundamental differences between the two villages with respect to proximity of rivers, vegetation cover, population density, and economic make-up of the population. However, there may be limitations in the statistical analysis due to the extremely high seroprevalence found in Parkarin and the small size of this village. Our data suggests strategies for targeted control and for prioritization of scarce resources. A community-based VL

![Figure 2](www.ajtmh.org)
Figure 3. Spatial risk patterns for visceral leishmaniasis in Parkarin, Kenya, 2001. This figure appears in color at www.ajtmh.org.

Figure 4. Map showing location of the most likely spatial cluster of visceral leishmaniasis in Loboi, Kenya, 2001. This figure appears in color at www.ajtmh.org.
control or suppression program could be formulated on educating residents of this endemic area about the risk associated with house construction and the proximity of domestic animals to one’s living quarters. These points are likely related to sand fly habitat, whereby the sand flies seek out a favorable habitat in this arid setting that would be supportive of egg laying and survival of immature forms. Most residents kept their animals at night in corrals no more than 30 m from their homes. A higher and statistically significant difference (P = 0.001) of the ratio of animals to people was found in Parkarin (median = 17.5) than in Loboi (median = 5). The animals serve as a blood source, and the accumulation of animal dung may be attractive to the sand fly, drawing the vectors into closer association with the humans and increasing the risk of being bitten. In addition, the edges of the corral constructed from cut thorn bushes provide good areas for rodent burrows and nesting sites. Positive correlation of disease and the presence of domestic animals have been shown in some studies, whereas others have shown no significant effect and have noted an inverse relationship between domestic animals and disease presence. However, the nature of the relationship between disease transmission and domestic animals is complex. Among the factors that can affect feeding behavior, host odor, heat loss, and CO₂ production are important stimuli for orientation of blood sucking insects in the open (i.e., nonforest) situations, diverting blood feeding from people to animals. Blood meal studies of P. martini, the vector of kala azar in the study area, has shown it to have a broad host range among domestic animals, with a preference for goats. Zooprophylaxis explains the situation in Parkarin where the higher ratio of goats appears to have a protective effect on the human population.

VL is a grave public health problem in this area that imposes an additional strain on the local health authorities and is unlikely to be resolved by the current strategies. Understanding the transmission dynamics of VL could lead to sustainable prevention and control measures. Alternative methods of vector control, other than the conventional indoor spraying of houses with residual insecticide (which can be prohibitively expensive) should be considered. Repeated pesticide applications to the whole community can reduce sand fly numbers, but as the reduction is short lived, this method is used only in epidemics.

The termite hills associated with leishmaniasis transmission in Kenya are common throughout the two villages. Although no association was shown in this study, the termite hill habitat is the favored breeding and resting sites of P. martini. This points to targeted control strategies such as insecticidal applications to resting habitats such as termite mounds and insecticide barrier spraying. The feeding propensity of P. martini on livestock and the efficacy of zooprophylaxis could be enhanced by using the livestock not only to divert the vector from humans to animals but to attract them to contact with insecticide-treated livestock. Because P. martini is active during the night when people sleep, the use of treated bed nets to block transmission could provide considerable protection. A prospective intervention study would be needed to evaluate the effectiveness of targeting control interventions at high-risk areas identified by this study.

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