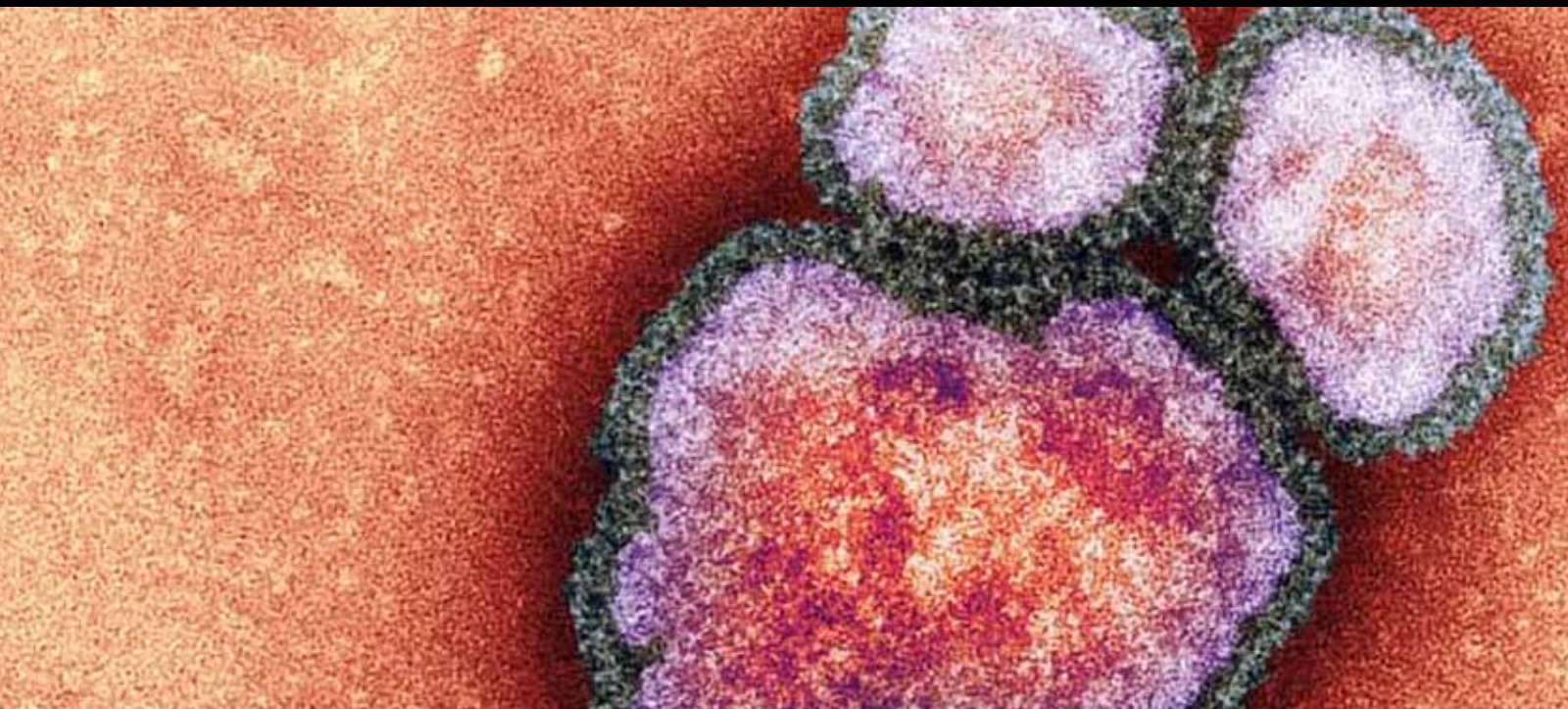


SPATIAL STUDIES ON VECTOR- TRANSMITTED DISEASES AND VECTORS

GUEST EDITORS: MARIA CORETI ROSA-FREITAS, NILDIMAR ALVES HONÓRIO,
CLÁUDIA TORRES CODEÇO, GUILHERME LOUREIRO WERNECK, AND NICOLAS DEGALLIER





Spatial Studies on Vector-Transmitted Diseases and Vectors

Journal of Tropical Medicine

Spatial Studies on Vector-Transmitted Diseases and Vectors

Guest Editors: Maria Goreti Rosa-Freitas,
Nildimar Alves Honório, Cláudia Torres Codeço,
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Contents

Spatial Studies on Vector-Transmitted Diseases and Vectors, Maria Goreti Rosa-Freitas, Nildimar Alves Honório, Cláudia Torres Codeço, Guilherme Loureiro Werneck, and Nicolas Degallier
Volume 2012, Article ID 573965, 5 pages

Survey of Water Bugs in Bankim, a New Buruli Ulcer Endemic Area in Cameroon, Solange Meyin A. Ebong, Sara Eyangoh, Estelle Marion, Jordi Landier, Laurent Marsollier, Jean-François Guégan, and Philippe Legall
Volume 2012, Article ID 123843, 8 pages

Using a Geographical-Information-System-Based Decision Support to Enhance Malaria Vector Control in Zambia, Emmanuel Chanda, Victor Munyongwe Mukonka, David Mthembu, Mulakwa Kamuliwo, Sarel Coetzer, and Cecilia Jill Shinondo
Volume 2012, Article ID 363520, 10 pages

A Predictive Spatial Model to Quantify the Risk of Air-Travel-Associated Dengue Importation into the United States and Europe, Lauren M. Gardner, David Fajardo, S. Travis Waller, Ophelia Wang, and Sahotra Sarkar
Volume 2012, Article ID 103679, 11 pages

Effects of Artificial Flooding for Hydroelectric Development on the Population of *Mansonia humeralis* (Diptera: Culicidae) in the Paraná River, São Paulo, Brazil, Marcia Bicudo de Paula, Almério de Castro Gomes, Delsio Natal, Ana Maria Ribeiro de Castro Duarte, and Luís Filipe Mucci
Volume 2012, Article ID 598789, 6 pages

Geographic Distribution of Chagas Disease Vectors in Brazil Based on Ecological Niche Modeling, Rodrigo Gurgel-Gonçalves, Cléber Galvão, Jane Costa, and A. Townsend Peterson
Volume 2012, Article ID 705326, 15 pages

Species Distribution Models and Ecological Suitability Analysis for Potential Tick Vectors of Lyme Disease in Mexico, Patricia Illoldi-Rangel, Chissa-Louise Rivaldi, Blake Sissel, Rebecca Trout Fryxell, Guadalupe Gordillo-Pérez, Angel Rodríguez-Moreno, Phillip Williamson, Griselda Montiel-Parra, Víctor Sánchez-Cordero, and Sahotra Sarkar
Volume 2012, Article ID 959101, 10 pages

Information System and Geographic Information System Tools in the Data Analyses of the Control Program for Visceral Leishmaniasis from 2006 to 2010 in the Sanitary District of Venda Nova, Belo Horizonte, Minas Gerais, Brazil, Lara Saraiva, Camila Gonçalves Leite, Luiz Otávio Alves de Carvalho, José Dilermando Andrade Filho, Fernanda Carvalho de Menezes, and Vanessa de Oliveira Pires Fiúza
Volume 2012, Article ID 254361, 9 pages

Distribution and Abundance of Phlebotominae, Vectors of Leishmaniasis, in Argentina: Spatial and Temporal Analysis at Different Scales, María Gabriela Quintana, María Soledad Fernández, and Oscar Daniel Salomón
Volume 2012, Article ID 652803, 16 pages

Use of Indicator Kriging to Investigate Schistosomiasis in Minas Gerais State, Brazil, Ricardo J. P. S. Guimarães, Corina C. Freitas, Luciano V. Dutra, Carlos A. Felgueiras, Sandra C. Drummond, Sandra H. C. Tibiriá, Guilherme Oliveira, and Omar S. Carvalho
Volume 2012, Article ID 837428, 10 pages

Editorial

Spatial Studies on Vector-Transmitted Diseases and Vectors

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Received 10 May 2012; Accepted 10 May 2012

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1. Space, the First Frontier

Space is the stage where factors leading to disease take place.

Landscape epidemiology, health geography, spatial epidemiology, and landscape ecology¹ are disciplines that share a fundamental concept: space.

Space as herein defined is the multidimensional extent in which elements and historical events—geological, physiological, ecological, climatic, economical, and cultural—concur and interact with humans to influence the presence, development, activity, and longevity of pathogens, reservoirs, and vectors, leading to different patterns of infection and disease.

Disease is a spatially-determined phenomenon [1–3]. The correct identification of spatial risk factors plays a key role in prediction, prevention, and control of disease [4–7].

The analysis of space and its role in diseases has been occupying thinkers since Hippocrates *circa* 400 B.C. Terms like tropical diseases, malaria, and American trypanosomiasis just to name a few, have geographically-oriented denominations that immediately refer to spaces where transmission occurs².

According to Hippocrates, an endemic disease is determined by the nature of a certain place. The term endemic³ entails in itself this concept. Hippocrates' ecological concept of disease was brought up again by Galen in the early Christian era and passed untouched through the modernization of science during the Renaissance.

2. The Study of Space

Spatial analysis is concerned with the geographic space, that is, observations that correspond to locations in space that capture their proximity in the real world. The interrelation between entities increases with proximity in the real world and their representation in geographic space and assessment using spatial analysis techniques are appropriate (Tobler's first law of geography, [8]) and in accordance with the concept of spatial dependence that forms the foundation of spatial analysis.

In 1939, Pavlovsky structured the theory of the natural nidity⁴ of transmissible diseases, known outside the Soviet Union only by late 1950s [2, 3]. Pavlovsky's theory consisted of 3 axioms: (1) diseases tend to be limited geographically; (2) this spatial variation arises from underlying variation in the physical and/or biological conditions that support the pathogen and its vectors and reservoirs; (3) if those abiotic and biotic conditions can be delimited on maps, then both contemporaneous risk and future change in risk should be predictable [9].

A landmark on spatial analysis was Snow's 1854 map of cholera deaths and the water pumps that supplied the city. The superposition of cholera cases and a main water pump gave support to his hypothesis that cholera was spread by the water [10].

Spatial analysis comprises a set of generic exploration methods and the visualization of data in the form of maps.

Nonetheless, spatial analysis is neither a technology for making maps nor a map is the sole objective to be achieved. The interaction with mapped variables resulting from spatial analysis is more challenging than the interaction with traditional maps and spatial records. Spatial analysis has changed our perspective of viewing a map. It has moved mapping from a historical role of input provider to a dynamic and essential element in the decision-making process [11].

Spatial analysis exploration and visualization methods allow not only the visual description of the distribution of variables but also the identification of patterns in the spatial distribution making it easier to comprehend the phenomena underlying these observations. Through these procedures it is possible to select the most accommodating inferential model [12], to choose the best explanatory hypothesis and propose control scenarios supported by the spatial relationships observed.

3. Spatial Studies Tools

Visual inspection of spatial distribution of data allows apprehending existing patterns. The translation of these patterns into a system of theoretical significance is an important tool in the investigation of the disease process.

Currently, spatial studies of disease and vectors involve the use of computational analyses and representation of geographic data using the so-called geographic information systems-GIS or spatial analysis tools, as in the broader sense used in this text. These spatial analysis tools perform the computational treatment of georeferenced data points, lines, and areas and store their attributes in relation to the earth surface and represented in a cartographic projection [12]. Advances in remote sensing, global positioning systems, computer software, and hardware led to the creation of powerful data exploratory tools.

A spatial-related geographic database is composed of georeferenced data input and integration, graph, and image processing functions, visualization, and plotting, spatial analysis tools, data storage, and retrieval in an organized form.

After being subjected to visualization techniques, hypotheses on spatial behavior of data are challenged to validation and corroboration through spatial analysis and theoretical models.

Besides being able to create static risk maps based on distributions of vectors, reservoirs and disease incidence, spatial analysis can model spatiotemporal dynamics and show how the spatial distribution of infectious diseases changes through space and time. Where field data are unavailable, predictors of disease risk can be applied.

Spatial analysis is both quantitative and qualitative. Neither quantitative nor qualitative methods are end tools. Quantitative spatial analyses with predictive models and qualitative scrutiny and theories help to gain a better understanding of visualized data and the underlying processes within.

Nature of spatial data affects the type of spatial analysis to be employed and interpretation.

Data selection, data cleaning, and preprocessing and, moreover interpretation of results are all part of the subjective qualitative interpretation. Subjective analysis should be ascertained as an important tool. The testing of different analyses should be extensively used to aggregate knowledge on the problem. Spatial analysis results should be used to corroborate or reject findings and applied to mathematical models for interpretation and theory construction. These in turn, will be used for building up knowledge on disease dynamics and ultimately for practical control issues like policy and management decisions and, for checking particular interventions.

4. In This Issue

The papers comprised in this special issue contain up-to-date methods of spatial analyses applied to the study of diseases and vectors. These papers investigate spatial and temporal scales, and develop risk maps and models aiming to increase our understanding as well as supporting decisions in control programs.

In order to reduce malaria transmission in Zambia, a spatial study was developed to guide the implementation of effective vector control measures, and increase the understanding of the interactions between epidemiological and entomological malaria transmission determinants and the impact of interventions (E. Chanda et al. in this special issue). Monitoring the impact of vector control through a spatial-based decision support system revealed spatial variations in the prevalence of infection and vectors which are susceptible to insecticides. It also enabled the characterization of the spatial heterogeneity and the identification of areas with reduced parasitaemia and increased insecticide resistance. The spatial-based decision support system proposed by E. Chanda et al. provided opportunity for rational policy formulation and cost effective utilization of limited resources for enhanced malaria vector control.

Major spatial changes induced by man may lead to disequilibrium that in turn may result in human disease. This is the hypothesis raised by Paula et al. (in this special issue). The closure of a dam in São Paulo, Brazil, favoured the proliferation of aquatic weeds, the main habitat of *Mansonia* mosquito species (M. B. de Paula et al. in this special issue). This event led to a dramatic increase of the *Mansonia humeralis* population, from 3 to >50%. *Ma. humeralis* is a persistent biter provoking nuisance in the human population and potentially facilitates the transmission of arboviruses. A spatial-oriented sustainable entomological control was advised for this area.

Although Brazil was declared free from Chagas disease transmission by the domestic vector *Triatoma infestans* by the World Health Organization in 2006, vector-transmitted human acute cases are still being registered (R. Gurgel-Gonçalves et al. in this special issue). In order to assess Chagas disease transmission risk, distribution models for 62 Brazilian triatomine species were generated (R. Gurgel-Gonçalves et al. in this special issue). Although most actual occurrences were recorded in open areas of the Brazilian savannah (*cerrado* and *caatinga*), spatial analyses and

distribution models showed that Brazil is, as its most, vulnerable to Chagas vector-borne transmission.

Spatial analysis was applied in a study of *Biomphalaria* snail species, which are the intermediate hosts of *Schistosoma mansoni* in Minas Gerais, Brazil, to optimize resource allocation (R. J. P. S. Guimarães et al. in this special issue). Kriging showed to be a rather robust tool since its results presented a good agreement with the field findings. This tool allowed the delimitation of the *Biomphalaria* distribution, characterizing the uncertainty of areas at risk.

Buruli ulcer is a debilitating human skin disease with an unknown transmission mode with epidemiological data linking it to swampy areas. Data available suggest that aquatic insects play a role in the dissemination and/or transmission of this disease. However, aquatic insect biodiversity and biology in Africa remain poorly documented. Entomological survey in Bankim, Cameroon, an area recently described as endemic for Buruli ulcer was conducted in order to identify the commonly occurring aquatic bugs and document their relative abundance, diversity, and spatial distribution (S. M. A. Ebong et al. in this special issue). Abundance, distribution and diversity of aquatic bugs varied according to type of aquatic environments and maybe used for future risk maps assessment.

Control actions for visceral leishmaniasis in Minas Gerais, Brazil, showed that the use of an automated database with geoprocessing was important to guide control measures (L. Saraiva et al. in this special issue). In fact, the use of spatial analysis tools promoted greater efficiency in the decision making and planning activities especially for urban areas where many of the disease characteristics are unknown.

Spatial-temporal analysis of the abundance of phlebotomines vectors of tegumentary and visceral leishmaniasis was performed in Argentina compared spatio-temporal scales (M. G. Quintana et al. in this special issue). Microscale, mesoscale, and macroscale analyses resulted in different spatial observations. These observations raised the awareness of scale choice and consistency in conclusions from spatial studies. Scales from microfocal to regional, although within each other in increasing order, require questions, resolution, data quality, and different analytical tools, to support the conclusions appropriate to each scale.

5. Space in the Future

Simple interactions among proximal entities can lead to complex, persistent, and functional spatial entities at higher aggregate levels. Spatial studies of diseases and vectors seem to be an excellent platform from which to explore these issues.

Spatial data comes in many varieties and it is not easy to arrive at a system of classification that is simultaneously exclusive, exhaustive, imaginative, and satisfying [13].

Innovative research on spatial analysis has been building newer insights into fundamental issues as structure of theories, models, technologies, and new methods of representation that go beyond earlier GIS models, giving birth to new techniques for addressing uncertainty [14].

The increasing ability to capture and handle geographic data means that spatial analysis is occurring within

increasingly data-rich and growing analytical power environments. This wealth of new processing capabilities provides an opportunity to address complex spatial issues in entirely new ways [11].

The use of spatial models to generate potential distribution and risk maps, followed by careful assessment of models, could lead to increment in knowledge of the different properties of a system at different levels of aggregation and in different study fields to target interventions to prevent, manage, and control disease [9].

In the search for valid and reliable conclusions a variety of exploratory and confirmatory, qualitative and quantitative procedures are being developed daily.

The incorporation of spatial analytical functionality within commercial and open-source GIS, the linkage of specialized statistical and other analytical modules have attained a conspicuous progress in spatial studies [15].

Nonetheless, the pursuit for methods and tools that allow more specific and ever demanding treatment of space in empirical applications in many sciences, in measurement, in stressing space-time dynamics, in hypothesis development and in validation of theoretical constructs will never cease to exist. This pursuit was valid a decade ago [15] and still is today.

We have started witnessing new map forms and processing environments.

New maps forms go beyond the 2D paper map. Users often require being able to have spatial information on a 3D view of the terrain. Virtual reality can transform the information from polygons to objects near photographic realism [11]. A four dimension spatial analysis tool system (3D plus time) is currently the major challenge. Currently, visualization of time is only possible by animation of a series of map layers.

Among the new processing environment are full integration of the global positioning system and remote sensing imagery with spatial analysis in real-time applications.

Typical commercial GIS toolbox software products are being substituted by web spatial analysis services where users customize their own views.

High-level applications have been initiated by the free release of data in the web and the ability of using analytical tools and display capabilities from different sites resulting in complex data manipulation which however does not require specific GIS knowledge from the end user. In this new environment, the user focuses on the spatial logic of a solution and is hardly aware that spatial analysis is even involved [11].

A combination of spatial studies innovations derived from other sciences and interdisciplinary expertise might lead to advancements farfetched from currently used tools and observed outcomes. These spatial studies will most likely take us closer and closer to unveil the natural complexity of components of disease in space.

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Endnotes

1. *Ecology*. (Greek *oikos* = house, dwelling place, habitation; *logia* = study of) The study of the relationships between living organisms and their environment. The term *Ökologie* was coined in 1866 by the German scientist Ernst Haeckel (1834–1919) [16]. *Landscape*. A portion of land or territory which the eye can comprehend in a single view, including all the objects it contains [17]. A heterogeneous land area composed of a cluster of interacting ecosystems that is repeated in similar form throughout [18]; an area of land containing a mosaic of habitat patches, often within which a particular focal habitat patch embedded [19] relevant to the phenomenon under consideration at any scale where each species is likely to respond to more than one scale [20]. Originally introduced as a painters' term c. 1600 as a painting representing natural scenery, old English *landscape*, Germany *landschaft*, old Norwegian *landskap*; the meaning tract of land with its distinguishing characteristics is from 1886 [16]. *Geography*. (Greek *geo* = earth, *graphia* describe-write) The study of lands, features, inhabitants and phenomena of Earth. First used by Eratosthenes (276–194 bC) [21]. *Habitat*. Latin = it inhabits. An ecological or environmental area that is inhabited by a particular organism. The natural environment in which an organism lives, or the physical environment that surrounds (influences and is utilized by) a species population [22]. As a technical term in Latin texts on English flora and fauna, literally it inhabits in 1762. From the verb *habitare* = to live, dwell. General sense of dwelling place is first attested in 1854 [16]. *Environment*. The circumstances, objects, or conditions by which one is surrounded. The complex of physical, chemical, and biotic factors (as climate, soil, and living things) that act upon an organism or an ecological community and ultimately determine its form and survival. The aggregate of social and cultural conditions that influence the life of an individual or community [23]. State of being envired or surrounded c. 1600. Nature, conditions in which a person or thing lives, first recorded in 1827, used by Carlyle to render German *Umgebung*. Specialized ecology sense first recorded in 1956 [16].
2. *Malaria*. Italian. mal'aria, from mala aria, literally bad air, from *mala* = bad (feminine of *malo*, from Latin *malus*) + *aria* = air. Probably first used by Italian physician Francisco Torti in 1740 (1658–1741). The mosquito-borne disease was once thought to have been caused by foul air in marshy districts [16]. *American Trypanosomiasis*. An anthrozoosis due to the protozoan *Trypanosoma cruzi* transmitted to man by infected faeces of a blood-sucking triatomine bug, skin break or through mucous membranes, contaminated food, blood transfusion, pregnancy and delivery, organ transplantation or laboratory accident endemic of Central and South Americas [24].
3. *Endemic*. ἐνδημία *en in*, within, inside + *demos* people or population and also place, home.

4. *Nidality*. Nidus according to Pavlovsky, a translation of Russian *ochag* = hearth, breeding ground. Thus a nidus of a disease is its nest, home, or habitat, equivalent to the Latin focus [25].

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Research Article

Survey of Water Bugs in Bankim, a New Buruli Ulcer Endemic Area in Cameroon

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Received 15 July 2011; Revised 8 February 2012; Accepted 5 March 2012

Academic Editor: Nildimar Honório

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Buruli ulcer is a debilitating human skin disease with an unknown transmission mode although epidemiological data link it with swampy areas. Data available suggest that aquatic insects play a role in the dissemination and/or transmission of this disease. However, their biodiversity and biology remain poorly documented. We conducted an entomological survey in Bankim, Cameroon, an area recently described as endemic for Buruli ulcer in order to identify the commonly occurring aquatic bugs and document their relative abundance, diversity, and spatial distribution. Collection of aquatic bugs was realized over a period of one month by daily direct capture in different aquatic environments (streams, ponds, and rivers) and through light traps at night. Globally, the data obtained showed the presence of five families (Belostomatidae, Naucoridae, Nepidae, Notonectidae, and Gerridae), their abundance, distribution and diversity varying according to the type of aquatic environments and light attraction.

1. Introduction

Buruli ulcer is a debilitating human skin disease caused by *Mycobacterium ulcerans* [1, 2]. This infection is a neglected emerging disease that has recently been reported in some countries as the second most frequent mycobacterial disease

in humans after tuberculosis [3, 4]. The majority of cases are localized in Africa occurring mainly in poor local communities. Other cases have been reported in Asia, Australia, and South America [1].

Despite the increasing number of endemic areas, the exact mode of transmission of *M. ulcerans* remains unclear.

Buruli ulcer has always been associated with swampy areas [5–10]. The role of aquatic insects in Buruli ulcer transmission has been suggested by some studies in the past 10 years [11, 12]. Experimental laboratory studies have confirmed this possibility by showing that *M. ulcerans* was able to settle in glands of water bugs and transmitted to mice through bitings. Field investigations found that water bugs captured in endemic areas were positive for *M. ulcerans* [13–19]. Recently, viable *M. ulcerans* was detected in saliva of water bugs [20]. These studies allowed proving that water bugs are the host and a probable vector of *M. ulcerans*. However, the exact role of aquatic bugs remains to be clarified. Indeed, the etiological agent of Buruli ulcer may be introduced by bites of these insects or by trauma at skin sites [11, 12, 21].

All over the world, about 45000 species of insects are known to inhabit diverse freshwater ecosystems [22, 23]. These insects are involved in nutrient recycling and form an important component of natural food webs in aquatic ecosystems [24]. They also serve as reliable indicators of ecological characteristics of water [25]. Among aquatic insects, water bugs belong basically to two categories: semiaquatic bugs which live upon the water surface and true water bugs which live beneath the water surface. Most of them are carnivorous and can even feed on small vertebrates such as fishes and amphibians [26–28]. The majority of water bug species are also known to display flying activity, in their adult forms at night when attracted to light [29–32]. They may therefore also play a role in *Mycobacterium ulcerans* dissemination in the environment as suggested by [20, 33–35].

Bankim district has been described recently as a Buruli ulcer endemic site in Cameroon, and aquatic bugs collected in this region were positive for *M. ulcerans* [34]. But aquatic bugs' biodiversity and biology are poorly documented, making it hard to characterize the relations between *M. ulcerans* and these aquatic insects. In the above-mentioned context, the present study was carried out with 2 objectives:

- (1) to identify the commonly occurring medium and large size aquatic bugs fauna elements; to work out their relative abundance, diversity; to perform comparison between daytime square-net captures and night time light trap captures;
- (2) to provide a database and spatial distribution of aquatic bugs related with Buruli ulcer cases in this area.

2. Materials and Methods

2.1. Sites of Study. Capture of aquatic bugs was organized within June 2009 in Bankim (6.0405N 10.2737E), a rugged land in north-western Cameroon at an altitude of about 750 meters. This region represents a transition between forested south and savanna north. Its geography, tropical climate and population contexts differ from the forested Nyong River Basin, the endemic region of Central Cameroon. The building of a dam on the Mape River in 1989 profoundly modified the environment by creating an artificial lake of 3.2 billion m³ capacity. This study was carried out in selected

water bodies (5 streams, 3 ponds, 1 river) and in different geographic landscapes like the savanna around the dam, near the habitations, and the forest around the Mbam River. Water bodies were located around Bankim town, in Ngom and along the Bankim Mappé road (Figure 1(a)). The incidence of Buruli ulcer in this region is increasing [34]. Farming is the main activity with specific population groups raising cattle and other involved in commercial fishing. The population density is about 30 inhabitants/km². The prevalence of Buruli ulcers in this endemic area is represented in Figure 1(b).

2.2. Aquatic Bug Capture and Sampling. The medium and large size water bugs were collected using two sampling methods: direct method in aquatic environment and indirect method by using light trap to capture winged imagos.

Direct collection of insects was performed daily for a period of one month in water bodies. Sampling was made by hauling a square-net (32 × 32 cm and 1 mm in mesh size) from the surface to a depth of 1 meter and over a distance of 1 meter. A given sample corresponds to the mixture of all insects collected after 45 minutes. After collection, insects were transported in labelled plastic bottles containing freshwater from the site. Adults as well as nymphs were then selected, counted, and preserved in 70% ethanol for laboratory identification. For each site, GPS coordinates, nature, and intensity of human activities in water, type of water body were noted.

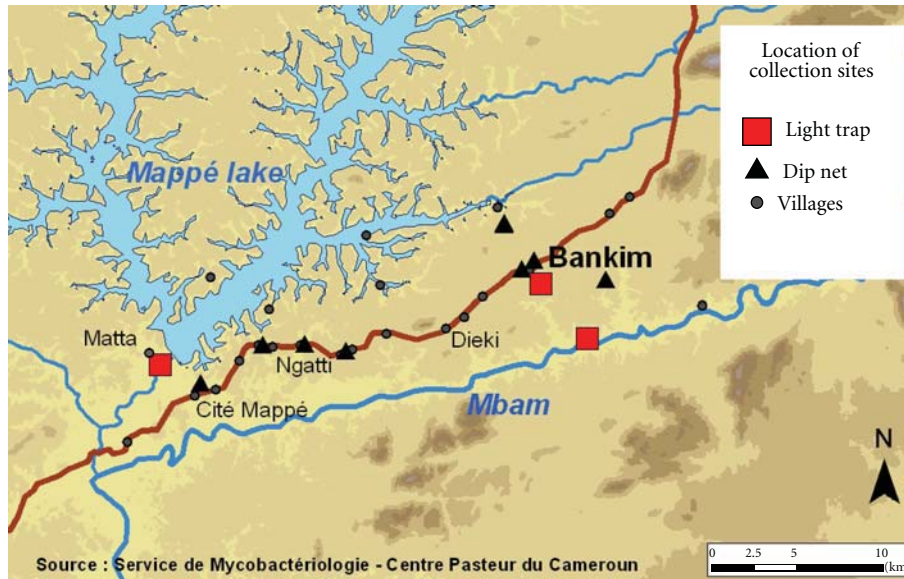
Night time light trapping was used for indirect insect collection. This mobile light trap consisted of a 250 W bulb connected to an electrical generator put in front of a white sheet. Light traps were installed, respectively, five times around the dam and the Mbam River and 4 times near habitations from 6:30 PM to 11:00 PM beginning at full moon and ending at the end of lunar cycle. All attracted insects were collected in labelled plastic bottles containing 70° ethanol and processed as indicated previously [20].

Three sites were selected for night time collection, one by the forest zone Matta, another in the savannah Bankim, and the last one near habitation.

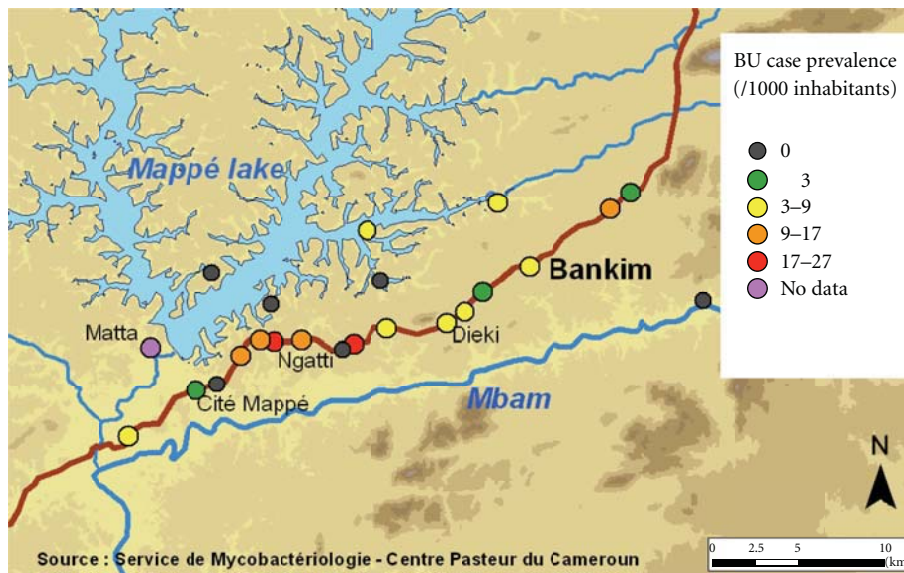
2.3. Water Bugs Identification. Aquatic Heteroptera, generally called water bugs, forms three infraorders of Hemiptera order (Leptopodomorpha, Geromorpha, and Nepomorpha) which belong to the Insect class and the Arthropoda phylum. Heteroptera is mainly identified by observing:

- (1) piercing-sucking mouthparts, with a segmented rostrum arising from the front of the head;
- (2) two pairs of wings in adults: partly membranous forewings; hemelytra and fully membranous hind wings.

Identification of water bugs took place in the entomological laboratory of the Institute of Agricultural Research for Development (IRAD) in Yaoundé Cameroun. Each collected specimen was attributed to a given family on the basis of the Heteroptera family determination criteria [36]. Currently, identification keys enable identification only for water bug families.



(a)



(b)

FIGURE 1: (a) Study site; (b) Buruli ulcer case prevalence per village.

3. Results

3.1. Direct Insect Collection. Globally, 728 water bugs were collected, within which 338 were collected directly in aquatic environments and 390 captured through light traps. Those collected in aquatic environment belong to five families (Belostomatidae 33.13% ($N = 112$), Naucoridae 27.81% ($N = 94$), Nepidae 28.09% ($N = 95$), Notonectidae 5.91% ($N = 20$), and Gerridae 5.02% ($N = 17$)). But their abundance, distribution, and diversity vary according to the type of water body. The river was poor in aquatic bug population, with only one family captured; Nepidae ($N = 9$) represented by two subfamilies Ranatrinae ($N = 3$) and Nepinae ($N = 6$). Generally, in the ponds and streams, the five families were present but their abundance and diversity

seem only dependent on the geographical location of the collecting site as on Figure 2(a).

3.2. Indirect Insect Collection. Light trap indirect collection yielded 390 specimens belonging only to 2 families; Belostomatidae represented 80.51% and Notonectidae 19.48% (Figure 2(b)). During the full moon, only the Notonectidae family come to light; Belostomatidae were absent at this phase of the, but they appeared a few nights after (Figure 2(b)). Belostomatidae family was very abundant mainly during the few nights that precede or follow the full moon, showing several peaks, which decreased progressively for rescinding at a few nights before full moon. Whatever the site of collection, the numerical variations of

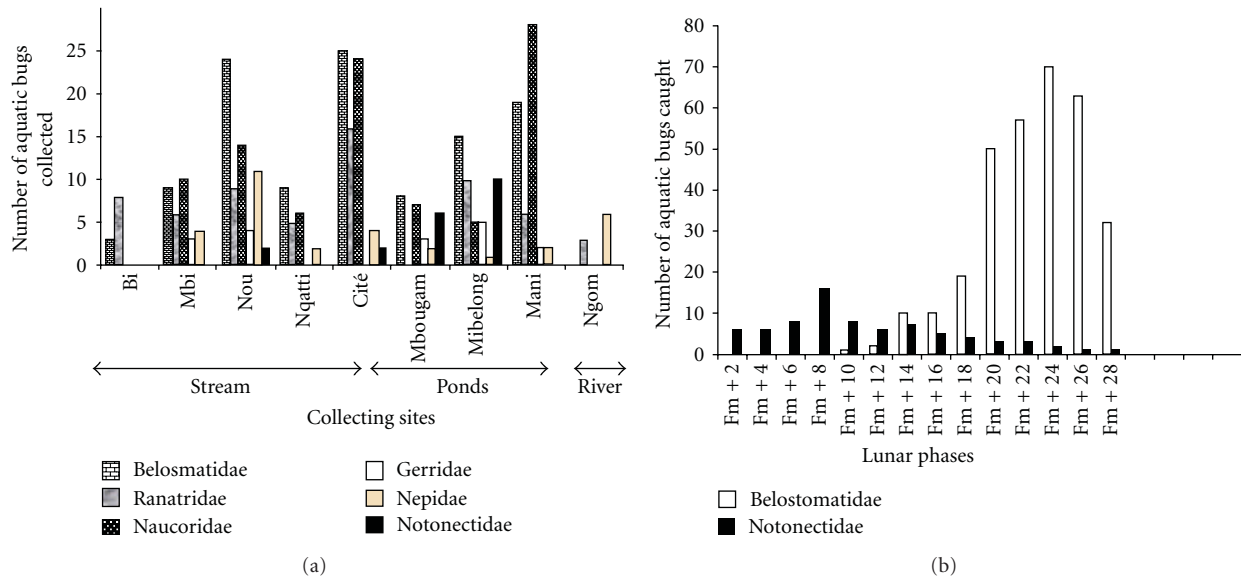


FIGURE 2: (a) Aquatic bugs per family and collecting water bodies in the aquatic environment. (b) Aquatic bugs caught in a light traps per families according to lunar phases.

TABLE 1: Identification of water bugs collected directly in aquatic environment and indirectly by light trap.

| Category | Family | Sub family | Genus | Direct collection | Indirect collection |
|------------------|----------------|----------------|------------|-------------------|---------------------|
| True water bugs | Belostomatidae | Belostomatinae | Appasus | 112 | 114 |
| | Belostomatidae | Lethocerinae | Lethocerus | 0 | 200 |
| | Nepidae | Rantrinae | ND | 63 | 0 |
| | Nepidae | Nepinae | ND | 32 | 0 |
| | Naucoridae | | ND | 94 | 0 |
| | Notonectidae | Anisopinae | ND | 14 | 46 |
| | Notonectidae | Notonectinae | ND | 6 | 30 |
| Semiaquatic bugs | Gerridae | Gerrinae | ND | 17 | 0 |

water bugs captured by light trap were almost consistent with Belostomatidae being prominent, that is, 33.33% of Belostomatidae and 5.12% of Notonectidae in the forest; 25.64% of Belostomatidae and 11.94% of Notonectidae in the savanna; 21.53% of Belostomatidae and 2.56% of Notonectidae near habitations (Figure 3(a)).

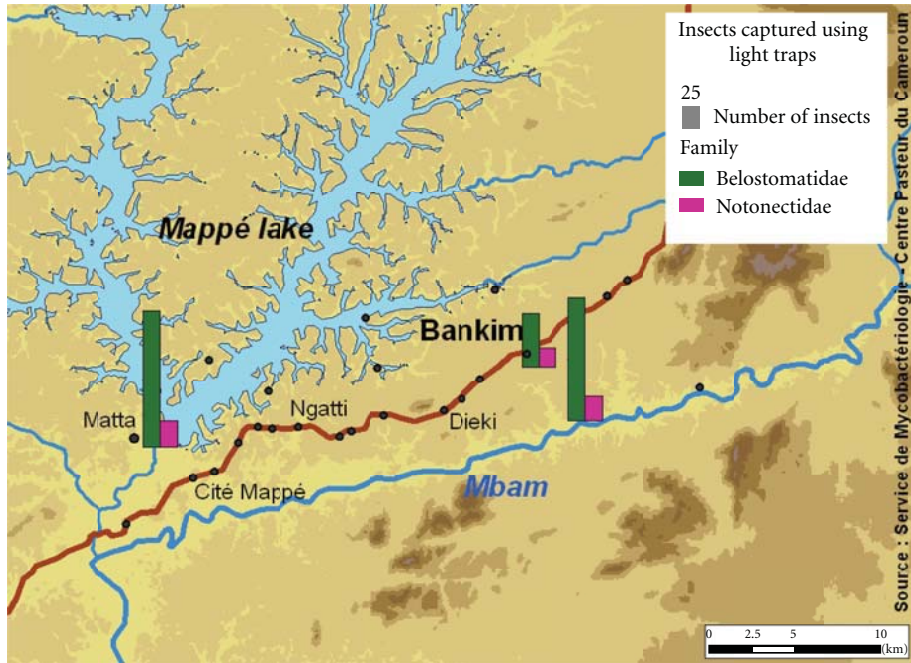
4. Discussion

4.1. Abundance Variation according to Type of Water Bodies. We noted that streams and ponds which were slow and stagnant showed the highest number of water bugs: 59.17% in the streams and 38.16% in the ponds. The number of individuals was quasinil in the river; only 9 (2.66%) water bugs were collected in the river perhaps because of its rapid flow. Furthermore, aquatic vegetation was abundant in streams and ponds, but it was scarce in the river. In all water bodies selected, we observed human activities but we cannot say if they influenced or not diversity of aquatic bugs. Nevertheless, it seems that diversity of water bugs was related with nature of water currents and presence of aquatic vegetation.

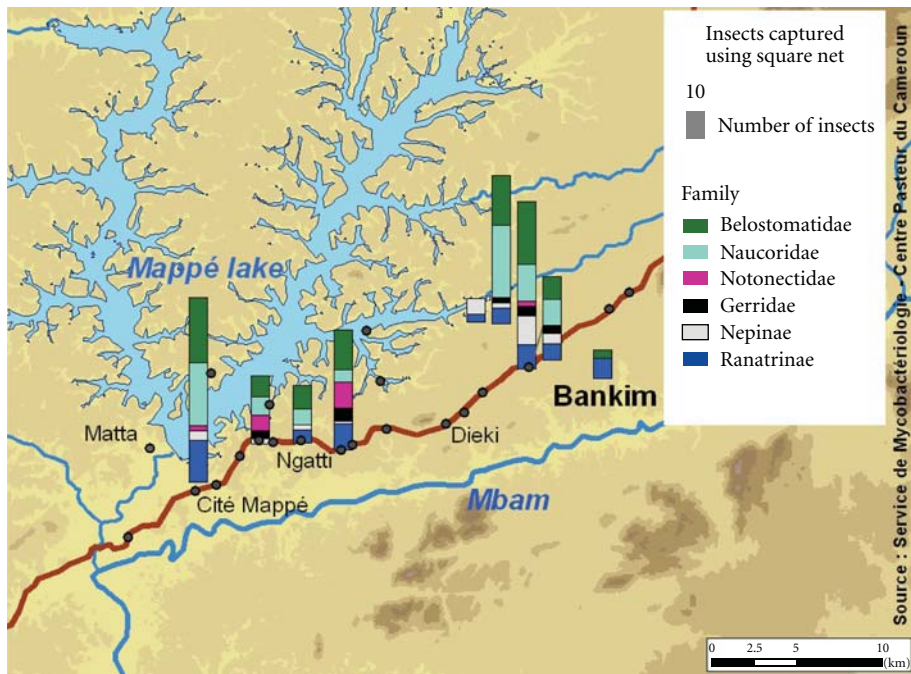
Spatial distribution of aquatic bugs is not uniform and does not depend on the type of water body but on the geographical location as illustrated in Figures 2(a) and 3(b). For example, Notonectidae family was met in only 2 of the five streams and 2 of the 3 ponds. Belostomatidae and Naucoridae were found in all ponds and streams. These last two families (Belostomatidae and Naucoridae) are carnivorous and suspected to play a role in the transmission of Buruli ulcer and in the ecological expansion of the *Mycobacterium ulcerans* niche.

4.2. Abundance Variation according to Moon Cycle. During the moon cycle, Notonectidae family was present at all times but in less important numbers. These results agreed with the results obtained concerning flight activity of Belostomatidae [26]. Light trapping proved to be an interesting method to obtain important numbers of Belostomatidae and Notonectidae but reflected poorly the overall diversity of water bugs.

The number of water bugs is more important around the Mbam River which was situated in the forest than the other sites installed light trap. The installation near the habitations showed the least number of aquatic bugs.



(a)



(b)

FIGURE 3: (a) Distribution of Hemiptera captured in the night by light traps; (b) distribution of Hemiptera collected directly in the water bodies by square-net.

These results concerning the abundance of water bugs in the area neighbouring the Mbam River are to be related with the results of an epidemiological survey performed simultaneously [10]. In this case-control study, having baths for hygiene in the Mbam River was shown to increase the risk of Buruli ulcer in the populations odds ratio (95% confidence interval) = 6.9 (1.4–35).

4.3. *Seasonal Variations.* Water bugs collection conducted in Bankim during the rainy season in June permitted us to identify five water bug families: four families of true water bugs and one semiaquatic bugs (Table 1). These families include many unknown species as determination keys for water bugs species are not yet available for West Africa. These results are low comparing with those in another study

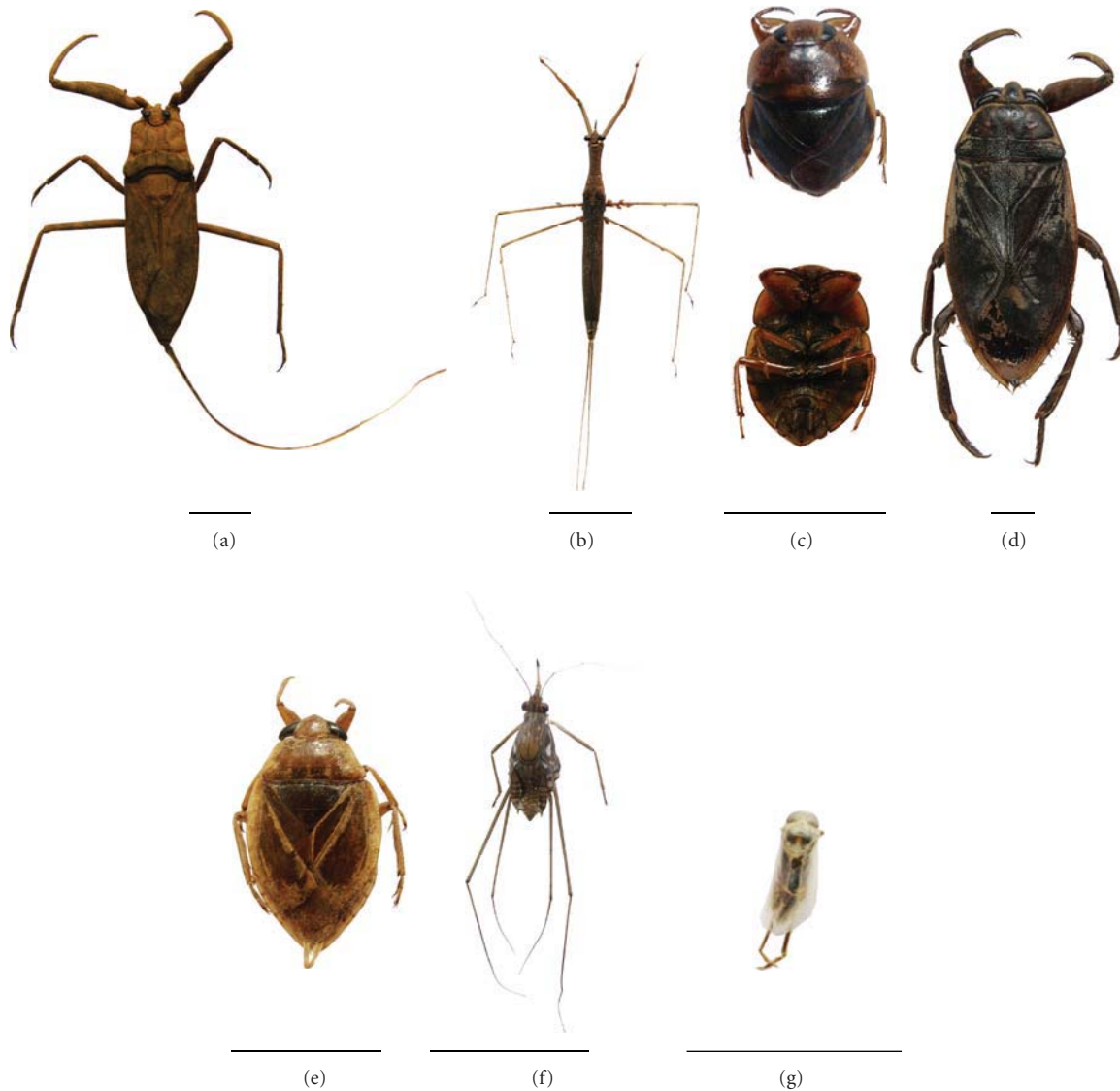


FIGURE 4: Specimens of water bugs collected during the study. (a) Nepinae, (b) Ranatrinae, the two subfamilies of Nepidae, (c) Naucoridae family (dorsal and ventral views), two morphotypes Belostomatidae, (d) giant Belostomatidae, (e) small size Belostomatidae, (f) Gerridae, and (g) Notonectidae.

in the same region during the long dry season in January which showed 1349 specimens belonging eight families [34]. In this study, *Mycobacterium ulcerans* molecular signatures were searched; among 244 insect pools (pool = group of ten insects belong the same family), 12 (5%) were *M. Ulcerans* positive. *M. ulcerans*-positive saliva was found in 11 (18%) of 61 insects in the family Belostomatidae and in 3 (8%) of 38 in the family Naucoridae. Beyond number of families in two studies, a large difference in numerical data of insect specimens was observed, 728 during the rainy season against 1349 in the long dry season. More sampling is required to confirm these results. All water bugs families collected in this study (Figure 4) were found in Akonolinga, the other Buruli ulcer endemic area in Cameroon [20].

5. Conclusion

This preliminary entomological survey in Bankim shows the distribution and diversity of aquatic bugs colonization of water bodies and reveals that their flight activity is influenced by light (direct and moon light). It also shows that the diversity of water bugs depends partly on the types of water bodies in the same endemic area, with streams and ponds seeming to be selective habitats offering best life conditions. Light attraction and the moon phases appeared to be influencing factor for aquatic bug's distribution. In prospective, this preliminary results need to be confirmed through monthly collection in endemic and nonendemic areas. Moreover, detection of *M. ulcerans* in salivary glands of the water bugs, in particular those are able to bite

humans (Belostomatidae and Naucoridae), will support their involvement in ecology and transmission of *M. ulcerans* [20].

Acknowledgments

The authors would like to thank all persons who helped in the realization of this study particularly communities in Bankim, staff of the entomological laboratory of the Institute of Agricultural Research for Development (IRAD) in Yaoundé, Cameroun, for helping in identifying water bugs. Thanks also to the field team Valentin Angoni and Daniel Gnavo for helping in collection of water bugs. Thanks to Afi Leslie for the help in translation and proofreading. This work was supported by Réseau International des Instituts Pasteurs (PTR 212, Inter-Pasteurien Concerted Actions A15/2008), Institut de Recherche pour le Développement (IRD) projet (Jeune Equipe Associée à l'IRD (JEA1)-ATOMyc), Fondation Française Raoul Follereau, Région Pays de la Loire, Institut National de Santé et de la Recherche Médicale (Inserm), and the French Agence Nationale de la Recherche (programme CEP&S). S. Meyin is a student in JEA1 team.

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Review Article

Using a Geographical-Information-System-Based Decision Support to Enhance Malaria Vector Control in Zambia

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Received 20 July 2011; Accepted 14 December 2011

Academic Editor: Maria Goreti Rosa-Freitas

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Geographic information systems (GISs) with emerging technologies are being harnessed for studying spatial patterns in vector-borne diseases to reduce transmission. To implement effective vector control, increased knowledge on interactions of epidemiological and entomological malaria transmission determinants in the assessment of impact of interventions is critical. This requires availability of relevant spatial and attributes data to support malaria surveillance, monitoring, and evaluation. Monitoring the impact of vector control through a GIS-based decision support system (DSS) has revealed spatial relative change in prevalence of infection and vector susceptibility to insecticides and has enabled measurement of spatial heterogeneity of trend or impact. The revealed trends and interrelationships have allowed the identification of areas with reduced parasitaemia and increased insecticide resistance thus demonstrating the impact of resistance on vector control. The GIS-based DSS provides opportunity for rational policy formulation and cost-effective utilization of limited resources for enhanced malaria vector control.

1. Introduction

In Sub-Saharan Africa, malaria remains a major cause of morbidity and mortality [1]. Its transmission is driven by a complex interaction of the vector, host, parasite, and the environment, and is governed by different ecological and social determinants [2, 3]. The survival and bionomics of malaria vectors are affected by climate variability, that is, rainfall, temperature, and relative humidity [4]. In this light, even minute spatial variations and temporal heterogeneities in the mosquito population can result in significant malaria-risk [5, 6] and its endemicity [7–9].

Since malaria distribution is not homogeneous, much effort needs to be expended towards defining local spatial distribution of the disease [2] precedent to deployment of interventions [10]. In resource constrained environments, monitoring, and evaluation is often incomprehensive and irregular and tend to lack the actual spatial and temporal

distribution patterns. If transmission determining parameters are to be harnessed effectively for decision-making and objectively plan, implement, monitor, and evaluate viable options for malaria vector control [11], they must be well organized, analyzed, and managed in the context of a geographical-information-system- (GIS-) based decision support system (DSS) [3, 12].

While vector control interventions are being deployed according to the World Health Organization-led Integrated Vector Management Strategy [10, 13, 14], prompt availability of relevant spatial and attribute data is vital to support malaria surveillance, management research, and policy initiatives. Different strategies coupled with new technologies such as mapping, GIS, and DSS, and spatial and temporal modelling are being harnessed to more effectively target limited surveillance, prevention, and control at research scale [15]. However, potential utilization of these approaches and their incorporation in the operational malaria vector control

programmes remains a significant constraint and continues to receive limited attention [16, 17].

Until recently, very few malaria endemic countries had incorporated the GIS technology into operational malaria control programmes, that is, in South Africa and the Lubombo Spatial Development in Mozambique in Southern Africa, where it has been harnessed for case mapping and monitoring of vector control coverage [18, 19]. In India, it has been used to monitor malaria transmission attributes as well as social-economical and social cultural aspects of malaria [3]. To achieve enhanced utilization of mapping and GIS technologies in operational malaria control, sharing of experiences with GIS and emerging technologies by malaria control programmes is critical [15, 17]. Herein is provided a review of data related to the operational use of a GIS-based DSS [12, 20] for optimal deployment, monitoring, and evaluation of entomological interventions for malaria control in Zambia.

2. Materials and Methods

The integration of operational and logistical data for malaria control program planning with epidemiological data will serve to strengthen both the epidemiological analysis and the planning and execution of control programs. GIS facilitate the integration of quantitative malaria determination and control data with data obtained from maps, satellite images, and aerial photos. A comprehensive review of data collected through nationally representative malaria indicator surveys and insecticide resistance data in major malaria vectors: *An. gambiae s.s.*, *An. arabiensis*, and *An. funestus*, including the comparative impact of main stream vector control interventions, has been conducted in Zambia.

2.1. Intervention. The intervention consists of scaled-up indoor residual spraying (IRS) in urban and periurban areas and insecticide treated nets (ITNs) in rural areas [14, 21–23]. Indoor residual spraying is implemented through annual campaigns with 85% coverage of eligible households using pyrethroids at 25 mg/m² (Syngenta and Bayer) and DDT at 2 g/m² (Avima) at the beginning of the peak malaria transmission period [24]. Pyrethroid-impregnated ITNs, that is, PermaNet (Verstargaard frandsen) and Olyset (Sumitomo Corporation), are deployed through antenatal and child clinics, equity programme, community mass distribution, and commercial sector and strive towards attaining 100% coverage in eligible areas [25]. This effort is coupled with effective case management by provision of definitive diagnosis, using rapid diagnostic tests (RDTs) and microscopy, and treatment with artemisinin-based combination therapy (ACT), and intermittent preventive treatment (IPT) to expecting mothers [21]. This is further augmented with interactive information, education, and communication (IEC) and behavioural change and communication (BCC) strategies to enhance utilization of interventions [26]. There is strong operations research feeding into and guiding implementation.

2.2. Spatial Decision Support System. Zambia is situated in the Southern African region with a population of

approximately 12 million, 45% of whom are below the age of fifteen [27]. Malaria is endemic country-wide and transmission is throughout the year with peak in rain season. The disease is the leading cause of morbidity and mortality accounting for 40% of outpatient attendances, 45% of hospital admissions with 47% and 50% of disease burden among pregnant women, and children under five years of age, respectively. Current trends in the country indicate that malaria is responsible for at least 3 million clinical cases and about 6,000 recorded deaths annually, including up to 40% of the under five deaths and 20% of maternal mortality [28, 29]. Malaria stratification aids in the development of community-based malaria control programs, by accumulating past experiences with and solutions to different factors associated with malaria outbreaks. Stratification can also point to the existing inequalities in resources, allowing for a more equal and homogeneous distribution of available resources [30]. In this regard, to allow for adaptation of intervention policy, procedures and methods to better outcomes, nineteen GIS-based sentinel sites, distributed amongst nine districts within a 350 km radius of the capital Lusaka (Figure 1), were established for the continual monitoring and collation of key malaria data such as parasitaemia risk, insecticide resistance profiles in vectors and impact of interventions on malaria prevalence. The study region is characterized by reduced seasonality of transmission with extensive vector control through IRS at 6 sites and ITNs in all sites from 2003 to 2010 by the National Malaria Control Programme (Figure 1).

2.3. Spatial Monitoring of Interventions. The spatial and temporal impact of IRS and ITNs on human parasite prevalence and insecticide resistance status in major malaria vectors was monitored. At each sentinel site annual household surveys were carried out annually from 2008 to 2010 to measure *Plasmodium falciparum* prevalence in children aged 1 to 14 [31, 32]. In Zambia, three nationally representative malaria indicator surveys (MISs) were also conducted in children under five years of age in 2006, 2008, and 2010 [33]. The MIS have been used (i) to estimate an empirical high-resolution parasitological risk map in the country and (ii) to assess the relation between malaria interventions and parasitaemia risk [34]. By standard WHO protocol, spatiotemporal insecticide resistance profiles of major malaria vectors: *Anopheles gambiae s.s.*, *An. arabiensis*, and *An. funestus* were determined at sentinel sites and were extended to other regions of the country [32, 35, 36]. More data on spatial distribution of insecticide resistance to bendiocarb (0.01%), DDT (4%), deltamethrin (0.05%), lambda-cyhalothrin (0.05%), malathion (5%), and permethrin (0.75%) have been collected by different partners and collated by the National Malaria Control Programme (Figures 4 and 5).

3. Results

3.1. Spatial Prevalence of Malaria Infection. *Plasmodium falciparum* accounts for 98% of all malaria infections in the country, causing the severest form of disease, with a low

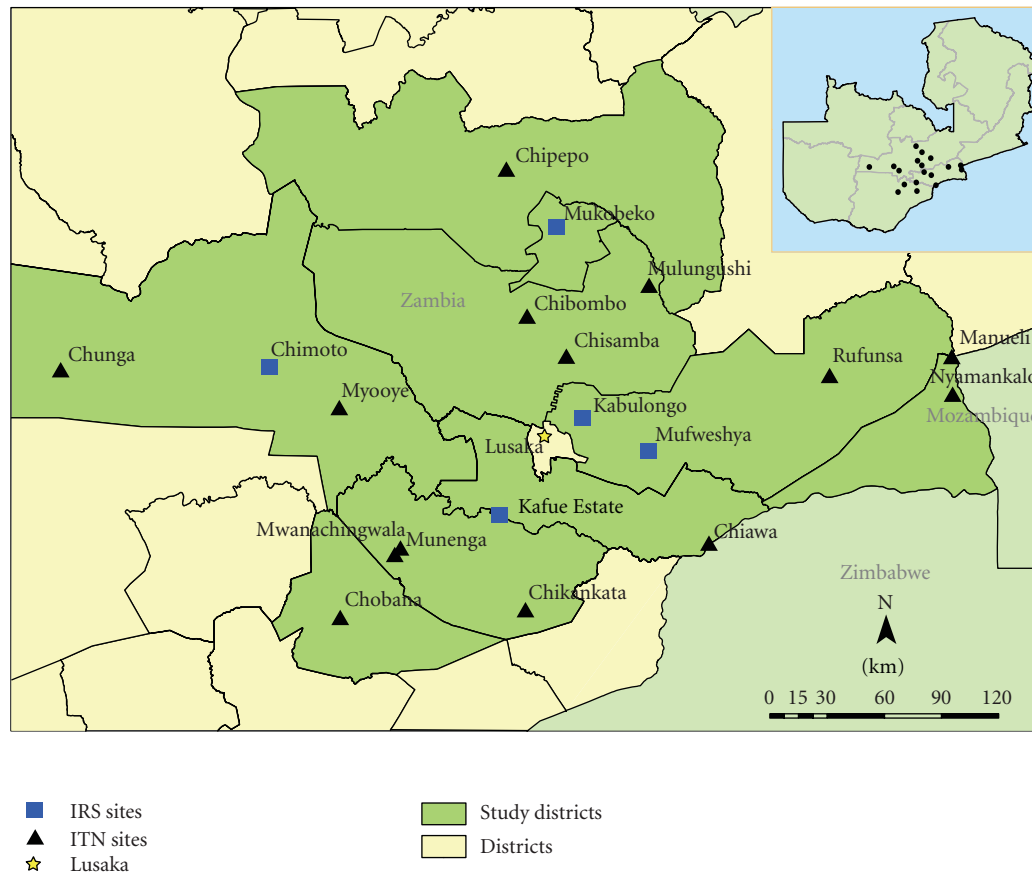


FIGURE 1: Map of Zambia showing the location and spatial distribution of GIS-based decision support system monitoring sentinel sites.

frequency of infections from *P. malariae* and *P. ovale*, and no transmission of *P. vivax*. The national malaria indicator survey for 2010 in children under the age of five years shows great spatial heterogeneity in prevalence of infection [37]. This has resulted in stratification of the country in three epidemiological categories: Type 1 areas with very low transmission and parasite prevalence of <1%, Type 2 areas with low transmission and prevalence of under 10%, and Type 3 areas with persistent high transmission and prevalence exceeding 20% at peak transmission season [33]. Cross-sectional surveys at sentinel sites (Type 2 areas) in children between 1 and 14 years across the study area (Figures 2 and 3) showed a combined prevalence of infection with *P. falciparum* to be below 10% albeit with great heterogeneity between IRS and ITN areas [32].

3.2. Spatial Distribution of Insecticide Resistance Profiles. By standard WHO protocol, suspected and overt resistance to insecticides being harnessed for vector control, pyrethroids, and DDT, has been detected in all the key vectors in operational settings of both IRS and ITNs (Figures 4 and 5). High levels of insecticide resistance have been detected in both *An. gambiae s.l* and *An. funestus* to pyrethroids and DDT. There is great variation in the level of resistance between IRS and ITNs localities, with exceptionally higher level resistance being detected in IRS areas compared to ITNs

areas ($P < 0.0001$). The west form of knockdown resistance (kdr) mutation has been detected in *An. gambiae s.s* in some areas of the country with crossresistance between pyrethroids and DDT [32].

3.3. Spatial Impact of Interventions on Malaria Prevalence. The overall prevalence of infection in children whose house had not been sprayed in the past year and did not sleep under a net the night before the survey was 6.8%. Children who slept under a net, but whose house had not been sprayed during the past year, had a prevalence of infection of 5.2%. Children whose house had been sprayed during the past year, but did not sleep under a net, had a significantly lower prevalence of infection of 3.2%. Children who slept under a net in a dwelling that had been sprayed had the lowest risk of infection with a prevalence of 2.6%. Thus incremental effect was observed for combined use of IRS and ITNs (Figure 6) [32].

4. Discussion

Given the spatial heterogeneity in the distribution of malaria vectors and variations in the inherent malaria risk, GIS has potential applications in deployment and monitoring of interventions. For resource-constrained malaria-endemic Sub-Saharan African countries, like Zambia, the need for a

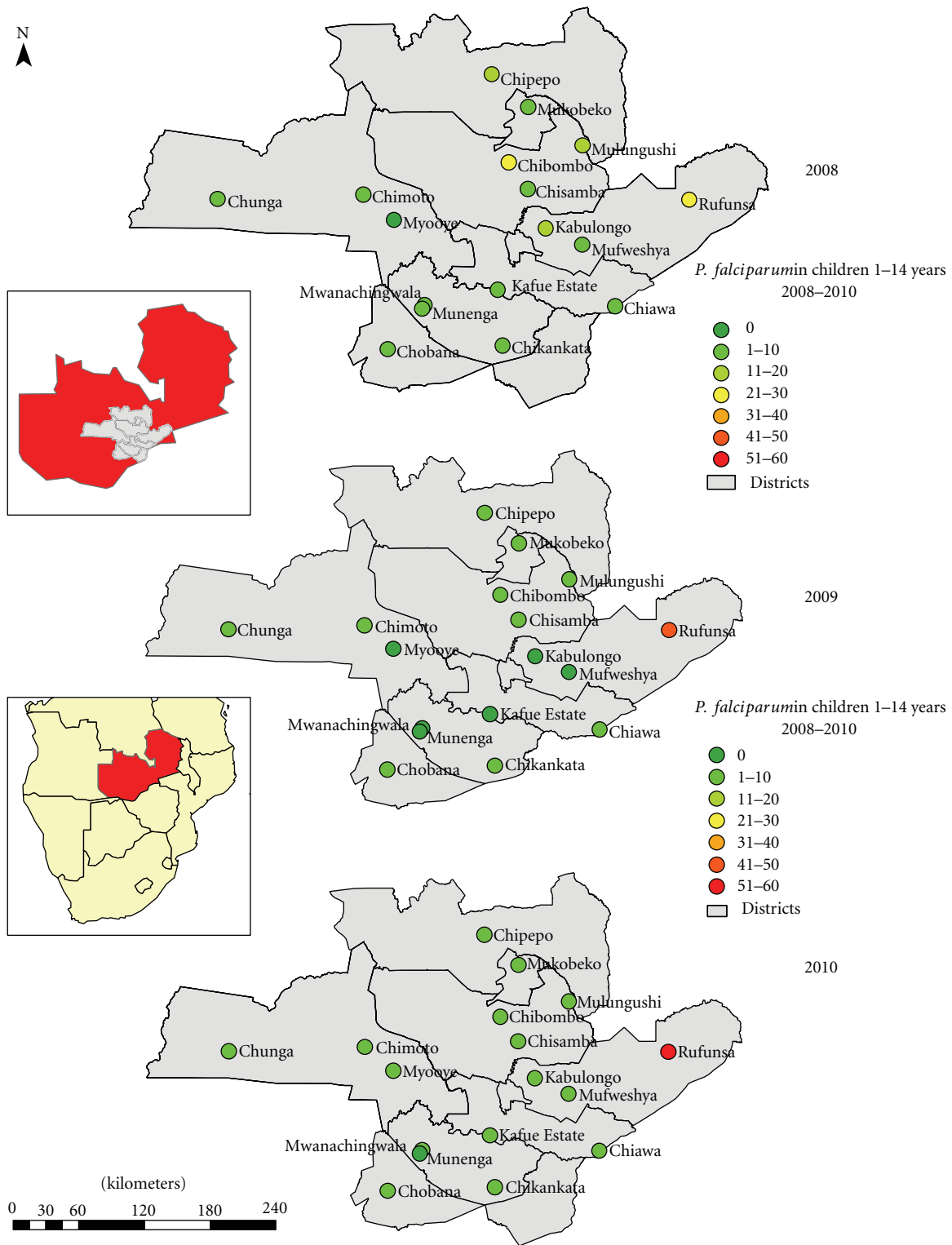


FIGURE 2: *P. falciparum* malaria parasite prevalence in children 1 to <15 years in monitoring sentinel sites from 2008 to 2010 surveys.

GIS-based malaria information system cannot be overemphasized. Until recently, decisions in the malaria control programmes were taken on an *ad hoc* basis driven by limited empirical evidence and undoubtedly resulting in misdirection of the limited resources available.

Following the increased funding for malaria control [38] particularly in Sub-Saharan Africa [39, 40], insecticide-based malaria vector control interventions are being scaled up in most endemic countries [41] albeit with limited empirical evidence on their impact and amenability to local

Zambia
Prevalence of infection with *Plasmodium falciparum*
in children 1 to 14 years of age by district, observed
during household surveys in 2008, 2009, and 2010

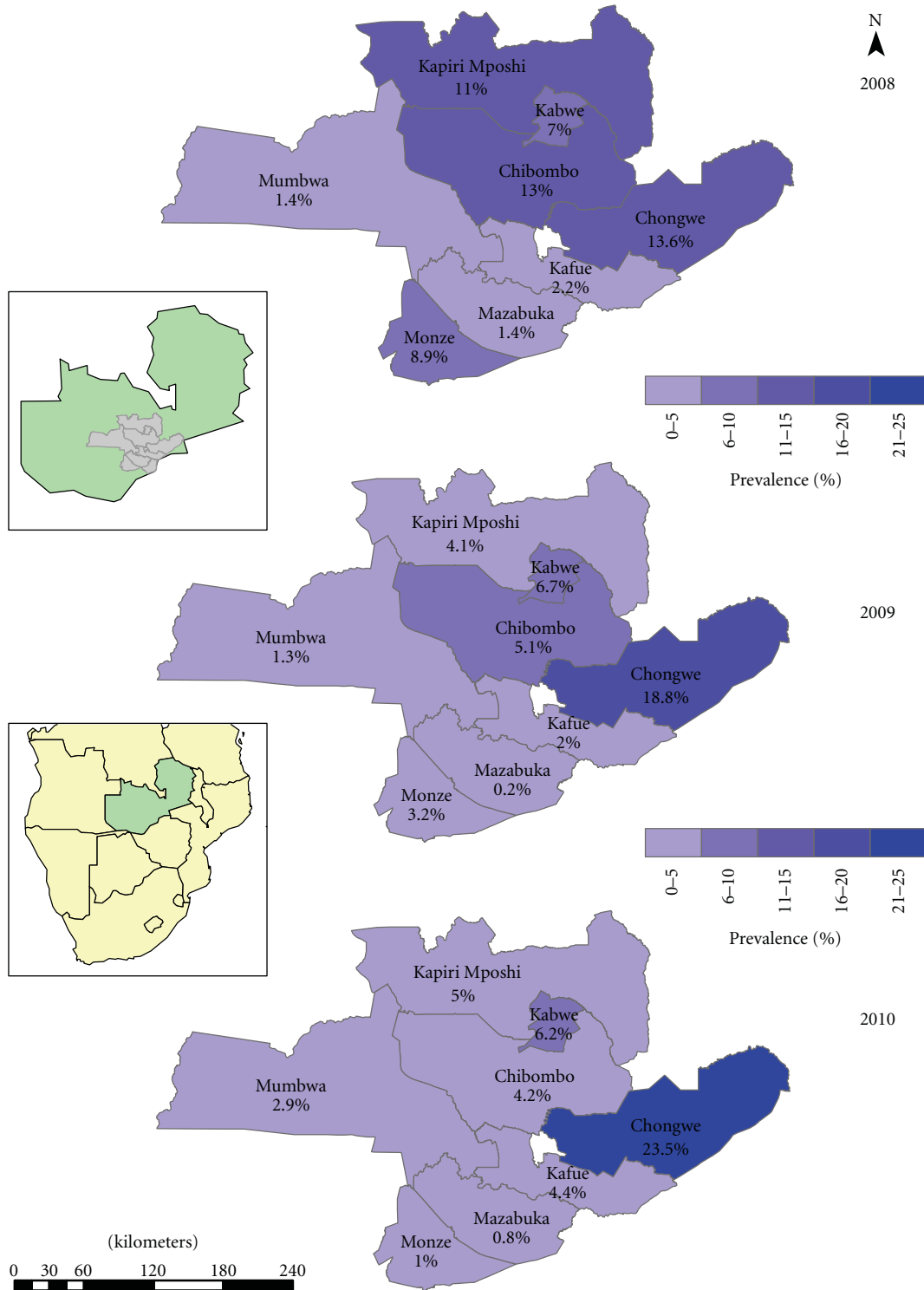


FIGURE 3: Prevalence of infection with *P. falciparum* in children 1 to <15 years as observed during the annual parasitaemia surveys from 2008 to 2010 by district.

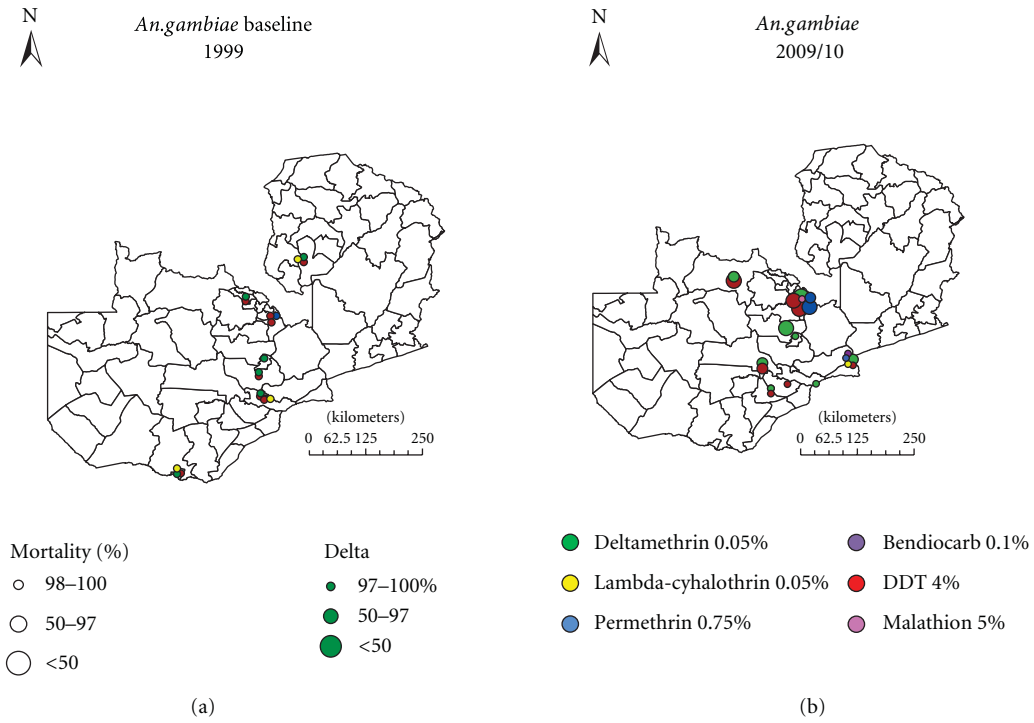


FIGURE 4: The spatial distribution of insecticide resistance in *An. gambiae s.l.* in 1999 compared to 2009/10 in Zambia.

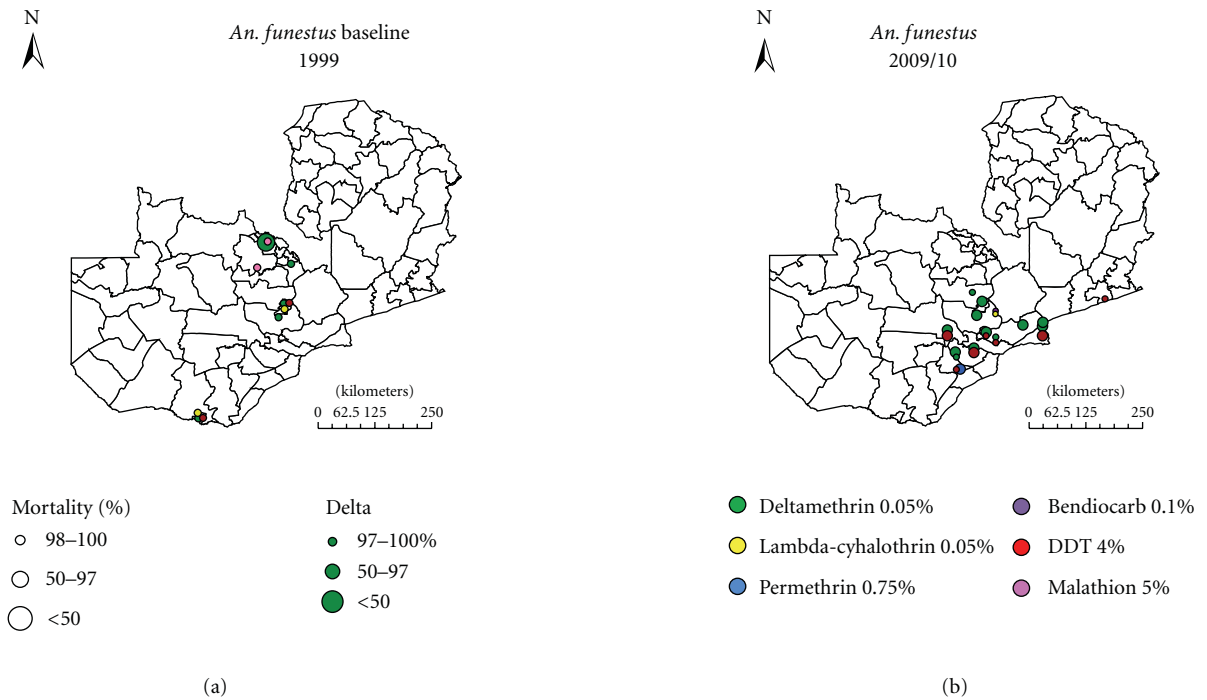


FIGURE 5: The spatial distribution of insecticide resistance in *An. funestus* in 1999 compared to 2009/10 in Zambia.

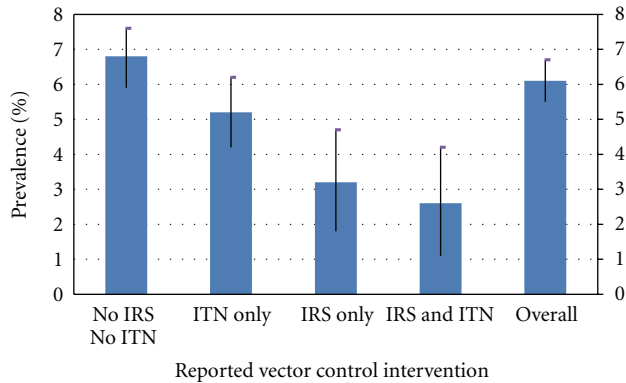


FIGURE 6: Prevalence of infection in children 1 to <15 years of age in Zambia by reported vector control intervention (2008, 2009, and 2010 combined). IRS: Indoor Residual Spraying; ITN: Insecticide Treated Net.

settings. Invariable monitoring, evaluation, and continuous surveillance of vector species abundance, infectivity, insecticide resistance status, and parasite prevalence in the population are imperative to ensure effective deployment of interventions and optimal utilization of limited resources [42]. The GIS-based decision support system is proving to be an invaluable tool to optimize impact assessment of malaria control interventions and thus rationalize resource utilization [1].

The use of GIS in Zambia has enabled detection of spatial trends of parasite prevalence following extensive deployment of front line vector control interventions. Cross-sectional prevalence surveys show continuous prevalence increase in children from 2008 to 2010 in Chongwe district. In Kapiri mposhi, Mumbwa, Mazabuka and Kafue districts, prevalence dropped between 2008 and 2009 but increased in 2010. However, progressive reduction in malaria prevalence was detected in Monze, Kabwe, and Chibombo districts from 2008 through to 2010 (Figure 3).

The GIS has introduced new dimensions to the understanding, prediction, analysis, and dissemination of spatial relations between disease, time, and space [43, 44]. It allows the integration of geographical referenced data, together with local knowledge in relational databases to accurately display complex interactions in simple formats [3]. The use of these data sets in a GIS provides an opportunity to integrate up-to-date information, local knowledge, and historical trends in a manner that draws attention to areas of change-associated problems and options for action. This makes GIS a tool not only for data analysis, but also for information management and decision-making thus facilitating policy formulation [18].

There was great heterogeneity in prevalence of malaria at sentinel sites relative to detected insecticide resistance in malaria vectors. At Chibombo, prevalence has been reducing despite high pyrethroid resistance detected in *An. funestus*. At Myooye and Chimoto, ITN deploying sites with high *kdr* mediated crossresistance to pyrethroids and DDT in *An. gambiae s.s.*, prevalence was reducing and remained at a low level across the three years. However, Rufunsa, another

ITN deployment area with high pyrethroid resistance in *An. funestus*, exhibited constant increase in parasitaemia despite high coverage of ITNs (Figure 2).

The usefulness of a GIS-based DSS for planning and managing control programmes is dependent on the availability of accurate and raw data on malaria transmission-related parameters. Monitoring and evaluation of malaria interventions and understanding of their true impact on disease burden is essential for measuring performance of a control programme. An effective system for monitoring and evaluation and continuous surveillance requires integration of spatially and temporally explicit data for entomological and epidemiological outcome indicators. This allows for identification of disease prevalence, planning of effective interventions, assessments of reduction of vector exposure and malaria burden resulting from implemented control measures. Continuous surveillance capturing real time data enables routine monitoring and evaluation of programme to demonstrate goals and impact on malaria burden. This is essential to increasing the efficiency and effectiveness of malaria control efforts [42]. The effective control of malaria requires programme managers to have access to the most up-to-date information on the disease in order to best direct interventions efforts against the vectors.

Effective implementation and monitoring and evaluation of malaria control interventions have resulted in redefinition of stratification of the country in three epidemiological zones for malaria transmission potential in Zambia [33, 45]. This necessitates appropriate targeting of interventions guided by entomological and epidemiological evidence of active malaria transmission. Although the quality of data collection and archiving kept on improving, most data bases have been vertical. An excel spread sheet contained ITN data base capturing quantities distributed by district and year. The IRS database captured quantities of commodities and equipment, and spraying coverage per district and year. The ITNs have been monitored through a two component system: (1) compilation of information on number of ITNs distributed and (2) tracking ITN coverage and/or ownership and utilization rates by householders. Since 2000, IRS has been monitored based on generic reporting forms for formal spraying management introduced by World Health Organization. This set comprised daily spray operator record, team leaders record, supervisors report, a weekly report, and a spraying completion report. By 2005, a computerized data base developed by Booman et al. was adopted [18].

In this case, the GIS-based DSS has not only streamlined evidence-based implementation of interventions, but has improved the tracking of entomological indicators: species characterization and insecticide resistance status, including parasite prevalence and impact assessment of ITNs and IRS. It has been greatly valuable in enabling the display of heterogeneities in malaria risk areas within low transmission intensities [42, 46, 47]. The marked insecticide resistance problem in IRS (Mufweshya, Kabulongo, Kafue Estates, and Mukobeko) and LLIN (Rufunsa, Myooye, Chipeco, Chibombo, and Chiawa) deploying sites (Figure 2), confirms other findings of resistance developing in the wake of extensive vector control [48–50]. This allows the malaria

control programme manager to better utilize the limited resources on insecticides to which the malaria vectors are still susceptible. Detection of high resistance levels has facilitated the planning of rational insecticide resistance management strategies and introduction of alternative noninsecticide-based vector control interventions. Due to low levels of transmission, malaria vector control interventions amenable to focalized implementation, such as larval source management using larvicides [51, 52] in the context of integrated vector management [13, 14], are being implemented.

The impact of main thrust vector control interventions on parasite prevalence in children between 1–14 years of age has been monitored through annual malaria surveys for three consecutive years at 19 sentinel sites (Figure 2). The use of the GIS-based DSS has facilitated for the assessment of the efficacy of IRS and ITNs either in combination or singly (Figure 6). In areas with high parasitaemia, this has allowed for the identification of areas that require replenishment of torn nets or areas that may require IRS instead of ITNs. Therefore, the value of any surveillance system for infectious disease is measured by its ability to provide timely, accurate “data for action” to people responsible for effective prevention and control activities and its ability to provide ongoing feedback to the primary gatherers of information [53, 54].

Although routine surveillance data have proved inadequate for monitoring control programmes [55], and have presently been supplanted by parasite prevalence surveys, vector-borne diseases demonstrate decided geographical heterogeneities and therefore require special tools for analysis [56]. The GIS with an inherent ability to manage spatial data provides an exceptional tool for continuous surveillance [57, 58] and provides a framework for harmonizing surveillance data and parasitaemia survey data. At a regional level, the ability of GIS to display data in an intuitively understandable manner has been harnessed to establish a continental database in Africa of spatial distribution of malaria [59]. The DSS has been used to collate data on insecticide resistance in Africa [20].

The ability of GIS-based DSS to deal with large data sets and to incorporate satellite images increases the feasibility of studying transmission determinants of malaria and has resulted in prompt availability of data to support surveillance and policy formulation. The epidemiological mapping of high-risk areas of malaria transmission and insecticide resistance profiles of major vectors has facilitated the recognition of those populations and geographic areas where it is possible to identify the main determinants of malaria morbidity and mortality. The revealed trends and interrelationships have allowed the identification of high risk areas and facilitated decision making and rational utilization of limited resources in a cost-effective manner.

5. Conclusion

In Zambia, an evidence-based decision support has created a more focused and purposeful approach to directing resources to areas of most need with reasonable returns for effort and resources invested. Monitoring the impact

of malaria vector control interventions through the GIS-based DSS on relative change in prevalence of infection and vector susceptibility to insecticides over time has enabled measurement of spatial heterogeneity of trend or impact. The revealed trends and interrelationships have allowed the identification of areas with reduced parasitaemia and increased insecticide resistance thus demonstrating the impact of vector control. Targeting interventions based on entomological and epidemiological evidence have not only contributed markedly to the success of the Zambian Malaria Control Programme, but also have provided opportunity for rational decision making in deployment of interventions and cost effective utilization of limited resources for enhanced malaria control.

Authors' Contribution

E. Chanda coordinated and participated in data collection, analyzed the data, and drafted the paper. V. M. Mukonka was responsible for management of project and contributed to the drafting of the paper and critically evaluated it. D. Mthembu and S. Coetzer participated in field survey data collection and mapped the sites. M. Kamuliwo was responsible for management of project and contributed to the drafting of the paper and critical evaluation. C. J. Shinondo contributed to the drafting of the paper, reviewed and critically evaluated it.

Acknowledgments

The authors would like to thank the Ministry of Health, Zambia, and all the staff at the National Malaria Control Centre whose assistance was essential to this study. They would like to acknowledge Dr. Louise Kelly-Hope (LSTM) for developing the resistance maps and the anonymous reviewers for helpful comments on the previous versions of this manuscript. This work was financially supported by the MoH Zambia and the Health Services and Systems Programme (HSSP).

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Research Article

A Predictive Spatial Model to Quantify the Risk of Air-Travel-Associated Dengue Importation into the United States and Europe

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Received 6 July 2011; Accepted 22 December 2011

Academic Editor: Nildimar Honório

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The number of travel-acquired dengue infections has been on a constant rise in the United States and Europe over the past decade. An increased volume of international passenger air traffic originating from regions with endemic dengue contributes to the increasing number of dengue cases. This paper reports results from a network-based regression model which uses international passenger travel volumes, travel distances, predictive species distribution models (for the vector species), and infection data to quantify the relative risk of importing travel-acquired dengue infections into the US and Europe from dengue-endemic regions. Given the necessary data, this model can be used to identify optimal locations (origin cities, destination airports, etc.) for dengue surveillance. The model can be extended to other geographical regions and vector-borne diseases, as well as other network-based processes.

1. Introduction

Dengue is the most common mosquito-borne viral diseases in the world [1]. Although it is not currently endemic to either Europe or the continental United States, except along the Texas-México border and possibly Florida, an increase in dengue occurrence in many of the endemic regions worldwide, in conjunction with a significant rise in the volume of international air travel, has resulted in a greater likelihood of imported dengue infections among travelers returning to the United States and Europe from dengue-endemic regions [2]. It has also increased the potential for transport and establishment of the mosquito vector species in those regions of Europe and the US in which suitable habitat is available.

The causal agent for dengue is a virus that is transmitted from person to person through the bite of infected *Aedes* mosquitoes (mainly *Ae. aegypti* and *Ae. albopictus*), with humans serving as the main viral host [1]. The geographic

establishment of dengue is thought to be limited purely by the occurrence of its principal vector mosquito species, *Ae. aegypti* and *Ae. albopictus*. Both species have proven to be highly adaptable to human habitation and, as a result, the global spread of the vector has been difficult to contain [1]. Dengue is considered endemic to urban and suburban areas in parts of tropical and subtropical America, part of Australia, South and Southeast Asia, the Pacific, and eastern Africa. In addition, the number of imported cases of dengue in the U.S. and Europe is on the rise and further spread and establishment are anticipated [2, 3].

At present, there is no epidemiological surveillance on a national scale in Europe or at the state level in the U.S. [3]. Limiting the importation and establishment of dengue will require dedicated surveillance measures, ideally based on reliable models of vector presence and virus incidence. This paper presents an extendible preliminary model which prioritizes passenger air travel routes for high

likelihood of importing infection into the U.S. and Europe from dengue-endemic regions. Our network-level regression model uses air traffic volumes, travel distances, predictive species distribution models, and infection data, to quantify the relative likelihood of importing infection along air travel routes. More precisely, this paper has two goals.

- (i) Development of a model that allows quantification of the risk of dengue importation through specific air travel routes, thus identifying locations at which surveillance systems can optimally be implemented.
- (ii) Prioritization of the type of data collection efforts that must be undertaken to enhance the predictive accuracy of such models.

Given the requisite data, our model can be used as a prediction tool for assessing the risk of importing dengue-infected humans or vectors via air travel based on origin-destination pairs as well as to analyze the effects of changes in passenger travel routes and volumes on spatial patterns of infection spread.

The model compounds all modes of dengue infection which can be caused by four virus serotypes (DENV-1, DENV-2, DENV-3, and DENV-4), and can range in clinical manifestation from asymptomatic infection to severe systemic disease [1]. Dengue fever (DF) is the more common manifestation of the virus (with an estimated 50 million infections occurring annually world wide), while dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) are rarer and much more severe manifestations of the disease. The model presented in this paper does not distinguish between DF, DHF, and DHS cases because the data available do not permit a more fine-tuned analysis.

Although dengue is now rare in the U.S. and Europe, the mosquito vectors are still present. At least one of the two major vector species, *Ae. aegypti* or *Ae. albopictus*, is known to have established populations in many U.S. states [3]. The European Center for Disease Control [4] recently gathered entomological and environmental data to map the current distribution, as well as establishment risk, for *Ae. albopictus* in Europe in the event of its introduction. It concluded that temperate strains of this species already exist and are likely to spread with new populations becoming established in several parts of Europe [4].

Thus, imported cases of dengue via international travel may potentially result in establishment of an autochthonous disease cycle and new regional outbreaks in both the U.S. and Europe. This can occur in at least two ways: (i) locally established mosquito populations become infected from new hosts (infected travelers) and then spread the disease; or (ii) mosquitoes carrying the virus arrive at a new environment suitable for them. This threat was exemplified recently in Key West, Florida, which experienced sizeable local outbreaks of autochthonous dengue transmission in 2009–2010 [5]. There have also been dengue outbreaks in south Texas, along the Texas-Tamaulipas border, but air travel is unlikely to have had a role in these outbreaks [6].

Epidemics of dengue, their seasonality, and oscillations over time, are reflected by the epidemiology of dengue in

travelers [2]. Modern transportation bridges the natural barriers previously responsible for containing infected vectors to a specific geographic region. For example, the global movement of troops and cargo ships during World War II facilitated the dissemination of *Aedes* mosquitoes and resulted in substantial spread of the disease in Southeast Asia [7]. Transportation of used tires has been shown to be responsible for introducing *Ae. albopictus* into the U.S. from Brazil in the 1980s [2].

Various studies have been conducted to identify the highest travel risks. One survey conducted by the European Network on Imported Infectious Disease Surveillance program [8], analyzed 294 patients with DF for epidemiological information and clinical features. They found most infections were imported from Asia [9]. Tatem et al.'s [10] estimated the relative risk of the importation and establishment of *Ae. albopictus* by sea and air routes, based on normalized measures of traffic and climatic similarity, and found a strong positive correlation between the historic spread of *Ae. albopictus* (into new regions) and a high volume of shipping (routed from ports where the species was already established). The total volume of travel was determined by the number of ship visits for sea travel and passenger volume for air travel. The climatic similarity was calculated as a distance-based vector.

Tatem et al. [10] approach can be extended through quantitative validation of such models. While their work provided insight into the vector importation and establishment process, model validation remained qualitative. In this paper we extend that approach by complementing qualitative risk analysis with quantitative model calibration using infection data. Moreover, Tatem et al.'s approach addressed the risk of importation and establishment of the vector but not the likelihood of infection directly. Our analysis takes infected individuals into account. Additionally, we incorporate climatic factors using species distribution models which are more robust than statistical correlational analysis as relied upon by Tatem et al. This methodology has become standard in disease ecology and epidemiology [11–13].

2. Models and Methods

Our analysis quantifies the relative risk of dengue infected (air travel) passengers entering currently nonendemic regions in the U.S. and Europe at which dengue cases have been recorded. However, it does not include the importation of infected vectors since the influence of that possibility is yet to be established [1, 2, 4]. This section further motivates the problem and introduces a network-based regression model for the risk analysis.

2.1. Imported Dengue in the United States and Europe. Nearly all dengue cases reported in the 48 continental U.S. states were acquired elsewhere by travelers or immigrants. From January 1996 to the end of December 2005, 1196 cases of travel-associated dengue were reported in the continental U.S. [14] (most dengue cases in U.S. nationals occur in those inhabitants of noncontinental U.S. territories such as Puerto

Rico (with over 5000 cases reported in 2005), the U.S. Virgin Islands, Samoa, and Guam, which are all endemic regions). In 2007, an estimated 17 million passengers traveled between the U.S. mainland and dengue-endemic areas of Asia, the Caribbean, Central and South America, and Oceania [15]. Since 1999 there have been 1117 cases of dengue in European travelers reported to the European Network on Imported Infectious Disease Surveillance [8].

Further complications arise from the severe underestimation of dengue cases due to underreporting and passive surveillance in both endemic and non-endemic regions. In tropical and subtropical countries where dengue fever is endemic, under-reporting may be due to misdiagnosis, limitations of the standard World Health Organization (WHO) case classification, and lack of laboratory infrastructure and resources, among other factors [16]. In non-endemic regions such as the U.S. and Europe, the actual number of dengue infections is greatly underestimated due to unfamiliarity with the disease. Additionally, 40–80% of all dengue infections are asymptomatic and closely mimic flu symptoms for which they are mistaken. This lack of accurate infection data makes it difficult to assess the actual threat of the disease.

2.2. Data. The species distribution models required data on the geographical occurrence of *Ae. aegypti* and *Ae. albopictus* and a suite of predictive environmental variables which will be discussed in Section 2.3.

The required data for the network model were as follows.

- (1) Disease data: annual infection reports for dengue-endemic countries, non-endemic European countries and U.S. states.
- (2) Transportation data: passenger air traffic volumes for all flights originating from endemic regions and destined for Europe or the U.S.
- (3) Spatial data: the corresponding distances for all travel routes.

Unless explicitly indicated otherwise, the data used in this model were from 2005, and aggregated to the annual level.

The set of dengue-endemic countries was as identified by the CDC [17]. Country-level infection data for the endemic regions and for European countries were obtained from the regional offices of the World Health Organization [18]. U.S. state level infection data was taken from the CDC [14]. These data sets include the annual number of reported cases for 2005 and 2007, the average of which was used to calibrate the model. Infection data were incorporated in the model in two ways. The number of reported cases at an endemic region was treated as an independent variable in the model, while infection reports for the susceptible node sets (U.S. states and E.U. countries) were used to calibrate the model.

Difficulties were encountered in acquiring the necessary infection data. First, surveillance data for dengue in Africa were sparse. Even though all four dengue virus serotypes have been documented there [19], we were unable to secure country-level infection data for most African countries. Consequently, these endemic countries were ignored in the

model. Although model performance is likely to improve if such data could be incorporated, available reports indicated that Africa is responsible for a relatively small fraction of travel-acquired dengue infections [20]; thus these countries appear to be the unlikely to have a significant impact model predictions. We were also unable to gather infection data for certain endemic countries in the western Pacific region which were similarly ignored and presumed not very relevant.

Transportation data were obtained from two sources. The U.S. air traffic data were from the Research and Innovative Technology Administration (RITA), a branch of the U.S. Department of Transportation (U.S. DOT), which tracks all domestic and international flights originating or ending in the U.S. and its surrounding regions [21]. Passenger market data was aggregated by World Area Code (WAC) to determine the total volume of passengers traveling from each endemic country into any U.S. state in 2005. A similar analysis was done using passenger air traffic data from Eurostat [22] to determine the volume of passengers flying into each European Union country from each endemic country. The transportation data used in this paper focus on passenger travel volumes and do not include cargo flights on which vectors could potentially be transported because the latter mode of dengue spread was excluded from this model.

The average distances used in the model were calculated in ArcGIS 9.3. The average distances were computed for each route as the geodesic distance between the geographic centers of each region.

2.3. Species Distribution Models. The risk for the establishment of dengue and potential cases of disease in an originally non-endemic area depends fundamentally on the ability of a vector to establish itself in that area. If the vector can establish itself then the disease can become endemic in two ways: (i) if the vector is already established, it can become infected from a person infected with dengue arriving in that area; or (ii) infected vectors can be transported into such an area and establish themselves. For this process, habitat in that area must be ecologically suitable for that vector. A relative measure of the suitability of one area compared to another defines a measure of the relative ecological risk [11–13]. If the ecological risk is low, such an establishment is highly unlikely. If that risk is high, then other factors, such as the (temporally) immediate ambient environmental conditions and the size of the founder population or the availability of hosts, become critical for establishment.

The analysis here was based on habitat suitability for the two principal dengue vector species, *Ae. aegypti* and *Ae. albopictus*. It was assumed that these two species do not interact, that is, the probability of the presence of each is independent of that of the presence of the other. The relative ecological risk for the establishment for each species was estimated using a global species distribution model at a 1 arc-minute resolution [23, 24] based on a maximum entropy algorithm incorporated in the Maxent software package Version 3.3.4, [25]. Maxent was used because it has proven to be predictively superior to other species distribution modeling algorithm in a large variety of studies [24, 26]. As input, Maxent uses species occurrence points (presence-only

data) and environmental layers (the explanatory variables). The former were obtained from the Disease Vectors database [27]. The latter consist of four topographic variables (elevation, aspect, slope, and compound topographic index) and a standard set of 19 climatic variables all derived from the WorldClim database [28, 29]. Models were constructed using a variety of subsets of these environmental variables. All computations used default settings [26]. Averages over 100 replicate models were computed. The best model was judged using the Akaike Information Criterion (AIC) for species distribution models. The best model for *Ae. aegypti* is one that used all 23 explanatory variables; that for *Ae. albopictus* is based on elevation, slope, aspect, maximum temperature of warmest month, minimum temperature of coldest month, precipitation of wettest month, and precipitation of driest month. Details of the species distribution models will be published separately in the epidemiological literature.

The output from Maxent consists of relative suitability values between 0 and 1 which, when normalized, can be interpreted as the probabilistic expectation of vector presence of a species in a cell. The probabilistic expectation of at least one of the vector species being present in a cell was calculated as the complement of the probability that neither is present, assuming statistical independence. Because the infection and travel data used in this work are at the state level for the U.S. and the country level for Europe, the expectations are aggregated to the same level by averaging them over all the cells in the relevant geographical units. These expectations define the relative ecological risk for dengue in each cell.

2.4. Mathematical Network Model. The network model predicts the expected number of dengue cases in each non-endemic region that can be attributed to a particular endemic region connected to it by travel. Two previous mathematical models quantifying risk estimates for acquiring arboviral infection are by Massad and Wilder-Smith [30] and Codeço et. al. [31]. Massad and Wilder-Smith's model was intended to evaluate the risk of infection at a specific site as a function of human population size, the number of infected mosquitoes, and estimated parameters for the biting rate and the probability that an infectious mosquito will infect a susceptible human. The model did not incorporate travel patterns or species distribution data; moreover, model predictions were not quantitatively validated using infection data. Codeço et. al. assessed the risk of yellow fever (YF) emergence in the city of Rio de Janeiro, Brazil, by estimating the probability of infected individuals arriving from YF-endemic areas via air and bus travel, and the probability of infective individuals triggering an epidemic (by using a stochastic transmission model). While this model accounted for travel patterns and local transmission probabilities, the model predictions were again not quantitatively validated.

Our model has similarities to a feedforward artificial neural network (ANN). Feed-forward ANNs have been used to model learning input-output systems, and can be calibrated through a "back-propagation" algorithm that minimizes a cost function representing output error [32]. The approach taken in this paper differs from traditional implementations of ANNs insofar as, not only is a response

function calibrated, but the function itself must be chosen to suit the process.

2.4.1. Network Structure. In the proposed network structure, geographic areas were represented as nodes, belonging to either the set G of endemic nodes, or one of the sets N_U or N_E of susceptible nodes in the United States and Europe, respectively. The links in the network represent directed air travel connections between geographic areas (originating from G), while the measure P_{ji} represents the number of predicted infections at a susceptible node i attributed to an endemic node j .

This directed bipartite network structure connected endemic countries to susceptible regions (U.S. states and E.U. countries). Initially a single model was developed which included all susceptible regions as a single set of destination nodes, N . However the significantly higher number of reported infections in Europe relative to the U.S. resulted in extremely poor predictions. The limited performance was likely a result of unobserved variables which differentiate the risk of importing infection into Europe versus the U.S, such as border control procedures, quality of healthcare and quality of disease surveillance. These variables were difficult to quantify directly, as we found through empirical testing, and were best accounted for by modeling the U.S. and Europe separately. Figure 1(a) provides an example of a bipartite network structure representative of the network structure modeled in this paper. The network modeled in this work was limited to the regions with available infection data. The resulting network included 56 endemic nodes, 42 total susceptible nodes (30 U.S. states and 12 European countries), and 664 links. The reason the network was not fully connected is because passenger travel did not occur between all pairs of nodes.

Figure 1(b) is a four-node extraction from the example network to illustrate the generalized link-based functional form used in our model. The function $f_{ji}(\lambda, x_j, y_i, z_{ji})$ represents the number of cases observed at i for which j is responsible, where λ represents a vector of calibrated parameters, x_j represents the characteristics of origin j , y_i represents the characteristics of destination i , and z_{ji} represents the vector of parameters specific to directed link (j, i) . The total predicted number of infections at i is $P_i = \sum_{j \in A(i)} f_{ji}(\lambda, x_j, y_i, z_{ji})$, where $A(i)$ represents the set of endemic nodes adjacent to i .

The most critical issue was determining the functional form of $f_{ji}(\lambda, x_j, y_i, z_{ji})$. Two complications arose: first, the process that $f_{ji}(\lambda, x_j, y_i, z_{ji})$ attempts to model was too complex to determine a functional form *a priori*, that is, the relative impact different variables will have is not clear ahead of time. Second, directional infection data (i.e., the source of infection for travel acquired dengue cases) was not available. Consequently, specifying the functional form of $f_{ji}(\lambda, x_j, y_i, z_{ji})$ was not feasible. Because the objective was to identify a link-based functional form that best replicated the number of reported cases at each susceptible region, a variety of functional forms were examined to identify the one with optimal performance.

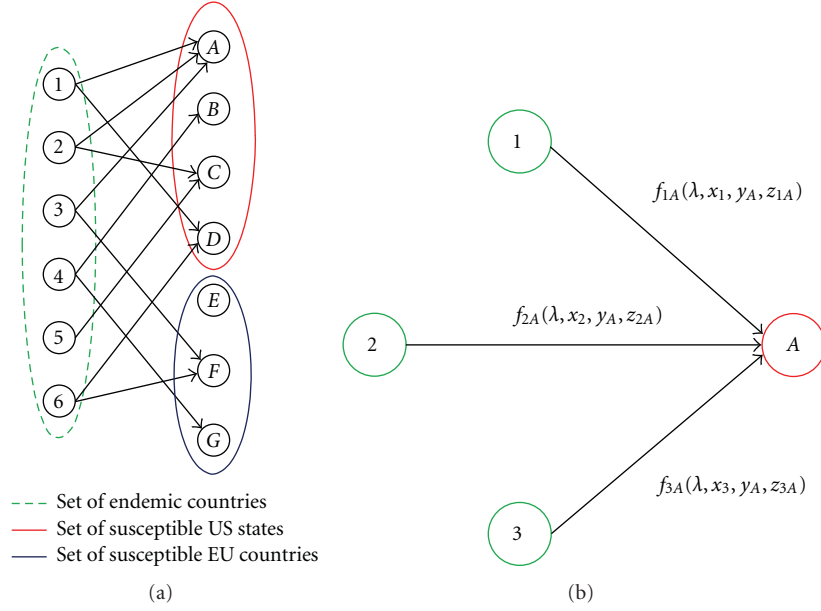


FIGURE 1: (a) Bipartite network connecting endemic regions to susceptible regions: the susceptible U.S. and Europe nodes represent mutually exclusive sets; (b) link-based functions: these predict the number of infections at susceptible node A, attributed to each adjacent endemic region (1, 2, and 3).

TABLE 1: Problem Notation.

| | |
|-------------|--|
| N_U | Subset of susceptible nodes in the United States |
| N_E | Subset of susceptible nodes in Europe |
| N | Complete set of susceptible nodes ($N_U \cup N_E$) |
| G | Set of nodes in the endemic region |
| I_i | Number of reported infections at node i |
| P_i | Total number of predicted infections at node i |
| P_{ji} | Number of predicted infections at node i attributed to node j |
| λ | Vector of parameter to be optimized |
| x_j | Vector of characteristics of infecting node j |
| y_i | Vector of characteristics of susceptible node i |
| z_{ji} | Vector of parameters specific to link (j, i) |
| V'_{ji} | Normalized passenger air travel volume between nodes j and i , ranging from 0 to 1 |
| S_i | Climate suitability of node i , ranging from 0 to 1 |
| I'_i | Normalized reported infections at node i |
| D'_{ji} | Normalized distance between nodes j and i , ranging from 0 to 1 |
| $A(i)$ | Set of endemic nodes adjacent to susceptible node i |
| α, b | Parameters to be optimize |

2.4.2. *Problem Formulation.* The notation used in the formal problem formulation is shown in Table 1.

The purpose of this analysis was to examine a variety of families of functions, further explore the most suitable member of each family, and examine the results from a qualitative perspective. The objective was to find the parameter vector λ for a given $f_{ji}(\lambda, x_j, y_i, z_{ji})$ such that the difference between I_i , the observed number of infections at

susceptible node i , and P_i , the predicted number of infections at i , was as small as possible. To ensure this, we formulated a nonlinear convex program to find the unknown parameter vector λ which minimized the sum of the squared difference between observed and predicted infection values over all susceptible nodes in the set. The problem formulation is as follows:

$$\begin{aligned} \min_{\lambda} \quad & \sum_{i \in N} (I_i - P_i)^2 \\ \text{s.t.} \quad & P_{ji} = f_{ji}(\lambda, x_j, y_i, z_{ji}) \quad \forall i \in N \quad \forall j \in G \quad (1) \\ & P_i = \sum_{j \in A(i)} P_{ji} \quad \forall i \in N. \end{aligned}$$

The characteristics of the resulting linear program depend on the role of the parameter vector λ in the function, $f_{ji}(\lambda, x_j, y_i, z_{ji})$. If the function is linear in respect to λ , the resulting program can be solved analytically for the optimal decision parameters through a system of linear equations. In other cases, however, the resulting function may be non-convex, and as such solvable only through simulation.

2.4.3. *Functional Forms.* Depending on the functional form of $f_{ji}(\lambda, x_j, y_i, z_{ji})$, namely, the behavior of $f_{ji}(\lambda, x_j, y_i, z_{ji})$ with respect to λ , the tractability of the resulting mathematical program will vary. In developing a sensible link function, we considered several factors such as the highly nonlinear response of the explanatory variable with respect to the dependent variables considered and concerns about overfitting the data. Various functional forms were examined

and compared, and the best performing function was found to have the following form:

$$P_{ji} = \beta + \alpha * \frac{V'_{ji} * S_j * S_i * \sqrt{I'_j}}{\sqrt{D'_{ji}}} \quad \forall i \in N, \forall j \in G. \quad (2)$$

The motivation for the final functional form, P_{ji} defined above, came from the Gravity Model for Trip Distribution. The function is the sum of two terms: the first term on the RHS is equivalent to the constant term in a standard regression model; while the second term bears a strong resemblance to the Gravity Model used for trip distribution. In the Gravity Model the fraction of trips attracted to zone j from zone i is proportional to the population of both zones, and inversely proportional to some measure of generalized cost of travel between them. Similarly, in the second term of the RHS of the equation above, the numerator accounts for the travel volume, the relative ecological risks of the origin and destination (from the species distribution models), and the number of cases reported at the source, while the distance is included in the denominator.

The square root of I'_j represents the concave relationship between the predicted number of infections at a susceptible location and the number of reported cases at an endemic source. For the denominator, the lowest value for the sum of squared errors was obtained by taking the square root of the distance. While proximity to endemic countries showed a positive correlation to the reported cases, the differential effect of distance was higher for areas closer to endemic regions. The concavity of the term can be attributed to the relationship between travel time and distance, which is certainly not linear. In order to normalize the data, the values for travel volume, distance, and number of reported cases at endemic regions were rescaled by the maximum value across all observations for their respective category.

2.4.4. Model Parameter Estimation. By rewriting the original mathematical program in terms of the node based variables P_i , it is evident that it holds the same structure as a multiple linear regression. The model was solved using the Ordinary least squares estimation procedure:

$$\min_{\lambda} \sum_{\forall i \in N} (I_i - P_i)^2, \quad (3)$$

where

$$P_i = \beta * \xi(i) + \alpha * \varphi(i) \quad \forall i \in N, \\ \varphi(i) = \sum_{\forall j \in A(i)} \frac{V'_{ji} * S_j * S_i * \sqrt{I'_j}}{\sqrt{D'_{ji}}} \quad (4) \\ \xi(i) = |A(i)|.$$

In order to estimate the values of α and β , we solved the system of equations that resulted from the first-order

optimality conditions of the convex program shown above. The system of equations reduced to:

$$\sum_i I_i \xi(i) - \alpha \sum_i \xi(i) \phi(i) - \beta \sum_i \xi(i) \xi(i) = 0, \\ \sum_i I_i \phi(i) - \alpha \sum_i \phi(i) \phi(i) - \beta \sum_i \phi(i) \xi(i) = 0. \quad (5)$$

Solving the system of equations yielded as estimates for α and β :

$$\alpha = \frac{\sum_i I_i \xi(i) \sum_i \xi(i) \phi(i) - \sum_i I_i \phi(i) \sum_i \xi(i) \xi(i)}{\sum_i \xi(i) \phi(i) \sum_i \xi(i) \phi(i) - \sum_i \xi(i) \xi(i) \sum_i \phi(i) \phi(i)} \\ \beta = \frac{\alpha \sum_i \phi(i) \phi(i) - \sum_i I_i \phi(i)}{\sum_i \xi(i) \phi(i)}. \quad (6)$$

3. Results and Discussion

The main objective of the model was to quantify the relative risk of various international travel routes. This was accomplished by first predicting the number of dengue cases specific to each travel route, and then calibrating the network model at a regional level using infection data. Therefore, there are two sets of results presented. Section 3.1 includes the total number of dengue cases predicted for each susceptible region based on the calibrated model output, and Section 3.2 includes the corresponding relative risk of each travel route, ranked based on their likelihood of transporting infected passengers.

The results included in this section are representative of filtered data. The filtering process was applied to the susceptible node set to remove outliers. The outliers were classified differently for the European and U.S. node sets. In the European data set any region with less than 5 cases was considered an outlier, while only states with one reported case were considered outliers in the U.S. node set. A lower threshold was implemented for the U.S. as there were fewer reported cases on average. The procedure resulted in five nodes being removed from N_E and 12 nodes being removed from N_U . After the filtering process there were 18 U.S. states and seven European countries included in the model.

3.1. Susceptible Node-Based Predictions. The model was able to predict closely the number of reported cases for the European countries, though it struggled to predict the number of reported cases for the U.S. states accurately. The results for the node-based predictions, P_i , are shown in Table 2(a) for European Countries and Table 2(b) for U.S. states.

The functional form introduced in Section 2.4.3 was used in both models, while the resulting regression parameters, α and β had different estimates. For Europe the optimal α and β were 271.52 and 5.08, respectively; for the U.S. 5.54 and 0.595. The combination of the low constant (β), high α value, and good fit of the European model signifies that the majority of variability in the data was accounted for by the independent variables included in the model. This was not the case with the U.S. model. On average, the European model predictions diverged from the reported cases by 24,

TABLE 2: Model output and actual reported infections for (a) Europe and (b) the U.S.

| (a) Infections for susceptible European countries. | | |
|--|----------------------------|---------------------------|
| E.U. country | Actual reported infections | Model reported infections |
| Belgium | 25 | 31 |
| Czech Republic | 9 | 31 |
| Finland | 12 | 40 |
| France | 300 | 247 |
| Germany | 204 | 231 |
| Sweden | 61 | 55 |
| United Kingdom | 170 | 196 |
| Total | 781 | 831 |

| (b) Infections for susceptible U.S. states. | | |
|---|----------------------------|---------------------------|
| U.S. State | Actual Reported Infections | Model Reported Infections |
| Hawaii | 11 | 6 |
| Massachusetts | 14 | 12 |
| New York | 55 | 22 |
| Pennsylvania | 3 | 11 |
| Florida | 22 | 24 |
| Georgia | 7 | 16 |
| North Carolina | 5 | 9 |
| Virginia | 5 | 6 |
| Illinois | 3 | 14 |
| Ohio | 4 | 6 |
| Wisconsin | 2 | 4 |
| Minnesota | 11 | 6 |
| Texas | 24 | 20 |
| Arizona | 5 | 4 |
| Nevada | 2 | 7 |
| California | 4 | 22 |
| Oregon | 4 | 4 |
| Washington | 6 | 5 |
| Total | 187 | 196 |

where 112 actual cases were observed on average per node. The U.S. model predictions diverged from the reported cases by an average of 6.2, where an average of 10.4 cases were reported per node.

Several factors contributed to complicating the task of identifying a function to perfectly fit the case data. Firstly, the limited size of the susceptible node set made it difficult for the model to differentiate between variability and noise. Secondly, the amount of noise in the data due unknown factors such as variations in regional surveillance efforts could not be accounted for. Thirdly, prevention measures being implemented were not only difficult to determine, but also difficult to quantify. All these uncertainties restricted the model's ability to estimate parameters that resulted in good predictive properties at the node level. However, our

results show that, though the fit at the node level could be improved upon, the route-level risk measures do show promising results, and as such, provide some insight into the role the independent variables play.

3.2. Endemic-Susceptible Route-Based Risk. Although the node-based predictions can be validated based on the reported infection data, the resulting route-based predictions were not directly-verifiable due to the unavailability of route-based infection data. The best measures of validation were (i) to find route-based predictions that correspond to known regional infection data when summed across all incoming routes, and (ii) to compare the results with previous travel-based patient surveys conducted to determine the most likely place of origin for illness.

Table 3 identifies the 20 international travel routes with the highest probability of carrying dengue-infected passengers into (a) Europe and (b) the U.S., and their corresponding relative risk, as produced by the model. The initial ranking was determined based on the predicted number of infected passengers traveling on each route. The predicted number of infected passengers was then normalized to the highest ranked route. Although the results shown are specific to the filtered node sets, similar results were obtained for the full node sets, for both Europe and the U.S. In the model Burma, Cambodia, Laos, and Thailand were aggregated to a single "South East Asia" endemic region.

Figure 2 compares the highest traveled international routes with the highest risk international travel routes for carrying infected passengers (as predicted by the model). The links included in Figure 2(a) are representative of the twenty highest traveled routes entering the U.S. and the twenty highest traveled routes entering the E.U.; the line thickness is proportional to travel volume. Figure 2(b) provides a visual illustration of the model output, specifically the 20 international travel routes with the highest probability of carrying dengue infected passengers into Europe and the U.S. (the set of links listed in Table 3); the line thickness is proportional to the relative risk of the route. The difference between these two mappings illustrates the significance of the regional level input variables (vector suitability and infection data) included the model.

As stated previously, one way to verify the predicted route-based risk was by comparing the results with previous patient surveys conducted to identify the source of infections. A previous study found of the travel acquired dengue cases in Europe between 1999 and 2002 [33]:

- (i) 219 (45%) originated in South-East Asia, represented in the model as 3 of the top 6 highest risk routes,
- (ii) 91 cases (19%) originated in South and Central America, represented in the model as 3 of the top 10 highest risk routes,
- (iii) 77 cases (16%) originated in the Indian subcontinent, represented in the model as 2 of the top 15 highest risk routes,



(a)



(b)

FIGURE 2: (a) The 20 highest traveled routes entering the U.S. and E.U. There are 40 total links; the line thickness is proportional to the travel volume. (b) The top 20 travel routes with highest relative risk of carrying Dengue infected passengers into U.S. and E.U. The line thickness is proportional to the relative risk of the route.

- (iv) 56 cases (12%) originated in the Caribbean, represented in the model as 2 of the top 20 highest risk routes.

The model predicted Brazil-Germany and Brazil-France as the two highest risk routes into Europe (with nearly equivalent relative risk). This is expected, as Brazil reported the highest number of dengue cases in the world per year,

almost 3-times those of second place Indonesia, and the volume of traffic on the Brazil-France and Brazil-Germany routes were two of the top 40 in the world. Indonesia, reported a very high number of infections, but reported a very low level of air travel on any given route destined for Europe. Using similar logic, Southeast Asia reported a number of infections on par with Indonesia, though the travel volume from Southeast Asia into Germany and

TABLE 3: Relative risk of spreading travel acquired dengue infection via international travel routes from endemic countries into (a) Europe and (b) U.S.

(a) Route-based relative risk european countries.

| Rank | From | To | Relative Risk |
|------|--------------------|----------------|---------------|
| 1 | Brazil | Germany | 1.00 |
| 2 | Brazil | France | 0.99 |
| 3 | South East Asia | Germany | 0.71 |
| 4 | South East Asia | United Kingdom | 0.52 |
| 5 | Brazil | United Kingdom | 0.35 |
| 6 | South East Asia | France | 0.29 |
| 7 | Vietnam | France | 0.29 |
| 8 | Singapore | United Kingdom | 0.27 |
| 9 | Singapore | Germany | 0.19 |
| 10 | India | Germany | 0.19 |
| 11 | Malaysia | United Kingdom | 0.19 |
| 12 | India | United Kingdom | 0.17 |
| 13 | Dominican Republic | Germany | 0.16 |
| 14 | Venezuela | Germany | 0.16 |
| 15 | Dominican Republic | France | 0.16 |
| 16 | Mexico | France | 0.16 |
| 17 | Mexico | Germany | 0.15 |
| 18 | Venezuela | France | 0.15 |
| 19 | South East Asia | Finland | 0.14 |
| 20 | South East Asia | Sweden | 0.13 |

(b) Route-based relative risk for U.S. states.

| Rank | From | To | Relative risk |
|------|--------------------|------------|---------------|
| 1 | Mexico | Texas | 1.00 |
| 2 | Mexico | California | 0.56 |
| 3 | Puerto Rico | Florida | 0.34 |
| 4 | Brazil | Florida | 0.33 |
| 5 | Venezuela | Florida | 0.24 |
| 6 | Mexico | Illinois | 0.23 |
| 7 | Puerto Rico | New York | 0.21 |
| 8 | Costa Rica | Florida | 0.19 |
| 9 | Mexico | Florida | 0.19 |
| 10 | Mexico | Arizona | 0.19 |
| 11 | Dominican Republic | New York | 0.17 |
| 12 | Colombia | Florida | 0.16 |
| 13 | Brazil | New York | 0.15 |
| 14 | Mexico | Georgia | 0.15 |
| 15 | Dominican Republic | Florida | 0.15 |
| 16 | Brazil | Texas | 0.14 |
| 17 | Brazil | Georgia | 0.12 |
| 18 | Honduras | Florida | 0.12 |
| 19 | Costa Rica | Texas | 0.12 |
| 20 | Mexico | Nevada | 0.11 |

the United Kingdom ranked among the world's top 25 travel routes; suggesting intuitively that travel volume is a dominant factor in assessing infection risk.

For the U.S. the model predicted the majority of U.S. infections were attributed to Central and South American countries, likely a result of the close proximity, high traffic, and high level of infection. More specifically, 19 of the top 20 highest risk routes into the U.S. (Nevada, ranked 20th not included) were destined for states which account for a very high fraction of incoming flights in the U.S.; accounting for 6 of the top 15 busiest American Airports by boardings [34].

As a destination, Florida accounted for 5 of the top 10 risk routes, which is supported by historical occurrence of the disease, exemplified in the 2009-2010 local outbreaks. Though it is possible that dengue was already present in the locality (Key West), and previously undetected, the results of this model suggest dengue was likely introduced via international travelers into a locality with environmental and social conditions ripe for transmission [5]; in the model Puerto Rico-Florida ranked as the third highest risk route. The travel volume on this route was among the top ten in the world, while the proximity and climate similarity were likely additional contributors to the infection risk.

Mexico-Texas and Mexico-California ranked as the two highest risk routes, and were also the top two traveled routes (by passenger volume) in the world [34]. The highest risk travel route predicted was from Mexico to Texas; with nearly twice the risk of Mexico-California. The high number of infections reported in Mexico, its proximity to Texas, and the high volume of travel between the two intuitively suggests this to be a high-risk pairing, which is supported by the model.

4. Conclusions

Dengue currently presents a serious risk to many parts of the U.S. and Europe where suitable environmental conditions for vector species' occurrence and establishment provide the potential for local outbreaks, were the virus to be introduced. The background to this analysis was the increasing number of dengue cases in the U.S. and Europe, coinciding with an increase in both the prevalence of dengue worldwide and increased volume of international passenger air traffic originating from dengue-endemic regions since the 1990s.

The model presented here was developed to explore the relationship between reported dengue infections and air travel. It used a network-based regression to quantify the relative risk from international air travel routes carrying infected passengers from endemic regions to non-endemic ones in the U.S. and Europe. Besides international passenger travel volumes, the model incorporated predictive species distribution models for the principal vector mosquito species. The model also incorporated travel distances and infection data. The following inferences follow from the model results.

- (i) The highest-risk travel routes suggest that the proximity to endemic regions is a dominant factor. Most high-risk routes into Europe originate in Asia (with

the exception of Brazil and Mexico), while all top 20 routes into the U.S. originate in South and Central America.

- (ii) Travel from dengue-endemic countries presents significant risk to Florida. Additionally, the high volume of domestic visitors to Florida in conjunction with established *Ae. aegypti* and *Ae. albopictus* populations, enhances Florida's potential role as a fulcrum spreading dengue to other parts of the continental U.S. The recent reemergence of dengue in Florida suggests that strong vector surveillance and control infrastructure is critically needed for identification and control of outbreaks of dengue.
- (iii) The high risk predicted for Mexico-Texas travel is further heightened by the risk of overland transmission (such as that from Tamaulipas into the Brownsville area [6]). Therefore surveillance along the Texas-Tamaulipas border should be complemented with surveillance at regions with airports connected to Mexico by regular or chartered flights.
- (iv) For many countries of Europe and U.S. states, if dengue gets introduced, the establishment of an autochthonous disease cycle is likely because many of these areas contain suitable habitats for *Ae. albopictus* and some contain established populations.
- (v) Some of the "source" areas indicate that dengue has yet to be brought under control in places where malaria has. This means that dengue may well replace malaria as the paradigmatic airport disease.

The results provided in this paper were obtained using existing (historical) data from the (recent) past and do not represent fully reliable predictions for relative risks in the future. However, the model introduced in this paper can be calibrated using epidemiological data from any time period. The calibrated model can be used as a predictive tool for quantifying route-based risk in the future provided that the necessary data are available, including real-time travel patterns, environmental conditions, and infection data. Moreover, the results in this paper are aggregated at the annual and regional (country or state) level due to the limitations of available data. Infection data proved to be the most difficult to gather because infection reports for many regions in the world are not available even at the annual level. Appropriate data will enable the extension of the model to allow analysis at finer spatial and temporal resolutions: the model can be regionally disaggregated to the city level, or disaggregated by month to account for seasonality. Moreover, this model can be deployed in other geographical regions, used for other vector-borne diseases, and modified to analyze other network-based processes. Finally, this model can potentially be extended to include other modes of transportation, such as freight and shipping networks.

The development of such models is an integral step in improving local and regional surveillance efforts. The quantitative results produced by the model can lead to more specific surveillance recommendations than the CDC is

currently able to make such as identifying (i) specific routes on which to implement surveillance and control strategies and (ii) optimal locations (origin cities, destination airports, etc.) for passenger surveillance efforts. As there is currently no vaccine for dengue; surveillance and intervention, along with vector control, are the only relevant options to prevent further geographic spread of the disease. The limitations of this analysis highlight the need for improving the quality of readily accessible disease data so as to enhance the prediction and control of epidemic episodes of vector-borne diseases in susceptible countries.

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Research Article

Effects of Artificial Flooding for Hydroelectric Development on the Population of *Mansonia humeralis* (Diptera: Culicidae) in the Paraná River, São Paulo, Brazil

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Received 11 August 2011; Revised 10 December 2011; Accepted 23 December 2011

Academic Editor: Nicolas Degallier

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The closure of two phases of the dam at the Porto Primavera Hydroelectric Plant on the Paraná River flooded a flawed system located in the Municipality of Presidente Epitácio, São Paulo state, favoring the proliferation of aquatic weeds. This study aimed to observe the population of *Mansonia humeralis* in the area, monitoring the richness, diversity, and dominance of this species both before and during different phases of reservoir flooding as well as evaluate its possible consequences concerning human and animal contact. Adult mosquitoes were collected monthly in the following periods: at the original level, after the first flood, and after the maximum level had been reached between 1997 and 2002. Collection methods used were an aspirator, a Shannon trap, and the Human Attractive Technique. A total of 30,723 mosquitoes were collected, *Ma. humeralis* accounting for 3.1% in the pre-flood phase, 59.6% in the intermediate, and 53.8% at maximum level. This species is relevant to public health, since the prospect of continued contact between *Ma. humeralis* and the human population enhances the dam's importance in the production of nuisance mosquitoes, possibly facilitating the transmission of arboviruses. Local authorities should continue to monitor culicid activity through sustainable entomological surveillance.

1. Introduction

Anthropic changes to the natural environment invariably impact biodiversity, and new habitats often become available after landscape alterations, particularly among culicids [1]. According to Forattini [2], this dynamic favors selective processes. Newly established ecotopes promote the proliferation of mosquito species that become nuisances or disease vectors for humans [3].

Female mosquitoes of the family Culicidae oviposit in several types and dimensions of reservoir water, where they cooccur with other species and are associated with plant matter. During immature stages, mosquitoes of the genera *Mansonia* Blanchard and *Coquillettidia* Dyar affix themselves

to the roots of aquatic macrophytes, from which they derive oxygen accumulated in the aerenchyma of the plant floating organs [4].

The eleven *Mansonia* species listed for Brazil [5, 6] depend on macrophytes during larval and pupal stages. As with most culicids, only adult females require blood meals (for oocyte maturation). Mosquitoes from the genus *Mansonia* are a nuisance to humans and animals in situations of high density and can become a public health concern due to their anthropophilic behavior [7].

Our group hypothesized that the damming of the Paraná River and construction of the Engenheiro Sérgio Motta Hydroelectric Plant, more commonly known as the Porto Primavera Hydroelectric Plant (PPHP), produced a favorable

environment for mosquitoes of the tribe *Mansoniini* owing to the propagation of macrophytes. The present study aimed to monitor the richness, diversity, and dominance of the *Ma. humeralis* Dyar and Knab population in different phases of reservoir flooding and assess its possible consequences regarding human and animal contact. Data concerning other species are also presented.

2. Materials and Methods

The present research was undertaken on the banks of the PPHP reservoir, formed by the damming of the Paraná River, 28 km upstream from its confluence with the Paranapanema River. Although the reservoir impacts several municipalities in Mato Grosso do Sul [8, 9] and São Paulo states [10], the study area was located in the Presidente Epitácio Municipality in São Paulo state, part of a larger project developed in this area.

Prior to the establishment of the PPHP, the study region originally comprised a complex of flooded areas known as the “Lagoa São Paulo Ecological Reserve”, previously formed by the São Paulo, Bonita, Comprida, Tremendal, and Jota lakes and consisting of a mosaic of rearing environments during rainy periods, with accumulated water [11].

After construction of the dam, both permanently submerged and seasonally flooded areas were inundated by the reservoir. The original level of the Paraná River was 247 m above sea level. Flooding was carried out in two stages: the initial phase in January 1999 raised the water level to 253 m, and in March 2001, the second flooding stage elevated waters to the current level of 257 m.

The area designated for mosquito capture, denominated site JB (52°00'25''W/21°38'45''S), is composed of flooded lowland, with the presence of floating macrophytes (*Eichhornia crassipes* (Mart.) Solms, *Pistia stratiotes* Linnaeus, *Salvinia* sp. Séguier, *Ricciocarpus natans* (L.) Corda, *Hidrocotyle umbellata* Linnaeus, *Cladium* sp. P. Browne, *Cyperus* sp. Linnaeus, *Egeria najas* Planch), in addition to rural plateau areas containing seven human settlements, with six aggregations of homes (Bairro Campinal and rural villages of Reassentamento Lagoa São Paulo [8]). Residents of these villages and rural landowners come into contact with the reservoir through leisure and/or fishing (Figure 1). Fragments and strips of remnant primitive semideciduous seasonal forest vegetation still occur.

Climate in the region is classified as Aw-Tropical, with a dry winter according to the Köppen system [8]. Average annual precipitation varies from 1,000 to 1,400 mm. Soil type is dark red latossol/sandy phase with low rainwater infiltration capacity [11].

Culicids were collected during each of the three flood stages: 247 m (07/1997 to 09/1998), 253 m (07/1999 to 09/2000), and 257 m (07/2001 to 09/2002). Adult mosquito collections were undertaken one day per month, using four capture methods: Mechanical aspiration (MA) in one 15 min sampling effort per capture, in the morning period in riparian areas; and Shannon Trap (ST), for 20 min of unit time in the evening at twilight, with intervals as

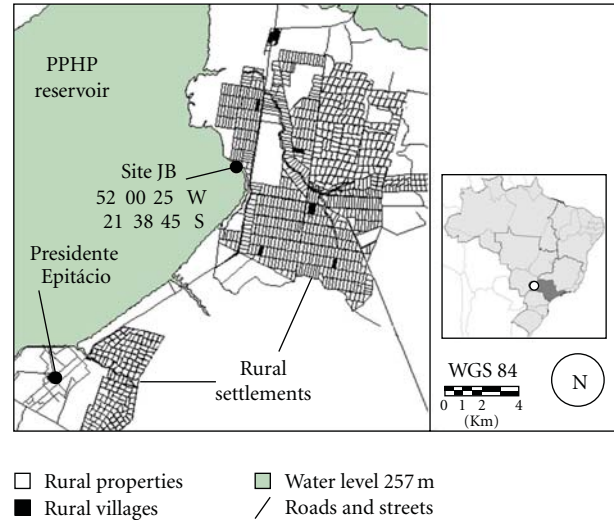


FIGURE 1: Site JB localization according to PPHP reservoir at water level of 257 meters upper sea level and distinct types of human distribution (rural properties, rural villages, and urban area). Cartographic data: CESP/ITESP/INCRA.

follows: first and second precrepuscular, crepuscular, and first postcrepuscular. There were two individual collectors.

In order to attract a greater number of mosquitoes for anthropophilic behavior, two collection methods were performed using humans: the Human Attractive Technique (HAT 24 h), involving collections over a period of 24 h, with hourly separation of the material gathered by two individual collectors; and the Human Attractive Technique (HATet), consisting of collections in the evening at twilight for 20 min of unit time, with increments divided into first and second precrepuscular; crepuscular; and first, second, third, and fourth postcrepuscular. There were two individual collectors. The last two capture methods were not used during the 257 m stage because of operational and infrastructural difficulties. Field collectors used personal protection to avoid bites.

Mosquitoes were identified in the Entomology Laboratory at the Public Health Faculty of the University of São Paulo.

Samples that were not *Ma. humeralis* were pooled into the category “other species” since our aim was to specifically evaluate the population of this taxon. Frequency of the species investigated was compared to “other species” at stages 247 m, 253 m, and 257 m.

Abbreviations used for species names were in accordance with Reinert [12] and identifications were determined following Forattini [13] and Lane [14].

Variations in temperature and rainfall were correlated with mosquito frequencies for each capture method. Monthly precipitation (mm) and air temperature (°C) data were obtained from the São Paulo Energy Company (CESP) and the Integrated Center of Agro-Meteorological Information, of the Campinas Agronomy Institute (CIIAGRO-IAC).

Statistical analyses were conducted on the mean monthly values of richness [15], diversity (Margalef) [16], and dominance (Berger-Parker) (exclusively for *Ma. humeralis*) [17].

Means between the periods before and after flooding were tested in order to evaluate frequency differences between *Ma. humeralis* and other culicids ($P \leq 0.05$). Comparative analysis was performed using Mann-Whitney, ANOVA, and Post Hoc (Tukey) tests for indices of richness, diversity, and dominance and comparison of means, with SPSS computer software, version 10.

3. Results

A total of 30,723 culicids were collected during the study, 22,181 were captured at flood stage 247 m (preflooding), and of these, *Ma. humeralis* accounted for 3.1% (695). At the 253 m flooding stage (first flooding), 7,982 mosquitoes were captured, 4,754 (59.6%) of which were *Ma. humeralis*. A total of 560 specimens were collected during the second flooding stage (257 m), *Ma. humeralis* corresponding to 53.8% (301) (Table 1).

During preflooding, mean monthly richness and diversity values varied among the capture techniques, as did the dominance of *Ma. humeralis* (Table 1).

Compared to *Ma. humeralis*, the mean richness and diversity values of other culicids decreased after the first flooding stage (247 m–253 m levels) (MA: richness $P = 0.08$, diversity $P = 0.04$, dominance absent; ST: richness $P = 0.05$, diversity $P = 0.06$, dominance $P = 0.002$; HAT 24 h and HATet: richness and diversity: $P < 0.001$, dominance $P = 0.001$). *Ma. humeralis* was not collected using the MA technique during stage 247 m. Following the second flooding stage (253 m–257 m levels), these lower richness and diversity values were maintained (MA: richness and diversity $P = 0.01$, dominance $P = 0.3$; ST: richness $P = 0.01$, diversity $P = 0.3$, dominance $P = 0.04$). Although *Ma. humeralis* dominance remained high in MA captures, samples obtained with ST were substantially reduced (Table 1).

Monthly richness and diversity values with the MA capture method were significantly different in comparisons between flood stages, except for richness between flood stage 247 m and 253 m. Means for ST captures were also reduced between flood stages. Richness and diversity values were statistically different in all comparisons, except for diversity between stages 247 m and 253 m, and 253 m and 257 m. Captures using HAT 24 h and HATet yielded significant declines in richness and diversity.

Although not statistically significant, dominance of this species was confirmed when the reservoir rose from 253 m to 257 m. Dominance values for *Ma. humeralis* between stages 247 m and 257 m were not significantly different with the ST method ($P = 0.5$). When using HAT 24 h and HATet, the mean dominance of *Ma. humeralis* at stage 253 m increased significantly from 247 m.

Monthly frequencies of other culicid species were correlated with the number of *Ma. humeralis* in all capture methods during the three flooding stages. In relation to the MA technique, no statistical difference was recorded in *Ma. humeralis* frequencies ($P = 0.3$) during stages 253 m and 257 m and for “other species” ($P = 0.2$) during stages 247 m and 253 m.

No statistical difference was observed between mean temperature prior to flooding (23.3°C) and that following flooding stages 1 and 2 (24.7°C) when compared with *Ma. humeralis* frequency after analysis of all techniques ($P > 0.1$). The same occurred for mean precipitation, which was higher (95.2 mm) during the preflooding stage than subsequent periods (62.6 and 50.6 mm), but not statistically different. *Mansonia humeralis* showed greater population density during winter and spring ($P > 0.1$).

4. Discussion

Mean richness and diversity values changed based on evaluation at each of the three reservoir levels. Immediately following initial flooding (flood stage 253 m), a 64% reduction occurred in the number of adult culicids, suggesting that many rearing sites were destroyed and immature forms did not survive the rising water.

Increased *Ma. humeralis* abundance (from 3.1% at 247 m to 59.6% at 253 m and 53.8% at 257 m) suggests that this species benefitted from the flooding in relation to other culicids. This supports previous results following the flooding of the Tucuruí Reservoir in Amazonia [7], where high nuisance levels were recorded in humans and animals due to larger *Mansonia* populations (97.1%).

Mansonia species were absent in forest fragments between the municipalities of Presidente Venceslau and Caiuá [18], near the study site sampled and in a similar ecological scenario. This contradicts our results in the preflooding stage, when an abundance value of 3.1% was recorded. The low frequency may be associated with the scarcity of macrophytes in the pre-flood water pools on the Paraná River floodplain.

With damming and the expansion of macrophyte assemblages, distribution of *Ma. humeralis* increased in the study area and it became the dominant species.

Differences in Culicidae richness and diversity were substantial for flood stages 253 m and 257 m. Diversity, evaluated by ST, did not exhibit the same effect, possibly because this method was applied during a period of low mosquito activity. Nevertheless, evening crepuscular captures with ST and HATet produced similar results in relation to total mosquitoes captured, although they were used at different times. When analyzed in regard to richness and diversity prior to flooding, the capture method suggested variability between richness and diversity, and between diversity and dominance. However, hematophagic activity of *Ma. humeralis*, measured by HAT 24 h, best represented local fauna. The low capture rate of the MA method may be due to the random selection of shelters visited, while the higher rates observed with human presence are likely related to the anthropophilic behavior of *Ma. humeralis*.

During immature collections along the São Domingos River (northern Paraná State), Lopes and Lozovei [19] concluded that culicids use forests adjacent to lakes as refuges. Their study area consisted of various forest fragments housing *Ma. humeralis*, while human settlements located within the dispersal radius of *Ma. humeralis* are permanent blood meal sources for females of these species.

TABLE 1: Total number of Culicidae collected and mean monthly values of richness, diversity, and dominance of *Mansonia humeralis*, according to the collection technique at the JB site in the municipality of Presidente Epitácio, for the preflooding (level 247 m) and postflooding (level 253 m and 257 m levels) phases of the Parana River.

| Taxonomic category | Level 247 m | | | | Level 253 m | | | | Level 257 m | | | | Total | % | | | | |
|--|-------------|--------------|---------------|--------------|---------------|--------------|------------|--------------|--------------|--------------|--------------|--------------|------------|-------------|------------|--------------|---------------|--------------|
| | MA | ST | HAT | HA/Tet | Subtotal | % | MA | ST | HAT | HA/Tet | Subtotal | % | | | MA | ST | Subtotal | % |
| <i>Ae. scapularis</i> | 134 | 701 | 6,463 | 1,304 | 8,602 | 38.8 | 6 | 186 | 88 | 12 | 292 | 3.7 | 23 | 38 | 61 | 10.9 | 8,955 | 29.1 |
| <i>Ma. humeralis</i> | — | 280 | 339 | 76 | 695 | 3.1 | 3 | 1,182 | 2,887 | 682 | 4,754 | 59.6 | 2 | 299 | 301 | 53.8 | 5,750 | 18.7 |
| <i>Ma. titillans</i> | 2 | 317 | 1,305 | 338 | 1962 | 8.8 | 2 | 326 | 245 | 120 | 693 | 8.7 | 1 | 15 | 16 | 2.9 | 2,671 | 8.7 |
| <i>Cq. nigricans</i> | 6 | 310 | 1,298 | 495 | 2,109 | 9.5 | — | 14 | 31 | 12 | 57 | 0.7 | — | — | — | 0.0 | 2,166 | 7.1 |
| <i>An. (Nys.) albitarsis</i> s.l. | — | 412 | 563 | 411 | 1,386 | 6.2 | 4 | 46 | 69 | 28 | 147 | 1.8 | 1 | 6 | 7 | 1.3 | 1,540 | 5.0 |
| <i>Cq. hermannoi</i> | 3 | 60 | 679 | 189 | 931 | 4.2 | — | 28 | 40 | 29 | 97 | 1.2 | — | — | — | 0.0 | 1,028 | 3.3 |
| <i>An. galvaoui</i> | — | 396 | 400 | 202 | 998 | 4.5 | 1 | 6 | 3 | 1 | 11 | 0.1 | — | 1 | 1 | 0.2 | 1,010 | 3.3 |
| <i>Ad. squamipennis</i> | 14 | 348 | 125 | 46 | 533 | 2.4 | 7 | 310 | 35 | 20 | 372 | 4.7 | — | 49 | 49 | 8.8 | 954 | 3.1 |
| <i>An. triannulatus</i> | — | 61 | 245 | 110 | 416 | 1.9 | 3 | 266 | 228 | 33 | 530 | 6.6 | — | 5 | 5 | 0.9 | 951 | 3.1 |
| <i>Ps. (Jan.) albigena</i> | 3 | 61 | 675 | 74 | 813 | 3.7 | — | 3 | 28 | — | 31 | 0.4 | — | 1 | 1 | 0.2 | 845 | 2.8 |
| <i>Ps. (Jan.) discrucians</i> | 1 | 106 | 411 | 37 | 555 | 2.5 | 1 | 4 | — | — | 5 | 0.1 | — | 3 | 3 | 0.5 | 563 | 1.8 |
| <i>Cx. (Aed.) amazonensis</i> | 2 | 30 | 303 | 85 | 420 | 1.9 | — | — | 5 | 2 | 7 | 0.1 | — | — | — | 0.0 | 427 | 1.4 |
| <i>Cx. (Mel.) ocosa</i> | 50 | 6 | 30 | — | 86 | 0.4 | 160 | 90 | 40 | 8 | 298 | 3.7 | 3 | 12 | 15 | 2.7 | 399 | 1.3 |
| <i>Cq. juxtamansonia</i> | 1 | 78 | 211 | 68 | 358 | 1.6 | — | 4 | 13 | 9 | 26 | 0.3 | — | 10 | 10 | 1.8 | 394 | 1.3 |
| <i>Ae. serratus</i> | 22 | 7 | 237 | 71 | 337 | 1.5 | — | — | 5 | 2 | 7 | 0.1 | — | — | — | 0.0 | 344 | 1.1 |
| <i>Cx. (Mel.) ribeirensis</i> | 1 | 10 | 314 | — | 325 | 1.5 | 2 | 6 | 1 | 9 | 9 | 0.1 | — | 1 | 1 | 0.2 | 335 | 1.1 |
| Total Culicidae (*) | 412 | 3,519 | 14,404 | 3,846 | 22,181 | 100.0 | 362 | 2,679 | 3,932 | 1,009 | 7,982 | 100.0 | 82 | 478 | 560 | 100.0 | 30,723 | 100.0 |
| Total of other species (except <i>Ma. humeralis</i>) | 412 | 3,239 | 14,065 | 3,770 | 21,486 | — | 359 | 1,497 | 1,045 | 327 | 3,228 | — | 80 | 179 | 259 | — | 24,973 | — |
| Richness (mean monthly value) | 8.5 | 13.5 | 25.8 | 16.2 | — | — | 5.5 | 10.1 | 11.3 | 6.3 | — | — | 1.9 | 4.7 | — | — | — | — |
| Diversity (mean monthly value) | 2.4 | 2.4 | 3.7 | 2.8 | — | — | 1.6 | 1.8 | 1.9 | 1.3 | — | — | 0.6 | 1.4 | — | — | — | — |
| Dominance of <i>Ma. humeralis</i> (mean monthly value) | — | 8.2 | 3.3 | 2.1 | — | — | 1.7 | 44.4 | 59.8 | 60.2 | — | — | 2.2 | 25.2 | — | — | — | — |

* Amounts including mosquito species with 1.0% or less specimens: *An. rondoni*, *Cx. (Culex) spp.* (1.0%); *Cx. (Mel.) Melanoconion section, Ps. (Jan.) ferox* (0.7%); *An. darlingi* (0.6%); *Cx. (Mel.) Atratus group* (0.5%); *Cx. (Mel.) idotrus* (0.4%); *An. parvus*, *Cq. shannoni*, *Wy. melanocephala* (0.3%); *Cx. (Mel.) Intrincatus group, Ma. amazonensis* (0.3%); *An. (Nys.) spp., Cq. albicosta*, *Cx. (Cux.) bidens*, *Cx. (Cux.) declinator*, *Cx. (Cux.) Coronator group, Cx. (Mel.) clarki*, *Cx. (Mel.) oedipus, Ma. wilsoni, Sa. glaucodaemon, Ur. geometrica* (0.1%); *Ae. (Stc.) albopictus, Ae. fulvus, Ae. hastatus/oligopistus, Ae. nubilis, Ae. serratus/nubilis, Ae. aenigmaticus, An. braziliensis, An. deaneorum, An. evansae, An. strodei, Cq. chrysonotum/albifera, Cq. venezuelensis, Cx. (Cux.) camposi, Cx. (Cux.) chidestri, Cx. (Cux.) coronator, Cx. (Cux.) maxi, Cx. (Cux.) mollis, Cx. (Cux.) quinquefasciatus, Cx. (Mel.) spp., Cx. (Mel.) aureonotatus, Cx. (Mel.) bastagari, Cx. (Mel.) contzi, Cx. (Mel.) delpointei, Cx. (Mel.) dunni, Cx. (Mel.) eastor, Cx. (Mel.) flabellifer, Cx. (Mel.) pilosus group, Cx. (Cux.) pavlovsky, Cx. (Mel.) pilosus, Cx. (Mel.) rabelloi, Cx. (Mel.) sp. of Atratus group, Cx. (Mel.) theobaldi, Cx. (Mel.) vaxus, Cx. (Mel.) zetekii, Cx. (Mel.) adamesi, *Li. durhamii, Li. flavisetosus, Ma. indubitans, Ps. (Gra.) sp., Ps. (Jan.) albipes, Ps. (Pso.) ciliata, Ps. (Gra.) confinnis, Ur. apicalis, Ur. lowii, Ur. mathesoni, Ur. pulcherrima, Ur. spp., and Wy. chalccephala/roucouyana* (<0.1%).*

Thus, increased nuisance levels are expected during periods of higher *Ma. humeralis* activity. Similar observations were made in the Taquaruçu Reservoir, Paranapanema Bay [20]. More recently, Cruz et al. [21] found a predominance of *Mansonia* near the Madeira River hydroelectric plant in Amazonia, emphasizing the importance of monitoring activities in areas with new reservoirs.

A strong positive correlation was recorded in the present study between artificial flooding and population levels of *Ma. humeralis*. In addition to being an indicator of macrophytes, this species can become a nuisance in areas surrounding a reservoir owing to its highly anthropophilic behavior, as was the case near the Tucuruí Reservoir [7].

Frequencies for other species were significantly different from mean values for *Ma. humeralis* in both flood stages, except for *Ma. humeralis* captured by MA at 253 m and 257 m. Findings with MA may be explained by the fact that the species studied does not have continuous distribution, exhibited by few individuals during the 2nd and 3rd stages and absent in stage 1. A low MA capture rate was observed for species other than *Ma. humeralis* at 247 m and 253 m stages.

Overall, diversity and richness of mosquito fauna were substantially affected by flooding. However, there was a notable increase in the *Ma. humeralis* population with rising water levels. This species was present over the entire monitoring period, with peaks in winter and spring, a trend not observed for other culicids. Surface waters drain into the lake during flooding and enrich the reservoir with nutrients, thereby stimulating the proliferation of macrophytes [22] and favoring *Mansonia*. In addition, drainage channels may transport aquatic vegetation and disperse immature mosquitoes into other areas [3], making control measure a complex undertaking.

Other investigations have emphasized the vulnerability of areas near the dam in providing favorable conditions for the spread of arboviruses, a concern reinforced by our results (Wanderley et al. [23]).

Ma. humeralis was the primary focus of the present study due to the sharp increase in population dominance following flooding, which may generate a possible nuisance to the human population. However, a substantial amount of Culicidae relevant to public health was recorded, also reported in other research, including *Aedes scapularis* (Rondani) and *Anopheles albitaris* s.l. Lynch Arribáizaga [24, 25]. This research confirms the importance of monitoring Culicidae fauna.

5. Conclusion

The authors suggest that during the operational phase of this Hydroelectric Plant, local authorities should monitor culicid activity using sustainable entomological surveillance.

Acknowledgments

The authors would like to thank systems analyst Fernão Dias de Lima for his involvement in database management. This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil (96/10014-1;

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Research Article

Geographic Distribution of Chagas Disease Vectors in Brazil Based on Ecological Niche Modeling

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Received 16 July 2011; Accepted 23 October 2011

Academic Editor: Maria Goreti Rosa-Freitas

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Although Brazil was declared free from Chagas disease transmission by the domestic vector *Triatoma infestans*, human acute cases are still being registered based on transmission by native triatomine species. For a better understanding of transmission risk, the geographic distribution of Brazilian triatomines was analyzed. Sixteen out of 62 Brazilian species that both occur in >20 municipalities and present synanthropic tendencies were modeled based on their ecological niches. *Panstrongylus geniculatus* and *P. megistus* showed broad ecological ranges, but most of the species sort out by the biome in which they are distributed: *Rhodnius pictipes* and *R. robustus* in the Amazon; *R. neglectus*, *Triatoma sordida*, and *T. costalimai* in the Cerrado; *R. nasutus*, *P. lutzi*, *T. brasiliensis*, *T. pseudomaculata*, *T. melanocephala*, and *T. petrocchiai* in the Caatinga; *T. rubrovaria* in the southern pampas; *T. tibiamaculata* and *T. vitticeps* in the Atlantic Forest. Although most occurrences were recorded in open areas (Cerrado and Caatinga), our results show that all environmental conditions in the country are favorable to one or more of the species analyzed, such that almost nowhere is Chagas transmission risk negligible.

1. Introduction

Chagas disease or American trypanosomiasis is a chronic and potentially fatal infection caused by the protozoan *Trypanosoma cruzi* [1]. Contamination of mucosa by feces of blood-sucking infected insects (Hemiptera, Reduviidae, Triatominae) is the most important way of transmission, although transmission may also occur congenitally, by blood transfusion, from organ donors, and orally, via ingestion of food contaminated with *T. cruzi*. No vaccines, or effective antiparasitic treatments are available to cure the chronic phase of Chagas disease, so control of domiciliated vectors is the main strategy to prevent human infection [2–4]. Chagas disease, originally restricted to Latin America, is now becoming a global public health concern in nonendemic areas owing to human migrations to developed countries [5].

In Brazil, the Chagas disease national control program was implemented in 1975–1983, when *Triatoma infestans* infested domiciles of 700 municipalities in 12 Brazilian states [6]. At that time, 4.2% of the Brazilian population was estimated to be infected and around 100,000 new cases were recorded per year [7]. In 1991, Brazil joined the Southern Cone Initiative, an international consortium with the main objective of reducing vectorial transmission through insecticide spraying against *T. infestans*. After 10 years of effort, the project had a crucial impact on Chagas disease transmission in the Southern Cone countries, resulting in a 94% reduction of disease incidence [8]. Subsequent initiatives were also launched in the Andean region, Central America, and Amazonia [9]. The total prevalence of Chagas disease was reduced from >16 million to 8 million people, estimated in 2005 [10] and numbers of deaths were also

reduced drastically [11]. In 2006, the Intergovernmental Initiative of Southern Cone, OMS, certified Brazil as free of vectorial transmission by *T. infestans* [12, 13]. In Brazil, the current estimate is that 1.9 million people are infected [10], much lower than the 6 million estimated in the 1980s [8].

The last Brazilian seroprevalence inquiry was carried out in 2001–2008, including 104,954 children, in which only 32 cases were detected, indicating significant reduction of transmission in recent years [14]. Nonetheless, acute cases of Chagas disease are presently being recorded in Brazil, mainly in the Amazon region [15]. In those cases, transmission involves either sylvatic vectors invading houses, food contamination or domestic/peridomestic populations of native triatomine species. The occurrence of those triatomines represents a great difficulty for the achievements via vectorial control [16–18].

Currently, in the subfamily Triatominae, 142 species are grouped in 18 genera and five tribes [19–24]. Studies of the geographic distribution of these species are crucial for understanding epidemiologic aspects of *Trypanosoma cruzi* transmission, and must be considered to orient control and monitoring of the disease. The study of the potential geographic distribution of important vector species is crucial for understanding geographic dimensions of risk transmission of the disease. In this context, ecologic niche modeling (ENM) is a tool that permits exploration of geographic and ecologic phenomena based on known occurrences of the species [25, 26]. ENM has been applied broadly to understanding aspects of Chagas disease transmission in the last decade, including characterization of the niches of vector species, and analysis of relationships between vector and reservoir distributions [27–37]. The objective of the present study is to analyze the geographic distributions of the triatomine species in Brazil and the factors related to their areas of occurrence. Ecologic aspects of the most important species are discussed, contributing to the knowledge of Chagas disease vectors in Brazil.

2. Material and Methods

2.1. Distribution Data. Distribution data of triatomine species in Brazil were obtained mainly from Lent and Wygodzinsky [38], Silveira and colleagues [6], and Carcavallo and colleagues [39]. New records were obtained from more recent studies [32, 33, 36, 37, 40–65]. We also obtained distributional data from species described after 1999 [22, 66–71]. Additionally, we analyzed triatomine records in the collections of Rodolfo Carcavallo and Herman Lent in the Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Instituto Oswaldo Cruz, FIOCRUZ. Recent information on the distribution of triatomines provided by Brazilian State Health Departments was also included.

2.2. Ecological Niche Models

2.2.1. Input Data. We compiled 3563 records of triatomines in Brazil that could be referenced to geograph-

ic coordinates with a reasonable degree of confidence (i.e., with an uncertainty of ≤ 5 km, to a precision of $\pm 0.01^\circ$). All records were georeferenced based on consultation of <http://www.fallingrain.com/world/> and <http://www.ibge.gov.br/>. Eliminating duplicate records at this spatial resolution and removing a few records that presented obvious errors of georeferencing or identification, 3223 records remained, which documented occurrence of 62 triatomine species in the country. These data were organized in spreadsheets for analysis.

We set an occurrence data sample size criterion of 20 unique latitude-longitude points per species as a minimum to permit robust ENM development, based on previous analyses [72, 73] and extensive experience with such applications. This threshold left a total of 17 species: *Panstrongylus megistus*, *P. lutzi*, *P. geniculatus*, *Triatoma pseudomaculata*, *T. rubrovaria*, *T. sordida*, *T. tibiamaculata*, *T. petrochiae*, *T. brasiliensis*, *T. melanocephala*, *T. costalimai*, *T. vitticeps*, *Psammolestes tertius*, *Rhodnius neglectus*, *R. nasutus*, *R. pictipes*, and *R. robustus*. In view of the exclusively sylvatic nature of the species *Ps. tertius* [63], we did not include this species in subsequent analyses. Barve and colleagues [74] recently recommended that niche and distribution models be calibrated across areas coinciding with the dispersal area (termed “M” by them) in the Biotic-Abiotic-Mobility (BAM) framework for understanding species geographic distributions [75], an assertion with which we concur amply. As essentially all of the species in this study have broad geographic distributions, we are comfortable with a relatively broad definition of M. However, a modification of the BAM framework for definition of calibration areas is based on considering the area that was actually sampled to produce the occurrence data for a study, which we can term S. Because both M and S determine the area that can possibly produce a presence record, albeit for very different reasons, and because our occurrence data came only from the extent of Brazil, we define our calibration area as $M \cap S$, which in this case is the whole extent of Brazil.

To characterize environmental variation across Brazil, we used two very different environmental datasets: multi-temporal remotely sensed imagery and climatic data. The multitemporal (monthly) normalized difference vegetation index values (NDVI, a “greenness” index) were drawn from the Advanced Very High Resolution Radiometer satellite. These datasets have a native spatial resolution of ~ 1 km. The monthly nature of these greenness indices (April 1992–March 1993) effectively provides a detailed view of vegetation phenology across the country, which has proved quite useful and informative in recent analyses [76]. The years 1992–1993 coincide well with the temporal provenance of much of the occurrence data.

The second environmental dataset consisted of “bioclimatic” variables characterizing climates during 1950–2000 drawn from the WorldClim data archive [77]. To avoid the confounding effects of calibrating models in an overly dimensional environmental space [78], we chose only a subset of the 19 “bioclimatic” variables in the climatic data

archive: annual mean temperature, mean diurnal range, maximum temperature of warmest month, minimum temperature of coldest month, annual precipitation, precipitation of wettest month, and precipitation of driest month. Both environmental datasets were resampled to 0.0417° spatial resolution for analysis, to match the approximate precision of the georeferencing of the occurrence data.

2.2.2. Modeling Strategy and Methods. Different algorithms have different strengths and weaknesses in particular situations [79], which makes the choice of method an important consideration. In view of the particular characteristics of this study—that is, the area of interest is the area sampled, with no need for transfer of models over broader or other landscapes—Maxent appeared to be an excellent choice. Although recent comparative studies identifying Maxent as the “best” algorithm [80] are oversimplifications, our experience and analysis [81] indicate that it is indeed the ideal choice for this particular study. We used default parameters, except that we chose a random seed, with 5 replicate analyses based on bootstrap subsampling; we used the median output grids as the best hypothesis of potential range and imported them into ArcGIS 10 as floating point grids.

For model results, for each combination of species with environmental dataset, we thresholded raw Maxent output independently. To emphasize the fact that omission error takes considerable precedence over commission error in niche modeling applications, we used a modified version of the least training presence thresholding approach [82] that takes into account the fact that some degree of error may exist in a dataset (otherwise, model predictions thresholded using this approach will be broadened artificially by error). Specifically, we estimated E , the expected amount of meaningful error in occurrence data [81] at 5%, and thresholded models at the suitability level that included $100 - E = 95\%$ of the model calibration data to produce binary models summarizing likely presence and absence over the landscape. Finally, we combined the models from the two environmental datasets by multiplying them—this step had the effect of retaining areas as suitable only if they were judged as suitable by models based on both the AVHRR and climatic datasets. We note that because our initial hypothesis was that all of Brazil is available to each species for potential colonization, we took no specific steps to reduce model predictions from potential to actual distributional areas.

Analyses were focused on the species diversity of broadly distributed (i.e., included within our initial list of well-sampled species) and human-disease-important triatomine species. As a result, we took the final, thresholded, and combined map for each of the 16 species listed above and summed them—given the binary nature of each of the maps, the sums yield a hypothesis of numbers of species present across Brazil. To provide a view of species’ responses to environmental variation across Brazil, we plotted 1000 random points across the country, and assigned to each (1) the predicted presence or absence of each species and (2) the values of the first two principal components of the

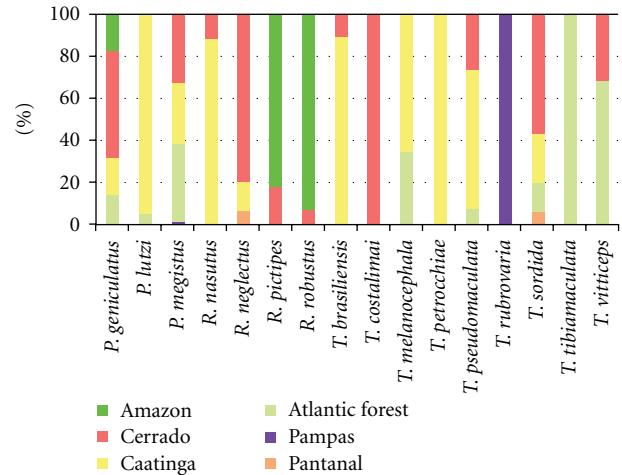


FIGURE 1: Relative occurrence of 16 species of triatomines across biomes, calculated based on proportions of known occurrences falling in each area.

bioclimatic data set, which summarize much of the variation in climatic dimensions in a readily accessible and visualizable space of two dimensions.

3. Results

3.1. Diversity Patterns. Sixty-two of the 142 known triatomine species have been found in Brazil (Table 1). Thirty-nine out of 62 species (63%) occur only in Brazil. The state of Bahia, in northeastern Brazil, has the largest number of species (25 spp.), followed by Mato Grosso (18 spp.) in the central-west, Para and Tocantins (15 spp.) in the north, and Minas Gerais (15 spp.) in the Southeast. Rio Grande do Sul is the state with the largest number of species in southern Brazil (11 spp.). Acre and Amapá are the states with the lowest number of recorded species (Table 1).

Grouping the 62 triatomine species by biome, we noted that the greatest number of species inhabits the Cerrado biome ($n = 24$; 39%), followed by the Amazon ($n = 16$; 26%), Caatinga ($n = 15$; 24%), and Atlantic Forest ($n = 15$; 24%). Fewer species were recorded in the Pantanal ($n = 9$; 15%) and Pampas ($n = 8$; 13%) biomes. The geographic distributions of some species coincide strongly with the distribution of particular biomes, while other species (e.g., *P. geniculatus* and *T. sordida*) occurred in at least four biomes (Figure 1). Most of these species occur in open areas in the cerrado and caatinga biomes, where the majority (70%) of occurrences were recorded.

3.2. Ecological Niche Models. We modeled the ecological niches of the 16 Brazilian triatomine species that are well sampled and present synanthropic tendencies. Of these species, *P. geniculatus* and *P. megistus* showed broad ecological and geographic distributions (Figure 2). The distributions of *R. neglectus*, *T. costalimai*, and *T. sordida* coincided with the Cerrado biome in central Brazil (Figure 3). On the other hand, distributions of *R. nasutus*, *P. lutzi*,

TABLE 1: Continued.

| Species | AC | AL | AM | AP | BA | CE | DF | ES | GO | MA | MG | MS | MT | PA | PB | PE | PI | PR | RJ | RN | RO | RR | RS | SE | SP | SC | TO | Total | Biome* |
|--------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----|--------------|--------|
| <i>T. brasiliensis</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 142 | Ca, Ce | |
| <i>T. carcavalloii</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 5 | Pa | |
| <i>T. circummaculata</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 10 | Pa | |
| <i>T. costalimai</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 21 | Ce | |
| <i>T. deaneorum</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 2 | Ce | |
| <i>T. delponteii</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | Pa | |
| <i>T. guazu</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | Ce | |
| <i>T. infestans</i> ** | | | | | | | | | | | | | | | | | | | | | | | | | | | 18 | ** | |
| <i>T. juareirensis</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | Ca | |
| <i>T. jurbergi</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | Ce | |
| <i>T. klugi</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | Pa | |
| <i>T. lenti</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 6 | Ce, Ca | |
| <i>T. maculata</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | A | |
| <i>T. matogrossensis</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 8 | P | |
| <i>T. melanica</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 2 | Ca | |
| <i>T. melanocephala</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 84 | Ca, F | |
| <i>T. oliverai</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 5 | Pa | |
| <i>T. petrocchiai</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 28 | Ca | |
| <i>T. platensis</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | Pa | |
| <i>T. pseudomaculata</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 378 | Ca, Ce, F | |
| <i>T. rubrofasciata</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 10 | *** | |
| <i>T. rubrovaria</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 30 | Pa | |
| <i>T. sherlocki</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | Ca | |
| <i>T. sordida</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 320 | Ce, Ca, F, P | |
| <i>T. tibiamaculata</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 30 | F | |
| <i>T. vanda</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 2 | Ce | |
| <i>T. vitticeps</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 81 | F, Ce | |
| <i>T. williami</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 18 | Ce, P | |
| <i>T. wygodzinsky</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 3 | F | |
| Total | 3 | 6 | 10 | 3 | 25 | 8 | 7 | 7 | 14 | 14 | 15 | 14 | 18 | 15 | 10 | 12 | 11 | 8 | 8 | 8 | 4 | 4 | 11 | 7 | 10 | 4 | 15 | 3563 | |

*Biomes: Amazon (A), Cerrado (Ce), Caatinga (Ca), Pampas (Pa), Pantanal (P), and Atlantic Forest (F). ***T. infestans* is a domestic species, nonendemic in Brazil. After control with insecticides this species is restricted to residual foci in two states. ****T. rubrofasciata* is a cosmopolitan species. In Brazil, its presence was detected in all the major harbors. Brazilian state abbreviations: AC: Acre, AL: Alagoas, AM: Amazonas, AP: Amapá, BA: Bahia, CE: Ceará, DF: Distrito Federal, ES: Espírito Santo, GO: Goiás, MA: Maranhão, MG: Minas Gerais, MS: Mato Grosso do Sul, MT: Mato Grosso, PA: Pará, PB: Paraíba, PE: Pernambuco, PI: Piauí, PR: Paraná, RJ: Rio de Janeiro, RN: Rio Grande do Norte, RO: Rondônia, RR: Roraima, RS: Rio Grande do Sul, SE: Sergipe, SP: São Paulo, SC: Santa Catarina, and TO: Tocantins.

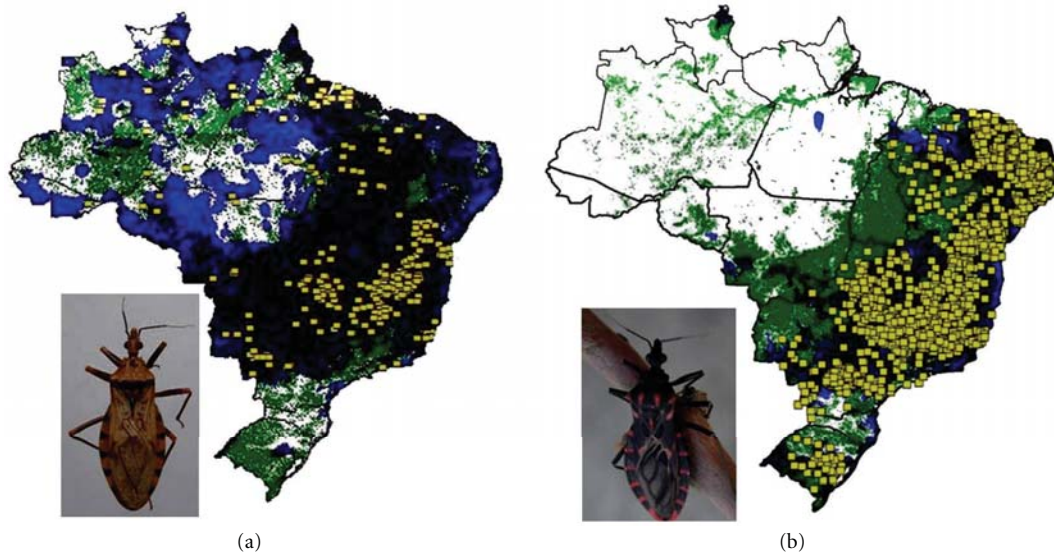


FIGURE 2: Ecological niche models projected as potential distributions for triatomine species with widespread distribution in Brazil. (a) *Panstrongylus geniculatus* and (b) *P. megistus*. Known occurrences of the species are shown as yellow squares, and the final consensus prediction is shown as black shading. Areas identified as suitable based on climatic grounds only are shown in blue, whereas areas identified as suitable based on normalized difference vegetation index (NDVI) only are shown in green.

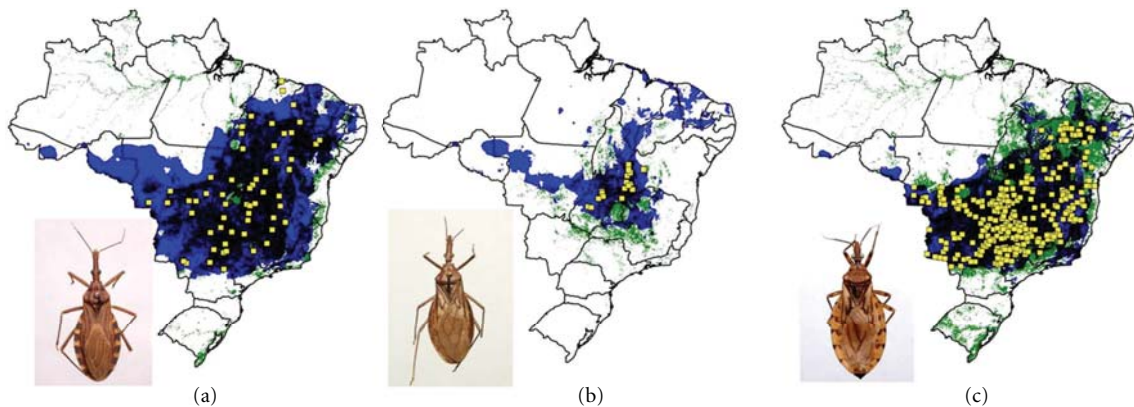


FIGURE 3: Ecological niche models projected as potential distributions for triatomine species in central Brazil. (a) *R. neglectus*, (b) *T. costalimai*, and (c) *T. sordida*.

T. brasiliensis, *T. pseudomaculata*, *T. melanocephala*, and *T. petrocchiai* coincided with the Caatinga of northeastern Brazil (Figure 4). We also noted higher probability of *R. robustus* and *R. pictipes* in northern Brazil (Figure 5), *T. rubrovaria* in the south (Figure 6), and *T. tibiamaculata* and *T. vitticeps* in the Atlantic Forest (Figure 7).

3.3. Factors That Determine Species' Distributions. As noted in 3.1 and 3.2, clear associations exist between species' ranges of occurrence and biomes. The overall map diversity among the 16 species modeled (Figure 8) indicates that the areas most favorable for the occurrence of these species are concentrated in the Cerrado and Caatinga, in the diagonal of open areas of eastern South America; other concentrations

were in open areas of the Amazon, especially in the state of Roraima.

The view of triatomine species' distributions across Brazilian environmental space shows the broad environmental diversity of the subfamily (Figure 9). Essentially all environmental conditions represented in the country are inhabited by one or more of the 16 species analyzed. Most of the species sort out by the region in which they are distributed: for example, *R. pictipes* and *R. robustus* in the Amazon, *Triatoma sordida* and *R. neglectus* in the Cerrado, *T. brasiliensis* and *P. lutzi* in the Caatinga, and *T. rubrovaria* in the far south. In sum, though, the view is of all of Brazil as having some suite of triatomine species present, such that almost nowhere is Chagas transmission risk negligible, although clearly some areas are at much higher risk than others.

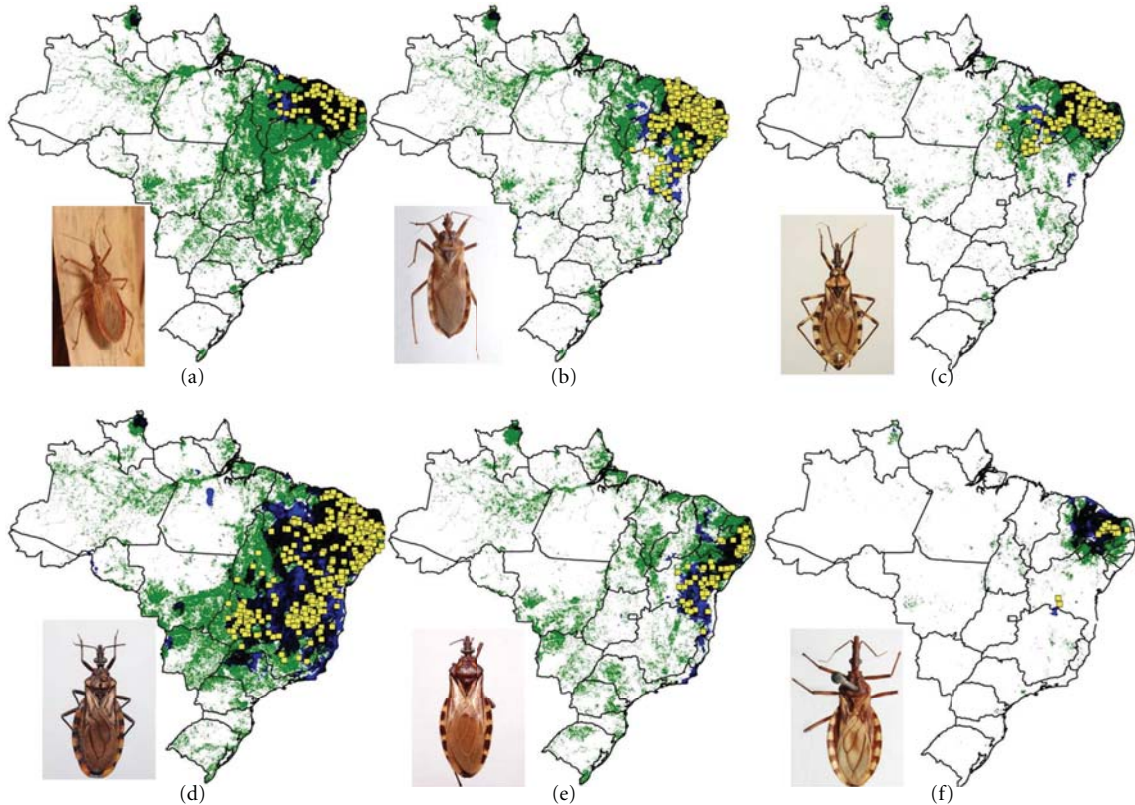


FIGURE 4: Ecological niche models projected as potential distributions for triatomine species in northeastern Brazil. (a) *R. nasutus*, (b) *P. lutzi*, (c) *T. brasiliensis*, (d) *T. pseudomaculata*, (e) *T. melanocephala*, and (f) *T. petrocchiae*.

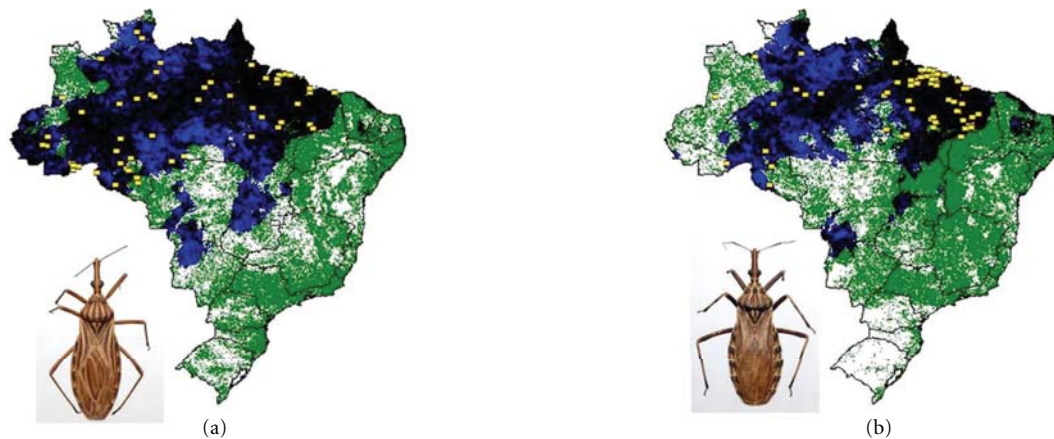


FIGURE 5: Ecological niche models projected as potential distributions for Amazonian triatomine species. (a) *R. robustus*, and (b) *R. pictipes*.

4. Discussion

This study analyses the geographic distributions of the 62 species of triatomines in Brazil. We found that the great majority of species has a distribution restricted to fewer than 20 municipalities across Brazil. The analysis of the distribution of 16 synanthropic species broadly distributed in Brazil shows clear associations with Brazilian biomes, indicating the Cerrado and Caatinga as the ones presenting the highest diversity. The Brazilian states presenting the

highest numbers of species are Bahia and Mato Grosso. The results suggest that essentially all environmental conditions represented across the country are inhabited by one or more of the 16 species analyzed.

Panstrongylus megistus was the species most broadly distributed throughout Brazil, as pointed out by Silveira [83]. The Atlantic Forest seems to represent the center of the range of *P. megistus* [84], although the species is also broadly distributed in humid areas of the Cerrado (“matas de galeria”) and Caatinga (forest remnants). *P. megistus* shows

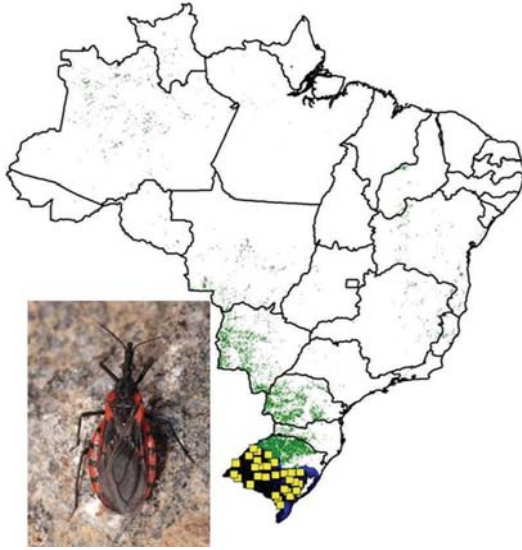


FIGURE 6: Ecological niche models projected as potential distribution for *T. rubrovaria*.

distinct levels of adaptation to domiciliary environments: in the south, it occurs mainly in sylvatic ecotopes [85] while in the southeast and in the northeast areas, where it is of epidemiologic importance, it occurs mainly in artificial ecotopes [84, 86, 87]. *P. megistus* seems to prefer hollow trees in arboreal habitats [88, 89] often associated with marsupials (*Didelphis* spp.), which are frequently infected by *T. cruzi*; this natural history explains the high levels of natural infection of *P. megistus* when compared to other triatomine vector species [90]. The broad geographic distribution, the acknowledged capacity to invade and colonize domiciles, and the high levels of *T. cruzi* infection indicate that *P. megistus* is the species of the greatest epidemiologic importance in Brazil after the control of *T. infestans*.

P. geniculatus is another triatomine species that is very broadly distributed in Brazil and the Americas, occurring in at least 16 countries [39, 48]. In sylvatic environments *P. geniculatus* preferentially inhabits armadillo nests (*Dasypos* spp.) [65, 88, 89] and domiciliary invasion by adults has been detected in several Brazilian states [6]; peridomiciliary colonies have also been found [91]. Nonetheless, its synanthropic behavior and vectorial competency are not as relevant as those of *P. megistus*. The bite of this triatomine is painful and causes allergic reactions in hosts, making blood meals in domestic environments difficult, consequently reducing the chances of colonization of artificial ecotopes [92]. The broad ranges of *P. geniculatus* and *P. megistus* could be facilitated naturally by mammals (marsupials and armadillos); future studies analyzing the geographic distribution of these triatomines in tandem with their potential hosts could clarify these possibilities.

In the Cerrado, the species most broadly distributed were *T. sordida* and *R. neglectus*. *T. sordida* occurs naturally under tree bark and also inhabits bird nests [93]. It is the most frequently captured species by entomological surveillance in Brazil [92, 94]. However, the risk of *T. cruzi* transmission by *T. sordida* is relatively low due either to the fact that

it inhabits mainly the peridomiciliary ecotopes or to its ornithophilic behavior [88, 95]. According to Forattini [84] the areas of higher occurrence of *T. sordida* are the ones related to the agricultural activities in the past, what could explain its presence in areas that suffered ecologic impact due to significant loss of vegetation. It is important to stress that this process has been documented in the last decades in the Cerrado [96].

Rhodnius neglectus is widespread in the Cerrado, and plays an important role in the enzootic transmission of the *T. cruzi* [97]. Besides the invasion of adults in domiciles [54], the domiciliary colonization of *R. neglectus* has also been recorded in the states of Minas Gerais, São Paulo, and Goiás [98, 99]. *R. neglectus* occurs mainly in sylvatic environments, inhabiting different species of palm trees in Brazil [32, 55, 100]. The role of the peridomiciliary palm trees as a source of synanthropic *R. neglectus* must be evaluated in future studies.

Triatoma sordida and *R. neglectus* have also been recorded in other biomes (e.g., Caatinga and Pantanal). One of the possible explanations for this broad distribution would be passive transportation by birds. The dispersal could be facilitated by the fact that the eggs could be attached on the feathers, as suggested for the Rhodniini. Another possibility would be the transport of nymphs among the feathers as already shown for *T. sordida* [101]. One of the lines of evidence of this kind of dispersal is the coincidence between the distribution of the *R. neglectus* and birds like *Phacelodomus ruber* and *Pseudoseisura cristata* [32].

Triatoma costalimai seems to be endemic in the Cerrado. This species has been frequently captured in sylvatic environments (calcareous rocks), and also in peri- and intradomiciliary ecotopes in the municipalities of northeastern Goiás, with high rates of *T. cruzi* infection [102–104]. The geographic distribution of *T. costalimai* in the Cerrado remains poorly known, so research is needed to clarify its synanthropic behavior and vectorial capacity. In addition, ecological studies of other triatomine species in the Cerrado should be carried out to improve knowledge of the species occurring in this biome.

Triatoma brasiliensis, *T. pseudomaculata*, *P. lutzii*, and *R. nasutus* are broadly distributed in the Caatinga. *T. brasiliensis* is the most important vector species in northeastern Brazil [86, 105]. In sylvatic environments, it frequently inhabits rock crops in association with rodents (*Kerodon rupestris*) presenting significant levels of natural infection by *T. cruzi* [106]. The occurrence of *T. brasiliensis* may be associated with the distribution of the rock formations and “chapadas” in the northeast region of Brazil. This species is frequently found infesting houses in five states (MA, PI, CE, RN, and PB) [17, 107, 108]. It can also be found in very low numbers in the border areas of its distribution in the states of Tocantins and Pernambuco. The other four members of the *T. brasiliensis* species complex are either less synanthropic (*T. b. macromelasoma* and *T. juazeirensis*) or exclusively sylvatic (*T. melanica*) [21, 22]. *T. sherlocki*, recently included in this species complex, seems to be in the process of adaptation to the domiciliary environment [109].

Triatoma pseudomaculata occurs under bark of trees and in bird nests [88], presenting low percentages of natural

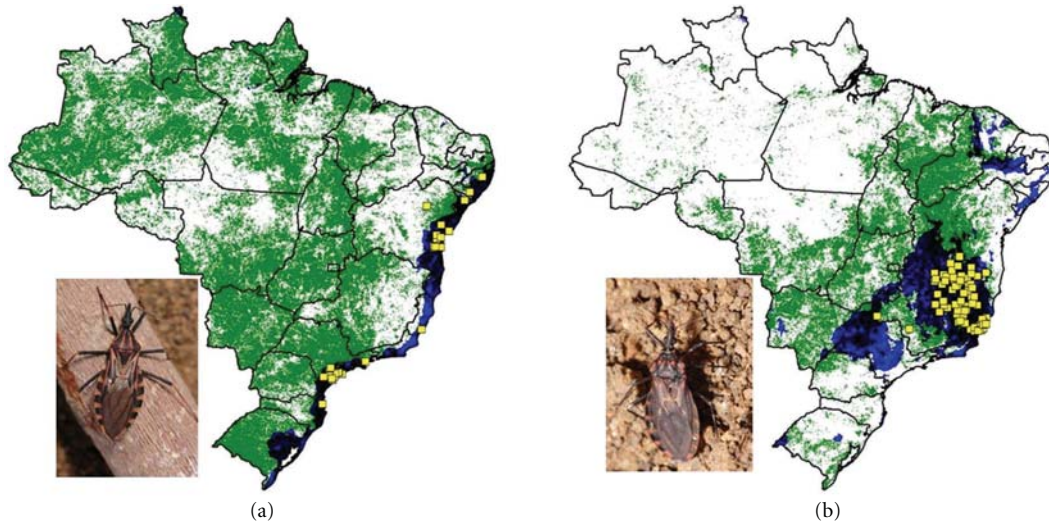


FIGURE 7: Ecological niche models projected as potential distributions for Atlantic Forest triatomine species. (a) *T. tibiamaculata* and (b) *T. vitticeps*.

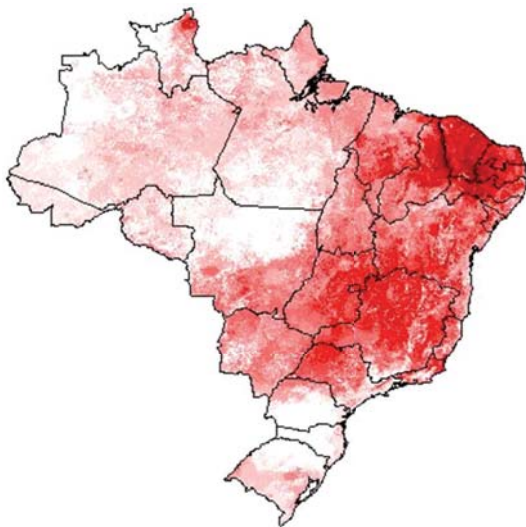


FIGURE 8: Triatomine species diversity map of Brazil, based on ecological niche models for 16 species of triatomine bugs. White areas have no species predicted as occurring, out of the 16 included in this analysis, while the darkest red areas have 13 species predicting as cooccurring.

infection by *T. cruzi* [86]. Although *T. pseudomaculata* can be found more frequently in peridomestic habitats, feeding generally on birds, in Ceará and Minas Gerais, full domiciliation has been observed [110, 111]. In some areas of the northeast, *T. pseudomaculata* has been the most common species after *T. brasiliensis*, so it must be kept under constant vigilance [86]. The presence of *T. pseudomaculata* in domiciliary environments has been linked to climate change and deforestation [111, 112]. One risk factor for domiciliation appears the passive transportation in wood for domestic use and construction of fences [92].

The epidemiologic importance of *Panstrongylus lutzi* has been increasing in the last years. This species was the fifth

most frequently found in captures across 12 Brazilian states in recent years [13, 105]. *P. lutzi* occurs in Caatinga and can be found inhabiting armadillo nests [113]; however, it has a much more eclectic diet in domestic environments and shows high levels of natural infection [45].

Rhodnius nasutus is found predominantly in Caatinga in the Northeast inhabiting palm trees of the species *Copernicia prunifera* [114, 115]. Nonetheless, *R. nasutus* may also occur in other palms and trees in the Caatinga [53, 116]. Although *R. nasutus* be considered as endemic to Caatinga, this species was registered in transitional areas with the Amazon forest (e.g., in the Maranhão Babaçu forests) and also with Cerrado [36].

The geographic distribution of *Triatoma melanocephala* and *T. petrochiae* was more restricted than the above-mentioned species. *Triatoma melanocephala* appears to occur in more humid areas than *T. petrochiae*. The natural habitats and food sources of these two species are little known [88], demanding further ecological study to clarify their vectorial potential in transmission of *T. cruzi* to humans.

Triatoma tibiamaculata and *T. vitticeps* present a geographic distribution more restricted to the Atlantic Forest. The former is frequently attracted by light but rarely colonizes houses [92]. This species was likely responsible for the contamination of the sugar cane juice that caused several cases of oral transmission recorded in the Santa Catarina [117]. In Bahia, *T. tibiamaculata* infected by *T. cruzi* has been found frequently in urban areas, mainly in the warmer months [118]. Adult specimens of *T. vitticeps* have been captured in rural areas of municipalities in Rio de Janeiro, Minas Gerais, and Espírito Santo [47, 62]. In contrast to *T. tibiamaculata*, *T. vitticeps* colonizes peridomestic areas, increasing risk of transmission of *T. cruzi* to humans [62, 92].

Rhodnius robustus and *R. pictipes* are broadly distributed in the Amazon region, as pointed out by Abad-Franch and Monteiro [119]. These species have several Amazonian palm trees species as their natural habitats, and present

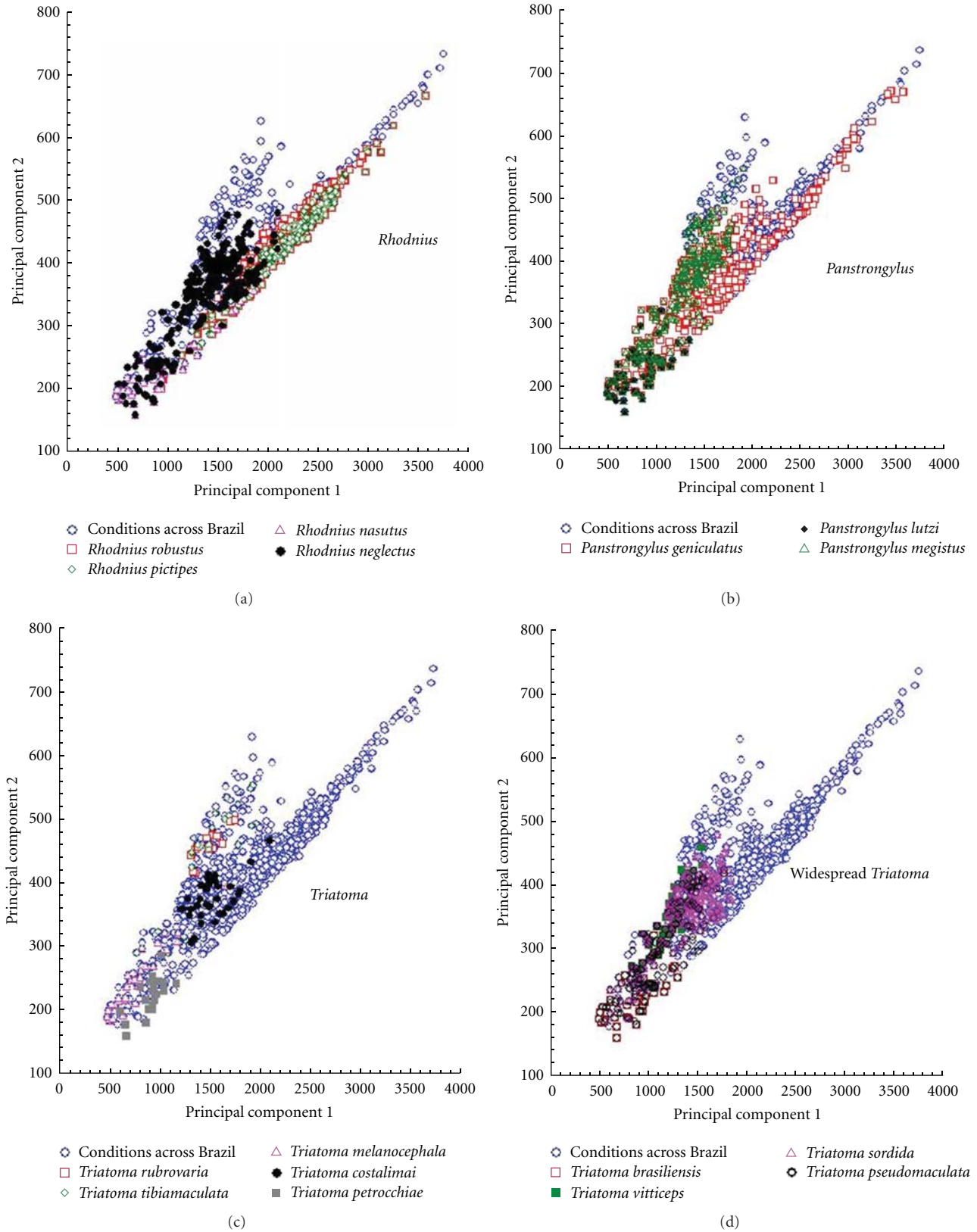


FIGURE 9: Distribution of 16 triatomine species in Brazil with respect to environmental variation summarized as the first two principal components summarizing variation in seven climatic dimensions. To provide a view of species' responses to environmental variation across Brazil, we plotted 1000 random points across the country, and assigned to each (1) the predicted presence or absence of each species and (2) the values of the first two principal components extracted from the bioclimatic data set.

high percentages of natural infection by *T. cruzi* [55, 120]. Invasion of houses by adults, apparently related to artificial light sources, should favor transmission of *T. cruzi* to humans either by direct contact or by food contamination. This last possibility has been recorded frequently in the Amazon, where Chagas disease has been considered emergent [60, 121, 122]. Other possible scenarios of transmission in the Amazon would be of triatomine invasion of peridomestic ecotopes, as for *P. geniculatus* in Pará [91] and *T. maculata* in Roraima [44].

Triatoma rubrovaria presents a geographic distribution restricted to the Pampas biome in the south. In natural environments, the species can be found under rocks (sometimes very near to domiciles), where it feeds on several animals, including insects [123]. After the control of *T. infestans*, occurrence of this species has been increasing in Rio Grande do Sul [13, 124].

The low occurrence of *T. infestans* detected in the present study is a further evidence for the effective reduction of this species in Brazil by the control programs [13, 125]. However, finding residual populations in Rio Grande do Sul and Bahia emphasizes the importance of constant entomological surveillance to avoid reinvasion by *T. infestans*.

5. Conclusions

Our results show that all environmental conditions in Brazil are favorable for one or more of the triatomine species analyzed, such that almost nowhere is Chagas transmission risk negligible. ENM presents a means of viewing species' ecology and biogeography across broad regions. It is by no means a substitute for detailed populational evaluations and natural history observations. Rather, used in tandem with local-scale studies, niche modeling provides complementary information. This study follows long years of previous exploration of these methodologies, and represents a step toward a complete distributional summary of the triatomines of Brazil.

Acknowledgments

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The authors are also grateful to all health agents of Brazilian states who provided information on the distribution of triatomines.

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Research Article

Species Distribution Models and Ecological Suitability Analysis for Potential Tick Vectors of Lyme Disease in Mexico

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Received 15 July 2011; Accepted 23 October 2011

Academic Editor: Nildimar Honório

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Species distribution models were constructed for ten *Ixodes* species and *Amblyomma cajennense* for a region including Mexico and Texas. The model was based on a maximum entropy algorithm that used environmental layers to predict the relative probability of presence for each taxon. For Mexico, species geographic ranges were predicted by restricting the models to cells which have a higher probability than the lowest probability of the cells in which a presence record was located. There was spatial nonconcordance between the distributions of *Amblyomma cajennense* and the *Ixodes* group with the former restricted to lowlands and mainly the eastern coast of Mexico and the latter to montane regions with lower temperature. The risk of Lyme disease is, therefore, mainly present in the highlands where some *Ixodes* species are known vectors; if *Amblyomma cajennense* turns out to be a competent vector, the area of risk also extends to the lowlands and the east coast.

1. Introduction

Lyme disease, the most frequently reported tick-borne infectious disease in the United States and Europe [1, 2], is increasingly being reported from Mexico [3, 4], where disease cases are more prevalent during warm-weather months when ticks are active. The etiologic agent, *Borrelia burgdorferi*, enters the skin at the site of the tick bite; after incubating for 3–30 days, the bacteria migrate through the skin and may spread to lymph nodes or disseminate through the bloodstream to other parts of the body. While *B. burgdorferi* infection might be endemic in Mexico [3, 4] it is relatively

rare in the southern USA making the question of its biogeography a matter of interest.

Additionally, in Mexico, the epidemiology and biogeography of Lyme disease are not well understood [5]. Several tick species have recently been identified as containing *B. burgdorferi* using a DNA polymerase chain reaction and, therefore, may be considered as candidates that may be involved in the enzootic transmission cycle in both Mexico and South America. These include tick species from the genus *Ixodes* [3, 4] as well as *Amblyomma cajennense* [5, David Beck, personal communication]. While detection of *B. burgdorferi* DNA by polymerase chain reaction is not

indicative of vector competence, the presence of *B. burgdorferi* in the molecular surveys does indicate a benefit from modeling the distribution of *A. cajennense* since it has been shown to feed on reservoirs for *B. burgdorferi* in Mexico. Additionally, the South American *A. cajennense* has been shown to be a competent vector for *Rickettsia rickettsii* [6], the causative agent of Rocky Mountain spotted fever, and has been shown to carry additional *Rickettsia* species which belong to the spotted fever group [7].

Ixodes ticks are hematophagous parasites during all active life stages. They have great importance from economic, veterinary, and human health vantage perspectives because of their capacity to transmit a variety of diseases to humans and animals [8]. These species are parasites of birds or mammals. In Mexico, 26 *Ixodes* species have been identified; these were collected from 20 of Mexico 32 states [9]. The distribution of *A. cajennense* extends from the southern regions of the United States (Texas) to the Caribbean Islands, and across Central and South America to northern Argentina, excluding the mountain regions [10, 11]. As a consequence, if *A. cajennense* was to contribute to maintenance of *B. burgdorferi* in the zoonotic cycle in any way or be a competent vector for a variety of spotted fevers in Mexico, the health impact could be significant. Thus far, *A. cajennense* has not been found north of latitude 27°N or south of latitude 29°S and its geographic range may be limited by temperature [10]. Low temperatures in mountainous areas such as the Mexican Sierra Madre and the Andes may be an obstacle for its establishment. With this restriction, the species is known to survive in regions with very different ecological conditions, spanning from arid grasslands to tropical forests [10].

The purpose of this paper is to explore the biogeography of *Ixodes* ticks and *A. cajennense* in Mexico and the suitability of different ecoregions and habitat types to their potential establishment using species distribution models (SDMs). This technique has been systematically developed to explore vector-borne zoonotic disease ecology and biogeography during the last 15 years [12, 13], and several studies have applied it to Mexico and nearby regions [14–16]. The goal was to determine the ecological variables that best predict georeferenced distributional data of a species collected through fieldwork, from museum collections, and so forth. These predictive variables are interpreted as identifying the potential geographical distribution of a species [17] and are sometimes also interpreted as identifying its fundamental niche [14, 18–20]. When biogeographic, behavioral, and other limitations to dispersal are taken into account, the potential distribution is refined to a predicted (realized) distribution.

For species that are relevant to the transmission of a disease, the relative suitability of different regions within the predicted distribution, as measured on a continuous scale, establishes the relative spatial ecological risk [13, 16, 17]. For vector-borne zoonotic diseases, a composite measure for this risk must include the SDMs of all relevant vector and reservoir species. This risk can then be combined with other measures of risk, including socioeconomic factors and disease case prevalence. A variety of techniques have been

developed to carry out such increasingly sophisticated disease risk analyses [17].

Because of a lack of data on other factors, this study is restricted to SDMs for potential tick vectors of Lyme disease. The aim was to analyze the predicted biogeography and habitat suitability for the *Ixodes* species, treated jointly, and *A. cajennense*. *Ixodes* species seem to be the most likely candidates for the transmission of Lyme disease in Mexico, and *A. cajennense* has been shown to be a competent vector for multiple tick-borne rickettsioses. Besides establishing the relative risk of the transmission of these diseases from these taxa, these SDMs will also permit prediction of the distributions of potentially epidemiologically relevant vector and reservoir distributions. This will allow the identification of the most likely candidates to transmit *B. burgdorferi* infections so that future studies can be guided by a better theoretical understanding of the underlying ecology of Lyme disease in Mexico.

A wide variety of techniques exist for SDM construction [21]. If presence-only (rather than presence-absence) data are all that are available, as is typically the case (including this study), machine-learning algorithms provide the most reliable results [21, 22]. These use georeferenced data on species occurrence points and environmental layers as input variables; as output they either provide binary predictions of presence or absence or a continuous measure than can be interpreted as relative habitat suitability. For risk analysis the latter is preferable. For this study, we chose a maximum entropy algorithm implemented in the MaxEnt software package [23–25] because, besides providing continuous output, its performance has been established as being as good or better than available alternatives [21, 22]. This choice has also become standard in constructing SDMs for systematic conservation planning [26–28].

2. Materials and Methods

2.1. Data

2.1.1. Tick Occurrence Data. Tick occurrence data were compiled from various sources including new field collections and information from prior publications. The field data were obtained from the University of North Texas Health Science Center, The University of Texas at Austin, the Texas Department of State Health Services (TX DSHS) and the Instituto de Biología, UNAM, Mexico. Specimens were identified by morphologic examination and by PCR amplification of 12S rDNA followed by sequence determination of the amplification products using the method of Williamson et al. [29]. All points were georeferenced using the MaNIS protocol (<http://manisnet.org/GeorefGuide.html>, last accessed 19 June 2011). Additional data came from Dergousoff et al. [30].

SDMs were constructed for an area including Mexico and Texas, both of which had sparse occurrence records; there were insufficient data to construct reliable models for Mexico or Texas alone. Table 1 lists all the data that were available for all species in Mexico and Texas and is restricted to those used in this analysis, along with the number of points that satisfied the error constraint (see Section 2.2) and the number of such

TABLE 1: Total number of records and final number of records used to generate the analysis.

| Tick species | Mexico | | | Texas | | |
|-----------------------------|--------------|--------------------------------|-------------------|--------------|--------------------------------|-------------------|
| | Total number | Number with adequate precision | Independent cells | Total number | Number with adequate precision | Independent cells |
| <i>Amblyomma cajennense</i> | 10 | 10 | 9 | 269 | 269 | 69 |
| <i>Ixodes boliviensis</i> | 10 | 1 | 1 | 0 | 0 | 0 |
| <i>Ixodes conepati</i> | 2 | 2 | 2 | 0 | 0 | 0 |
| <i>Ixodes cookei</i> | 3 | 3 | 3 | 0 | 0 | 0 |
| <i>Ixodes eadsi</i> | 5 | 4 | 4 | 0 | 0 | 0 |
| <i>Ixodes kingi</i> | 1 | 1 | 1 | 0 | 0 | 0 |
| <i>Ixodes marxi</i> | 1 | 1 | 1 | 0 | 0 | 0 |
| <i>Ixodes scapularis</i> | 5 | 4 | 3 | 56 | 56 | 51 |
| <i>Ixodes sculptus</i> | 0 | 0 | 0 | 1 | 1 | 1 |
| <i>Ixodes texanus</i> | 2 | 1 | 1 | 0 | 0 | 0 |

TABLE 2: Environmental parameters for species distribution models.

| Parameters |
|---|
| Annual mean temperature |
| Mean diurnal range |
| Isothermality |
| Temperature seasonality |
| Maximum temperature of warmest month |
| Minimum temperature of coldest month |
| Temperature annual range |
| Mean temperature of the wettest quarter |
| Mean temperature of the driest quarter |
| Mean temperature of the warmest quarter |
| Mean temperature of the coldest quarter |
| Annual precipitation |
| Precipitation of wettest month |
| Precipitation of driest month |
| Precipitation seasonality |
| Precipitation of wettest quarter |
| Precipitation of driest quarter |
| Precipitation of warmest quarter |
| Precipitation of coldest quarter |
| Elevation |
| Slope |
| Aspect |
| Compound topographic index |

points in independent cells. All data have been submitted to the Disease Vectors Database [31]. Given that the area of epidemiological interest for this paper was Mexico, the model results that were subjected to further analysis and are presented here are for Mexico.

2.1.2. Environmental Layers. The environmental layers used are listed in Table 2. These include four topographical variables (elevation, slope, aspect, and compound topographical index) and 19 bioclimatic variables. The latter were obtained from the WorldClim database [32] (<http://www.worldclim.org/>; last accessed 28 February 2010). Elevation data were obtained from the United States Geological Survey Hydro-1K DEM data set (http://eros.usgs.gov/#/Find_Data/Products_and_Data_Available/gtopo30/hydro; last accessed 28 February 2010). Slope, aspect, and the

compound topographical index were derived from the DEM using the Spatial Analyst extension of ArcMap 9.3.

2.2. Species Distribution Models. The study area of Mexico and Texas was divided into 3 429 052 cells at a resolution of 30 arcseconds. The average cell area was 0.77 km². Data were retained for this analysis only if the estimated error was less than 1 arcminute. Data prior to 1990 was excluded from the present analysis. Table 2 shows the number of data that were retained. A conservative threshold of independent data points (i.e., those falling in different cells at the resolution of this analysis) was used for model construction, namely, at least 10 independent cells [17].

SDMs were constructed separately for *A. cajennense*, but for together 10 *Ixodes* species (*I. boliviensis*, *I. conepati*, *I. cookei*, *I. eadsi*, *I. kingi*, *I. marxi*, *I. scapularis*, *I. sculptus*, and *I. texanus*) for three reasons: (i) though from this group only *I. scapularis* has so far been implicated as a vector for Lyme disease, other *Ixodes* species (e.g., *I. pacificus* and those of the *I. ricinus* complex) are also confirmed vectors. Consequently, it remains possible that these others may be competent vectors. (ii) The provenance of data points suggested that several of these species often cooccur (e.g., *I. scapularis* and *I. sculptus* in Texas). Given the sparse data points available, this meant that the geographical range of these potential vectors may be significantly underestimated if the SDMs were constructed separately for each species. (iii) Treating the data points together allowed much more reliable SDM construction because of the higher number of data points available for input.

Following a standard protocol [17], MaxEnt (Ver. 3.3.4) was run with the threshold and hinge features and without duplicates so that there was at most one sample per pixel; linear, quadratic, and product features were used. The convergence threshold was set to a conservative 1.0×10^{-5} . For the AUC, that is, the area under the receiver-operating characteristic (ROC) curve, averages over 100 replicate models were computed. For each model the test : training ratio was set to 40:60 following Phillips and Dudik [25] which means that models were constructed using 60% of the data and tested with the remaining 40%. An acceptability threshold of 0.90 was used for both the test and training AUCs, well above the standard 0.60 used in the literature.

TABLE 3: Size (area) of predicted range of *Amblyomma cajennense* and the *Ixodes* group.

| State | <i>Amblyomma cajennense</i> | | <i>Ixodes</i> | |
|---------------------|-----------------------------|-------------------------|---------------|-------------------------|
| | No. cells | Area (km ²) | No. cells | Area (km ²) |
| Aguascalientes | 0 | 0 | 1226 | 944.02 |
| Baja California | 1995 | 1536.15 | 3081 | 2372.37 |
| Baja California Sur | 2467 | 1899.59 | 1230 | 947.1 |
| Campeche | 21243 | 16357.11 | 7602 | 5853.54 |
| Chiapas | 2238 | 1723.26 | 17743 | 13662.11 |
| Chihuahua | 70 | 53.9 | 2380 | 1832.6 |
| Coahuila | 11578 | 8915.06 | 41080 | 31631.6 |
| Colima | 114 | 87.78 | 152 | 117.04 |
| Distrito Federal | 0 | 0 | 785 | 604.45 |
| Durango | 986 | 759.22 | 37042 | 28522.34 |
| Estado de Mexico | 8 | 6.16 | 18010 | 13867.7 |
| Guanajuato | 654 | 503.58 | 20401 | 15708.77 |
| Guerrero | 37 | 28.49 | 4747 | 3655.19 |
| Hidalgo | 997 | 767.69 | 19004 | 14633.08 |
| Jalisco | 542 | 417.34 | 20600 | 15862 |
| Michoacán | 39 | 30.03 | 22676 | 17460.52 |
| Morelos | 0 | 0 | 490 | 377.3 |
| Nayarit | 6087 | 4686.99 | 3415 | 2629.55 |
| Nuevo León | 43863 | 33774.51 | 42073 | 32396.21 |
| Oaxaca | 11002 | 8471.54 | 32519 | 25039.63 |
| Puebla | 2594 | 1997.38 | 18786 | 14465.22 |
| Querétaro | 204 | 157.08 | 10271 | 7908.67 |
| Quintana Roo | 6338 | 4880.26 | 1307 | 1006.39 |
| San Luis Potosí | 13836 | 10653.72 | 17477 | 13457.29 |
| Sinaloa | 2683 | 2065.91 | 3716 | 2861.32 |
| Sonora | 1409 | 1084.93 | 12211 | 9402.47 |
| Tabasco | 7571 | 5829.67 | 147 | 113.19 |
| Tamaulipas | 80607 | 62067.39 | 29230 | 22507.1 |
| Tlaxcala | 0 | 0 | 4571 | 3519.67 |
| Veracruz | 63260 | 48710.2 | 14420 | 11103.4 |
| Yucatán | 7966 | 6133.82 | 99 | 76.23 |
| Zacatecas | 4 | 3.08 | 17027 | 13110.79 |

Obtaining predicted ranges for the sake of comparisons required the conversion of SDM outputs, which were relative probabilities (specifying habitat suitability) into binary predictions of presence or absence. This was done using a threshold of 0.10 for *A. cajennense* and 0.12 for the *Ixodes* group which corresponded to the lowest probability predicted by the SDMs for an occurrence point used in model construction. The threshold was used after normalization of the MaxEnt output in Mexico so that the highest predicted value for occurrence in each model was 1 for at least one cell in the landscape.

3. Results and Discussion

3.1. Species Distribution Models. Figure 1 presents the species distribution model for *A. cajennense* Figure 2 and that for the present *Ixodes* group. For the 100 replicate models, for *A. cajennense*, the average test AUC was 0.91, the training was 0.99; for the *Ixodes* group, the corresponding numbers were 0.93 and 0.98. Figure 3 presents both distributions together showing their almost complete nonconcordance, which we will refer to as their “complementarity.”

Table 3 presents the areas occupied by the predicted distributions for the states of Mexico (see, also, Figures 4 and 5). The *Ixodes* group is predicted to be present in all states, while *A. cajennense* is predicted for all of them except Aguascalientes, Distrito Federal (Mexico City), Morelos, and Tlaxcala, all of which are located in central Mexico. The main distribution predicted for *A. cajennense* is in Veracruz (21.8%) and Tamaulipas (27.8%) (Figure 4), both in the northeast coast of Mexico and both having lowlands and warm temperatures [32]. In contrast, the *Ixodes* group is predicted mainly in Durango (8.7%), Coahuila (9.6%), Nuevo León (9.9%) (Figure 5), and all of the northern states characterized by the presence of high altitudes and temperate vegetation (see below).

3.2. Ecological Suitability. Table 4 presents the altitudinal dependence of the two SDMs. Although the complete predicted *A. cajennense* range is between 0 and 2800 m, most of it (95%) occurs between 200 and 1000 m. This result agrees with Solís [33] who found this species only in areas with altitudes below 1000 m even though, geographically, the species is widely distributed in the warmer parts of Latin

TABLE 4: Altitudinal intervals and predicted ranges of *Amblyomma cajennense* and the *Ixodes* group.

| Interval | <i>Amblyomma cajennense</i> | | <i>Ixodes</i> | |
|-----------|-----------------------------|-------------------------|---------------|-------------------------|
| | No. cells | Area (km ²) | No. cells | Area (km ²) |
| 1–200 | 148 | 113.96 | 0 | 0 |
| 201–400 | 13833 | 10651.41 | 5382 | 4144.14 |
| 401–600 | 6189 | 4765.53 | 16702 | 12860.54 |
| 601–800 | 3296 | 2537.92 | 16647 | 12818.19 |
| 801–1000 | 1351 | 1040.27 | 13644 | 10505.88 |
| 1001–1200 | 476 | 366.52 | 12412 | 9557.24 |
| 1201–1400 | 145 | 111.65 | 13443 | 10351.11 |
| 1401–1600 | 106 | 81.62 | 15187 | 11693.99 |
| 1601–1800 | 86 | 66.22 | 17187 | 13233.99 |
| 1801–2000 | 131 | 100.87 | 17490 | 13467.3 |
| 2001–2200 | 92 | 70.84 | 22017 | 16953.09 |
| 2201–2400 | 38 | 29.26 | 26877 | 20695.29 |
| 2401–2600 | 65 | 50.05 | 26918 | 20726.86 |
| 2601–2800 | 56 | 43.12 | 19741 | 15200.57 |
| 2801–3000 | 8 | 6.16 | 8194 | 6309.38 |
| 3001–3200 | 0 | 0 | 3201 | 2464.77 |
| 3201–3400 | 0 | 0 | 1449 | 1115.73 |
| 3401–3600 | 0 | 0 | 761 | 585.97 |
| 3601–3800 | 0 | 0 | 450 | 346.5 |
| 3801–4000 | 0 | 0 | 234 | 180.18 |
| 4001–4200 | 0 | 0 | 108 | 83.16 |
| 4201–4400 | 0 | 0 | 59 | 45.43 |
| 4401–4600 | 0 | 0 | 18 | 13.86 |
| 4601–4800 | 0 | 0 | 8 | 6.16 |
| 4801–5000 | 0 | 0 | 3 | 2.31 |
| >5000 | 0 | 0 | 1 | 0.77 |

TABLE 5: Ecoregion occupancy by *Amblyomma cajennense* and the *Ixodes* group.

| Ecoregion | <i>Amblyomma cajennense</i> | | <i>Ixodes</i> | |
|-------------------------------|-----------------------------|-------------------------|---------------|-------------------------|
| | No. cells | Area (km ²) | No. cells | Area (km ²) |
| Pine and oak forest | 3112 | 2396.24 | 196510 | 151312.7 |
| Cloud forest | 179 | 137.83 | 8913 | 6863.01 |
| Chaparral | 1681 | 1294.37 | 777 | 598.29 |
| Mangrove | 10432 | 8032.64 | 0 | 0 |
| Tamaulipan scrub thorn forest | 84540 | 65095.8 | 46848 | 36072.96 |
| Submontane scrubland | 22664 | 17451.28 | 24305 | 18714.85 |
| Xeric scrubland | 9409 | 7244.93 | 74934 | 57699.18 |
| Marshes of Centla | 1135 | 873.95 | 0 | 0 |
| Tropical rainforest | 110791 | 85309.07 | 31043 | 23903.11 |
| Tropical deciduous forest | 46377 | 35710.29 | 42190 | 32486.3 |
| Tropical dry forest | 799 | 615.23 | 0 | 0 |

America and the Caribbean [33]. However, in Guatemala, an ecological and epidemiological study of ticks [34] recorded that the presence of *A. cajennense* occurs up to 1400 m in areas with a marked rainy season (six months of rain and six months for dry season) [35]. The SDMs predict an expanded altitudinal range while confirming that the best habitat is between 200 and 1000 m.

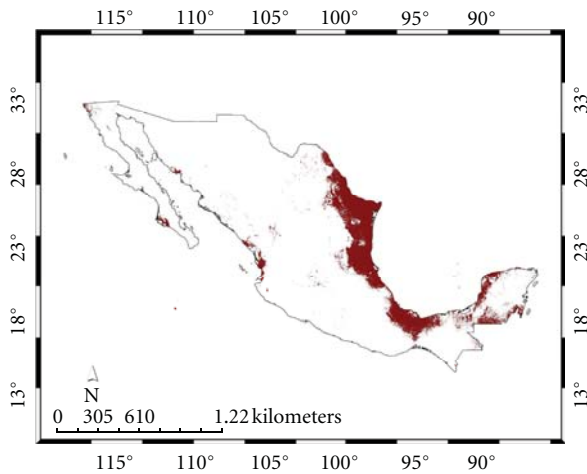
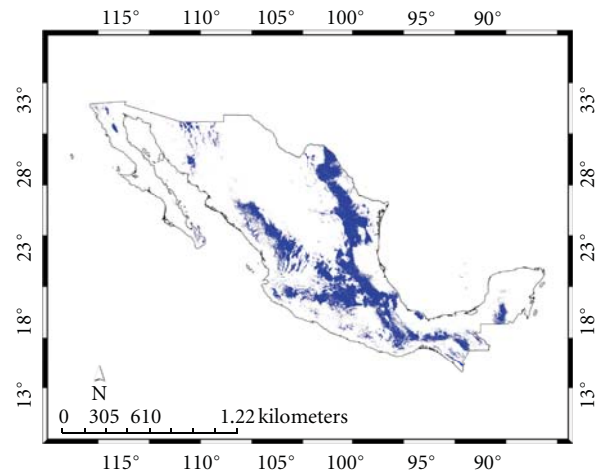
For the *Ixodes* group (Table 4), the complete altitudinal range goes from 200 m to over 5000 m though most of it (98%) is restricted to below 3600 m. The altitudinal range of the *Ixodes* group thus also complements that of *A. cajennense*,

partly accounting for the geographical complementarity noted earlier.

Table 5 shows the ecoregional distribution of the two SDMs (see, also, Figures 6 and 7). Although both SDMs share ecoregions, *A. cajennense* presence was primarily predicted for ecoregions such as mangroves and marshes along the coast of Mexico at low altitudes (Figure 6). In Mexico and the United States, this species is found in areas where the mean temperature is around 13°–16°C and the NDVI is high [36]. Relatively low mean temperatures and differences in the seasonal patterns of rainfall may limit this species

TABLE 6: Vegetation types for *Amblyomma cajennense* and the *Ixodes* group.

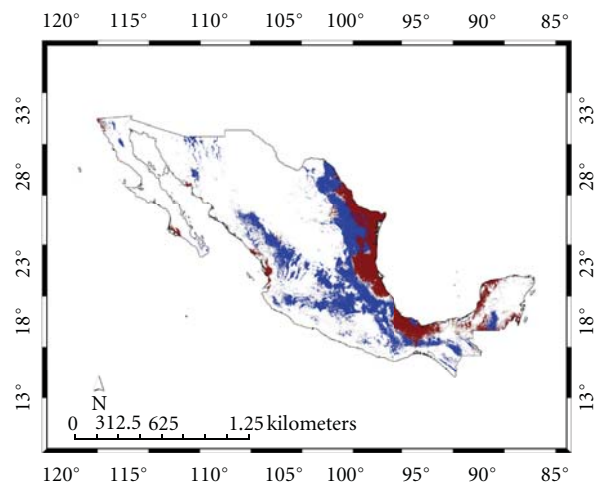
| Vegetation type | <i>Amblyomma cajennense</i> | | <i>Ixodes</i> | |
|----------------------------------|-----------------------------|-------------------------|---------------|-------------------------|
| | No. cells | Area (km ²) | No. cells | Area (km ²) |
| Grassland | 925 | 712.25 | 6602 | 5083.54 |
| Scrubland | 9560 | 7361.2 | 14920 | 11488.4 |
| Pine forest | 72 | 55.44 | 6478 | 4988.06 |
| Oak forest | 392 | 301.84 | 7602 | 5853.54 |
| Pine-oak forest | 76 | 58.52 | 9654 | 7433.58 |
| Tropical rainforest | 4116 | 3169.32 | 2622 | 2018.94 |
| Tropical deciduous forest | 4815 | 3707.55 | 6541 | 5036.57 |
| Aquatic inland vegetation | 1330 | 1024.1 | 14 | 10.78 |
| Cloud forest | 14 | 10.78 | 1501 | 1155.77 |
| Mangle | 473 | 364.21 | 0 | 0 |
| Palms/palm plantations | 30 | 23.1 | 18 | 13.86 |
| Savanna | 195 | 150.15 | 48 | 36.96 |
| Other vegetation types/not known | 28921 | 22269.17 | 17818 | 13719.86 |

FIGURE 1: Species distribution model for *Amblyomma cajennense*.FIGURE 2: Species distribution model for the *Ixodes* group.

colonization of areas to the north of its current distribution. Low temperatures are likely keeping the species out of elevated areas, such as the Sierra Madre in Mexico. The southern distribution of *A. cajennense* appears to be mainly restricted by relatively low temperatures and not by low humidity [36].

Table 6 shows the different vegetation types associated with both models (see, also, Figures 8 and 9). Although both SDMs share scrubland as the main vegetation type, 18.7 and 20.0%, respectively, for *A. cajennense* and the *Ixodes* group, the former is mainly associated with tropical deciduous and rainforest (17.4%), while the latter is associated with oak and pine-oak forest (23.3%). These predictions agree with Álvarez et al. [35] who collected *A. cajennense* in tropical wet forests and its transitions. It is likely that suitable *A. cajennense* habitat consists of warmer areas with moderate precipitation.

Moreover, suitable *A. cajennense* habitat is predicted to be restricted to areas with more dense or mixed vegetation and tall grass [37]. A study of horse farms showed that pastures

FIGURE 3: Complementarity of models for *Amblyomma cajennense* and the *Ixodes* group.

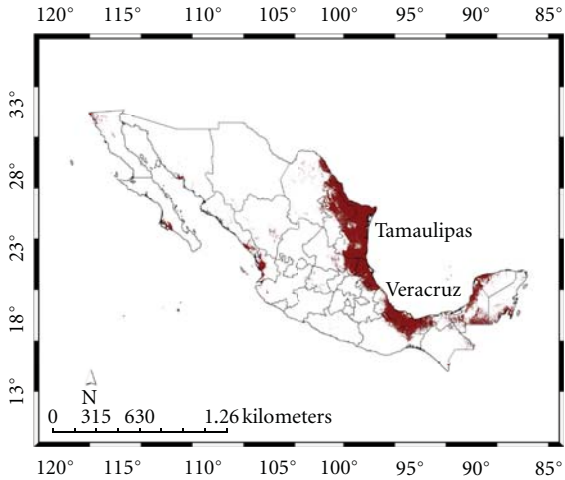


FIGURE 4: Species distribution model and Mexican states for *Amblyomma cajennense*. The principal states are shown (see text).

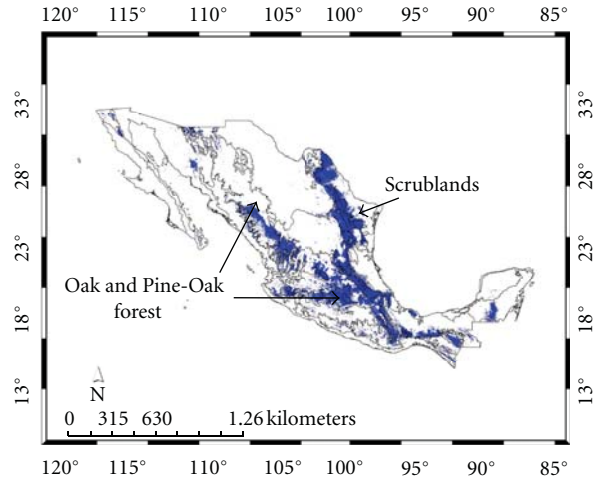


FIGURE 7: Species distribution model and ecoregions for the *Ixodes* group. The principal ecoregions are shown (see text).

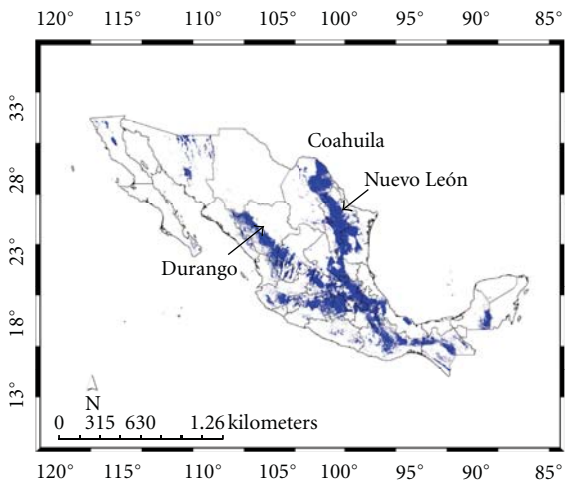


FIGURE 5: Species distribution model and Mexican states for the *Ixodes* group. The principal states are shown (see text).

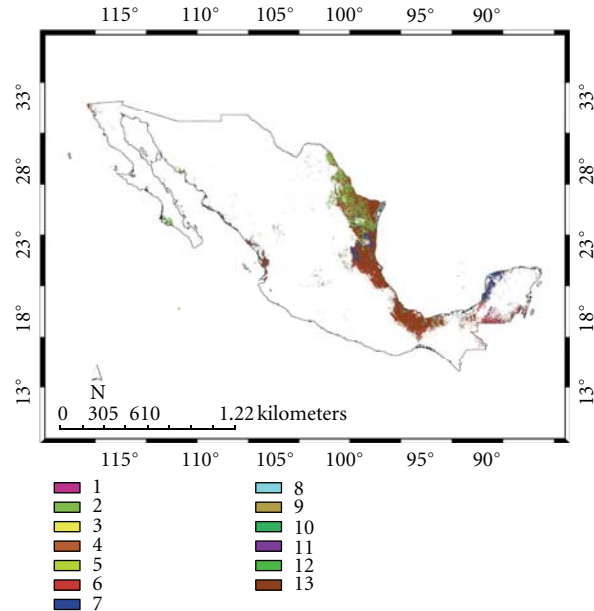


FIGURE 8: Species distribution model and vegetation types for *Amblyomma cajennense*: (1) grassland; (2) scrubland; (3) pine forest; (4) oak forest; (5) pine-oak forest; (6) tropical rainforest; (7) tropical deciduous forest; (8) aquatic inland vegetation; (9) cloud forest; (10) mangle; (11) palms/palm plantations; (12) savanna; (13) other vegetation types/not known.

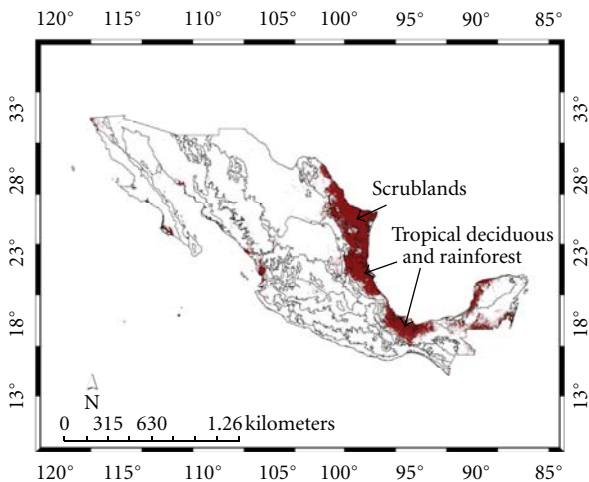


FIGURE 6: Species distribution model and ecoregions for *Amblyomma cajennense*. The principal ecoregions are shown (see text).

were most likely to be infested with *A. cajennense* when the pasture had mixed vegetation (grasses and shrubs) and was cut less than once per year [38]. In Argentina, *A. cajennense* was more abundant in forested areas than open areas [39]. In contrast, species from the *Ixodes* group are typically collected in heavily forested or dense brushy areas.

4. Conclusion

Species distribution models are potentially a powerful tool for assessing risk from vector-borne diseases [12, 17]. Even

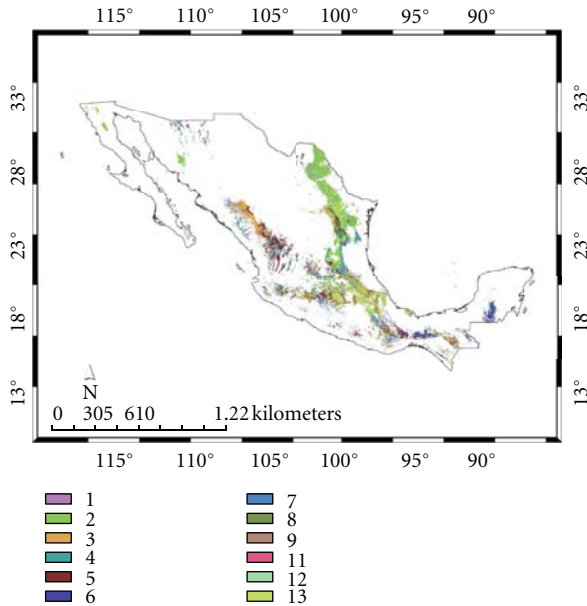


FIGURE 9: Species distribution model and vegetation types for the *Ixodes* group: (1) grassland; (2) scrubland; (3) pine forest; (4) oak forest; (5) pine-oak forest; (6) tropical rainforest; (7) tropical deciduous forest; (8) aquatic inland vegetation; (9) cloud forest; (10) mangle; (11) palms/palm plantations; (12) savanna; (13) other vegetation types/not known.

in systems as poorly understood as the one examined here, patterns of concordance in geographic or ecologic space can provide testable hypotheses for host, vector, and reservoir interactions besides their associations with habitat type, vegetation, or ecoregion. Such distributional hypotheses can form the basis for field studies, including analyses of specific parameters of species ecologic niches [40, 41], prediction of species distributions across scenarios of climate change [14, 42, 43], prediction of species invasions [9, 17, 44, 45], assessment of patterns of evolutionary change in ecologic parameters [46], and spatial/epidemiologic stratification of disease endemic areas.

Little is known about Lyme disease and its transmission cycle in Mexico. Assuming that the *Ixodes* group contains the vectors responsible for transmission, the results presented here identify the geographical regions and ecological characteristics of the regions with the highest potential for transmission: high-altitude low-temperature areas. The SDM also suggests why Lyme disease is relatively rare in the southern United States: the high temperatures of these areas make them relatively less suitable for potential *Ixodes* vectors.

Should *A. cajennense* affect the enzootic transmission cycle and assist with maintenance of *B. burgdorferi* in reservoir species, the area of high risk extends into the eastern lowlands of Mexico where the SDM for this species complements that of the *Ixodes* group. This result suggests that it is important to test *A. cajennense* for vector competence using appropriate laboratory methods.

Field efforts are currently under way to collect specimens of potential mammal reservoirs of *B. burgdorferi* and *R. rickettsii*. SDMs of these species will permit analysis of spatial

correlations between them and the vector SDMs which will permit the formulation of testable hypotheses about the Lyme disease cycle in Mexico.

Acknowledgments

For unpublished tick occurrence data, thanks are due to Kelly Pierce and Chuck Sexton. Thanks are due to Miguel Linaje for discussions. P. Illoldi-Rangel wants to thank the Faculty for the Future program for the grant given for her postdoctoral studies. Finally, thanks are due to the Texas Ecolab program and anonymous landowners for financial assistance and access to property. This project is partially funded by the Universidad Nacional Autónoma de México (PAPIIT IN202711) and the Consejo Nacional de Ciencia y Tecnología (CONACYT SALUD-2008-01-87868).

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Research Article

Information System and Geographic Information System Tools in the Data Analyses of the Control Program for Visceral Leishmaniasis from 2006 to 2010 in the Sanitary District of Venda Nova, Belo Horizonte, Minas Gerais, Brazil

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Received 15 July 2011; Accepted 13 October 2011

Academic Editor: Maria Goreti Rosa-Freitas

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The aim of this paper is to report a brief history of control actions for Visceral Leishmaniasis (VL) from 2006 to 2010 in the Sanitary District (DS) of Venda Nova, Belo Horizonte, Minas Gerais, Brazil, focusing on the use of information systems and Geographic Information System (GIS) tools. The analyses showed that the use of an automated database allied with geoprocessing tools may favor control measures of VL, especially with regard to the evaluation of control actions carried out. Descriptive analyses of control measures allowed to evaluating that the information system and GIS tools promoted greater efficiency in making decisions and planning activities. These analyses also pointed to the necessity of new approaches to the control of VL in large urban centers.

1. Introduction

According to the classification of World Health Organization [1], leishmaniasis are neglected diseases of great epidemiological importance, which require effective control measures. They present potential to epidemic outbreaks because of their transmission by vector insects [2].

These diseases present a wide variation in their geographical areas of occurrence, with a focal distribution. Leishmaniasis affect more than 100 countries worldwide, with tropical or subtropical climate occurring zoonotic and anthroponotic cycles [2–4]. VL, also referred as kala-azar, attacks the internal organs being the most severe pathology. VL is a zoonotic disease spread worldwide and the highest concentration of cases (90%) occurs in six countries including Bangladesh, India, Nepal, Sudan, Ethiopia, and Brazil [5].

In the Americas, around 90% of human cases of VL are registered in Brazil, where 21 (77.8%) of its 27 federative

units have reported autochthonous cases [6]. VL was originally a rural disease in Brazil, however, around 20 years ago, it has been spreading to urban areas of medium and large sizes [7].

Belo Horizonte (BH) City, the capital of Minas Gerais State, is located between the coordinates 19° 55' South and 43° 57' West, and at 858 meters above the sea level. The climate is tropical, average annual temperature is 21°C, and average rainfall is 1.450 mm/year. There are 2.412.937 inhabitants distributed in 331 km² with a population density of 7.209,08 inhabitants per km² [8]. The city is the main pole of services, knowledge, and technology of Minas Gerais. BH city is divided into nine sanitary districts (SD) which have defined geographical area, population, and administrative staff [9]. Each SD is subdivided into coverage areas (CAs), which correspond to the territory attended by a primary public health care unit (Public Health Centers), see Figure 1. Metropolitan region of BH is composed by 33 municipalities

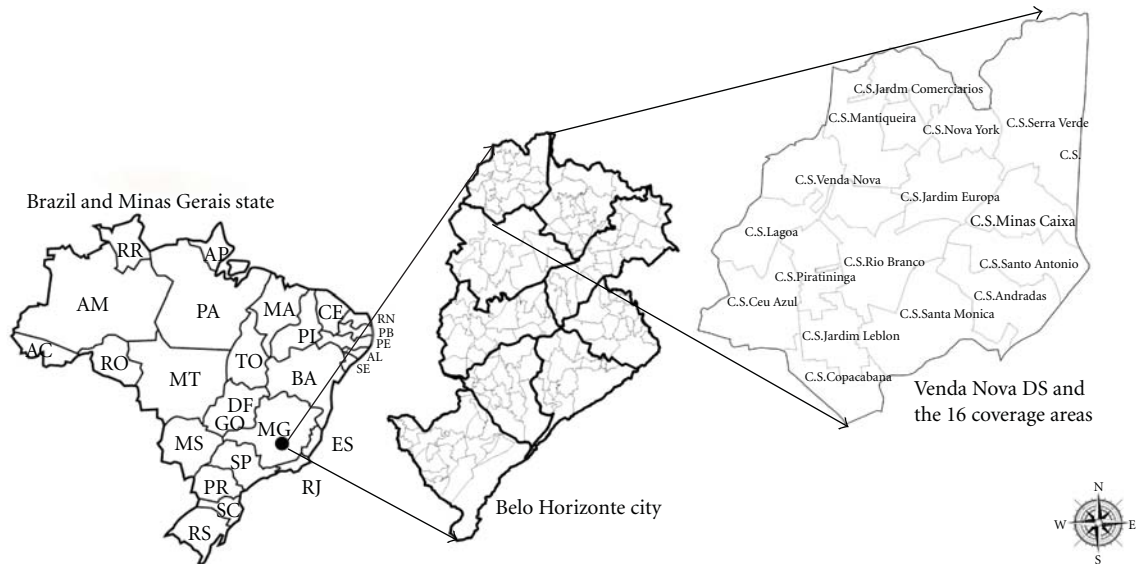


FIGURE 1: Administrative division of the Municipal District of Belo Horizonte and its location in Minas Gerais and in Brazil and Venda Nova Sanitary District location in the city of Belo Horizonte.

that also have geographical proximity (often being adjacent) and interchanging of inhabitants, products, and input with the capital. Autochthonous cases of VL have been registered by the official health service since 1989 in the metropolitan region of BH, Minas Gerais, which have a resident population predominantly urban [10].

The first autochthonous case of LV occurred in 1994, in Belo Horizonte City in an area bordering the municipality of Sabará. The disease has spread gradually in the following years to all regions of BH, presenting a pattern of transmission typically urban (domiciliary and peridomiciliary). VL has become a serious public health problem and a priority program of municipal government of BH City, due to the high incidence and lethality rates and geographic spreading. The Municipal Secretariat of Health of Belo Horizonte (MSH) reported 1,434 cases (partial data) of the disease with 161 deaths (11% of lethality), and VL cases have been reported in all sanitary districts from 1994 to 2010 [9].

Venda Nova SD—one of the nine SDs that comprise administrative division of BH—is the oldest one located in the north region of BH (Figure 1). Considering the period from 2006 to 2010, Venda Nova SD reported 104 cases, with 9 deaths (9% of lethality). The history of the human disease in Venda Nova SD shows that since the occurrence of the first autochthonous case of human VL in 1994, there were a territorial expansion and an increase in the number of cases. This also could be observed for canine infection rates [9].

The geographical expansion and increasing number of VL cases in the municipality have determined the rising demand of the program to control the disease leading to the need for agile and reliable analyses of data generated by performed actions. In this regard, the MSH in partnership with the Data Processing Company of Belo Horizonte (Prodabel) developed and implemented a computerized system to register the activities of the Programme for the Control

of Visceral Leishmaniasis—SCZOO LV in 2006. Data have been produced by the control activities of the canine reservoir (blood collection, laboratory processing, collection of serologically positive dogs, and euthanasia of seropositive dogs) and they are inserted into the Information System for Control of Zoonoses—component Visceral Leishmaniasis—subcomponent Canine Enquiry (SCZOO/LV/IC) [11].

The data of chemical control activities are inserted in the subcomponent of Insecticide Operation SCZOO/LV, which started in 2009, as a pilot project, being implemented in all the SDs from 2011. The data consolidation about the chemical measures was previously inserted on form called SD Weekly Leishmaniasis, centralized in the MSH. Both subcomponents of SCZOO/LV perform the automatic georeferencing of the data and generate the files in .dbf format which may be analyzed through the software Map Info, available in all SDs of Belo Horizonte.

2. Materials and Methods

This work aimed to report the experience of control of VL in Venda Nova SD, Belo Horizonte. The data analyzed were obtained from the Information Systems Control of Zoonoses—SCZOO/VL, subcomponent Enquiry Canine and Insecticide Operation, the Brazilian Information System for Reportable Diseases, and technical reports of the Management Control of Zoonoses of the SMH of Belo Horizonte and Management of Zoonoses Control (MZC) of Venda Nova SD.

The study analyzed data of control measures adopted by Venda Nova SD for Visceral Leishmaniasis from 2006 to 2010, considering the following aspects: epidemiological situation, the stratification of risk areas, annual programming of activities to control the canine reservoir and chemical control of vectors, geoprocessing of data, and new control strategy approaches.

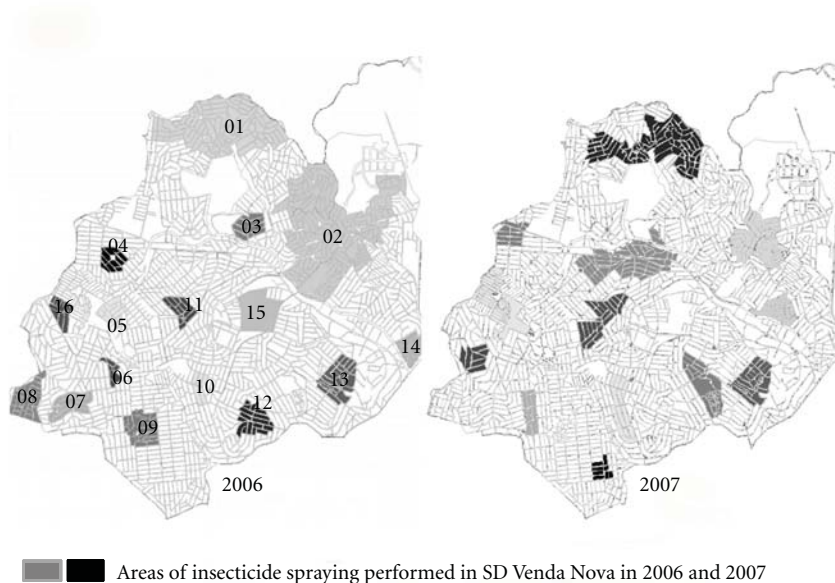


FIGURE 2: Example of the geographical pattern of areas scheduled for insecticide spraying in SD Venda Nova in the years from 2006 to 2010. The areas shown were planned for the years 2006 and 2007.

2.1. Study Area. Venda Nova SD is the oldest region of Belo Horizonte a thirty-year-old region. The origin and formation date back to 1711, when the troops of muleteers carried food and cattle to supply the district called “Curral D’el Rey.” The SD has a population of 263,930 inhabitants [12], an area of 28.30 km², and a population density of 8,670.58 inhabitants per km². The dog population is 36,706 dogs (1 dog: 7 inhabitants), according to the canine census of 2010 [Sanitary District Data, unpublished]. The region has 64,894 human dwellings, 89% are horizontal houses, however, there are prospects for verticalization. Characteristically, the occupation is heterogeneous with a variation between formal areas and peculiar environments such as houses with large gardens and terrain of great extension, places where individuals cultivate vegetables and raise livestock animals, and areas occupied by poverty pockets. The altitude ranges from 751 to 1000 meters above the sea level. The SD is subdivided into 16 coverage areas (AA): Céu Azul, Andradas, Venda Nova, Copacabana, Rio Branco, Santa Monica, Piratininga, Santo Antonio, Serra Verde, Minas Caixa, Jardim Europa, Jardim Leblon, Nova York, Mantiqueira, Minas Caixa, and Jardim dos Comercários (Figure 1).

2.2. Georeferencing. Databases georeferencing was performed using the coordinate system UTM and the datum SAD 69 (South American Datum). Geocoding was accomplished by SCZOO whether base did not have the coordinates of the address case, neighbours coordinates were used. Spatial analyses were carried out using MapInfo 8.5 Professional. These procedures are descriptive and based on overlaying maps and cluster analyses (hot spot) with a radius of 400 meters (average size of a city block in BH) and resolution of 80 meters. The procedures used are performed in the routine control of Management of Zoonosis Control (MZC).

2.3. Result Analyses. Files (in .dbf format) of SCZOO were organized through Excell 97/2003 generating compatible files with the software MapInfo and Prism. Excell 97/2003 was used to the descriptive statistics too. Graph Pad Prisma 4.0 was used for statistical analyses.

3. Results and Discussion

Evaluation of the annuals control measures for Visceral Leishmaniasis in Venda Nova SD is reported here.

3.1. 2006. Venda Nova SD carries out actions of control of VL in the priority areas (according the occurrence of human cases), but spraying of insecticides in buildings was also done where serologically positive dogs had been collected for euthanasia in 2006. A total of 31,639 buildings have been programmed for spraying, however, only 18,076 have been sprayed indicating the low coverage of insecticide spraying and the disparity between the number of buildings scheduled and those effectively sprayed. Sprayed areas have a dispersed profile without coverage of contiguous areas (Figure 2). In addition, the single insecticide spraying in buildings with serologically positive dogs presents low effectiveness in control. Whereas the insects can be only dispersed because places surrounding the sprayed building remain the same environment. A total of 12,072 serological tests for detection of *Leishmania* sp. were performed in serosurvey of dogs, with 11.57% positivity rate. Out of these positive dogs, 87% were euthanatized by the MCZ (Figures 2 and 7).

SCZOO was implemented in the SD in this year. This software automatically georeference all dogs examined and report their situation. The analyses of control measures were already available through the data of this database after 2006.

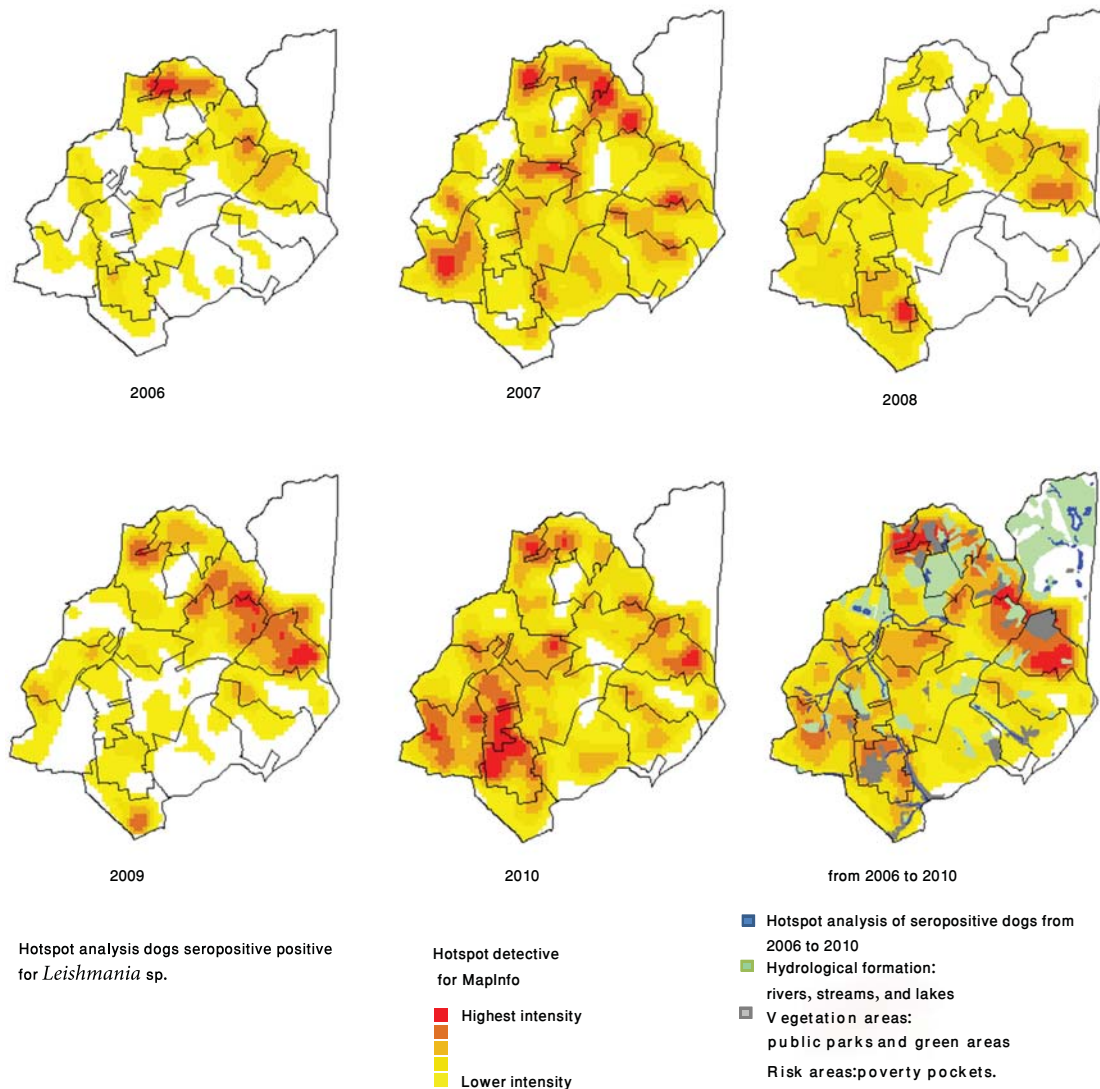


FIGURE 3: Dogs seropositive for *Leishmania* sp. in serosurveys carried out in SD Venda Nova from 2006 to 2010, the results shown georeferenced positive samples and clusters analysis for each year.

3.2. 2007. Program to 2007 remained the same pattern carried out for 2006, 16,716 buildings were planned to be sprayed and only 10,226 were sprayed, and buildings maintained the same pattern of geographic distribution. However, considering actions to control canine reservoir, there was an increase in number of examinations performed and the euthanasia rate remained high. A total of 24,782 examinations were done in the serosurvey with positivity rate of 11.57% and over 81% of dogs were euthanized by MCZ (Figures 3 and 7).

This higher number of tests carried out is due to the possibility of more robust analyses, with the software SCZOO. The concern about controlling the canine reservoir led to an increase in processing capacity of samples by the laboratory of the SMH.

3.3. 2008. The Ministry of Health drew up the Municipal Plan for Intensification of the Surveillance and Control of

Visceral Leishmaniasis due to the high number of cases of visceral leishmaniasis in 2008 (Figure 8). This plan gives priority to efforts to control VL in each covered area (CA). SMH carried out a stratification analysis of risk areas for VL in Belo Horizonte following the warning of Ministry of Health (Figure 4). This analysis was used to recast control measures of VL in Venda Nova SD in 2009. The stratification of incidence rates from 2003 to 2007 show clearly the worrying situation of the disease in Venda Nova SD.

Agents controlling endemic diseases, who work specifically in the control of VL, have been displaced to perform actions to control dengue transmissions due to an increase in number of cases in 2008 despite the Plan for Intensification. Dengue is a disease of epidemic patterns and great political appeal (Figure 9). This fact hindered the measures to control the VL.

Taking into account the 30,000 buildings planned for insecticide spraying, only 4,848 buildings have been sprayed.

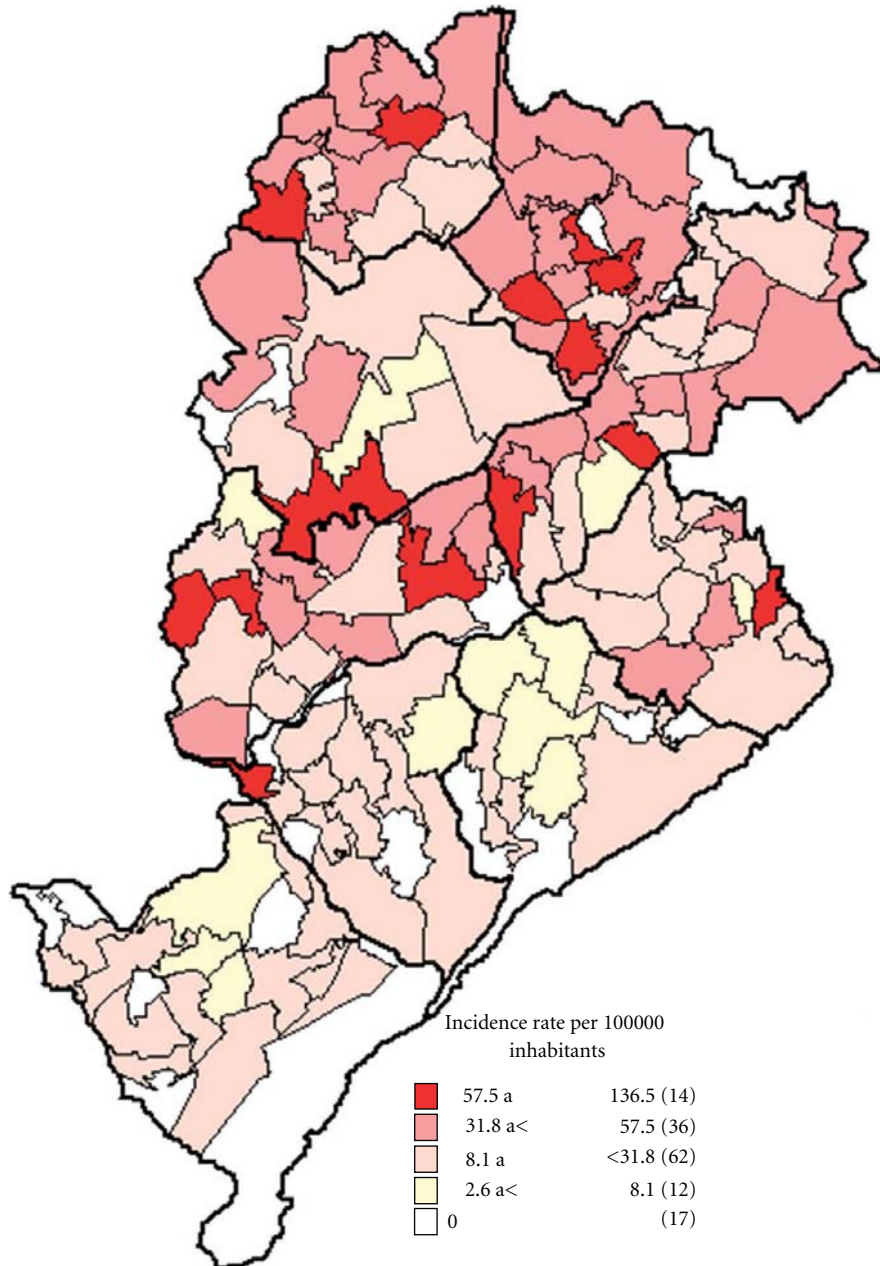


FIGURE 4: Areas of risk for the transmission of Visceral Leishmaniasis in Belo Horizonte City, considering the incidence rates from 2003 to 2007.

Insecticide spraying kept the profile geographically dispersed as occurred in 2006 and 2007. A total of 25,087 examinations for *Leishmania* sp. were conducted in a serosurvey of dogs, with a positivity rate of 8.39 and 87.56% of these dogs were euthanized by MZC. Venda Nova SD has had the improvements implemented by the plan of intensification only in 2009.

3.4. 2009 and 2010. The team of agents to control the VL received an increase of 24 employees in 2009 as a consequence of intensifying the plan implemented in 2008. The program of control measures has considered operating factors such as human and material resources, dislocation

of employees from the health centers to work field, among others in 2009 and 2010, through the analyses of data from previous years. Furthermore, the programs of VL control for 2009 and 2010 were based on the risk stratification (Figure 4) and on Venda Nova SD-specific data such as annual history of VL human cases and annual history rates of seropositive dogs. Analyzed variables were ranked, thus higher weights were attributed to the incidence rates of VL human cases and positivity of dogs exams in the previous year (2008 or 2009).

A serosurvey was carried out in 100% of the domiciled dog population of 11 CA in 2009 and the serosurvey was done in 16 CA (all area of Venda Nova DS) in 2010 considering the logical to work the areas more completely.

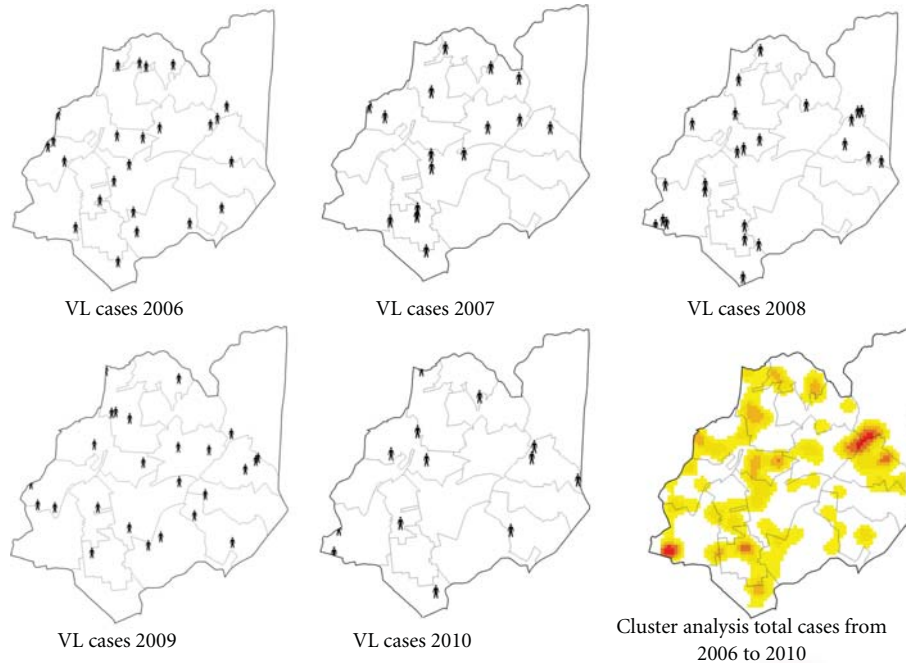


FIGURE 5: Human cases of Visceral Leishmaniasis SD Venda Nova from 2006 to 2010, cluster analysis considering all cases from 2006 to 2010.

Discrepancy between the number of buildings programmed for insecticide spraying and the number of buildings effectively sprayed was smaller than the previous years. Despite the possibility of analyzing, the areas of spraying remained the same profile to previous years, covering small and sparingly geographical areas.

A total of 8,000 buildings were scheduled for insecticide spraying in 2009, the procedure was performed in 8,530 buildings. Considering the actions aimed at controlling the canine reservoir, 21,351 tests were carried out in serosurvey, with positivity rates of 7.96% and 89.82% of seropositive dogs were euthanized. A total of 15,000 buildings were programmed for insecticide spraying in 2010 and the procedure was performed in 10,112 properties. The recruitment of employees for actions to control dengue impaired to achieve the goal. BH city has undergone great dengue epidemics in 2010 (Figure 9). Regarding the actions aimed to control the canine reservoir, 30,460 tests were carried out in serological screening, with a positivity rate of 9.36% and 85.70% of the seropositive dogs were euthanized.

3.5. Changes to the Control Programming in 2011. The analyses of the incidence rates of VL human cases, the lethality of the disease, and its geographic spread in Venda Nova SD revealed the necessity to employ new approaches in control of VL (Figures 2 and 5). Data of control measures referring to canine reservoir showed that Venda Nova SD performs a significant number of serological tests in its canine population (2006: 32.0% of the population was tested, 2007: 69.5%, 2008: 68.3%, 2009: 58.0%, 2010: 82.9%).

A large percentage (always higher than 80%) of the serologically positive dogs was euthanized in the years

analyzed (Figure 7). Despite these actions, the data point out that the rate of canine seropositive remains high with significant increase in 2010 (Figure 10). Seropositive dogs are dispersed over all areas of the Venda Nova SD, which is evident considering the increase in number of examinations performed and better spatial coverage of the SD, from 2006 to 2010 (Figure 3).

Serological canine positivity rate and VL human cases have low linear correlation ($r^2=0.028$), in the other words, a human case of VL is a complex event that cannot be explained only by the presence of infected dogs in an area. Overlaying the map of VL, human cases with maps of factors traditionally related to VL as vegetation, hydrography, and areas of poverty pockets. It is not possible to identify a strong spatial correlation between them. Cases of Visceral Leishmaniasis are spread in every coverage areas of Venda Nova SD and it is possible to observe areas of concentration of cases both in risk areas and outside them. Considering this data, we may infer that the VL presents new profile of occurrence in urban areas (Figure 6).

Considering these data, we can presume that only the actions for elimination of the vertebrate reservoir and insecticide spraying in areas with human cases and seropositive dogs are not sufficient for an effective control of this endemic disease.

These analyses have only been feasible through the use of SCZOO and by training in MapInfo for technical professionals of SD Venda Nova. These analyses made possible the changes of programmed actions for 2011. Areas were ranked according to geographical history of human cases and seropositive dogs. Canine serosurvey was programmed for 100% of the domiciled dog population and

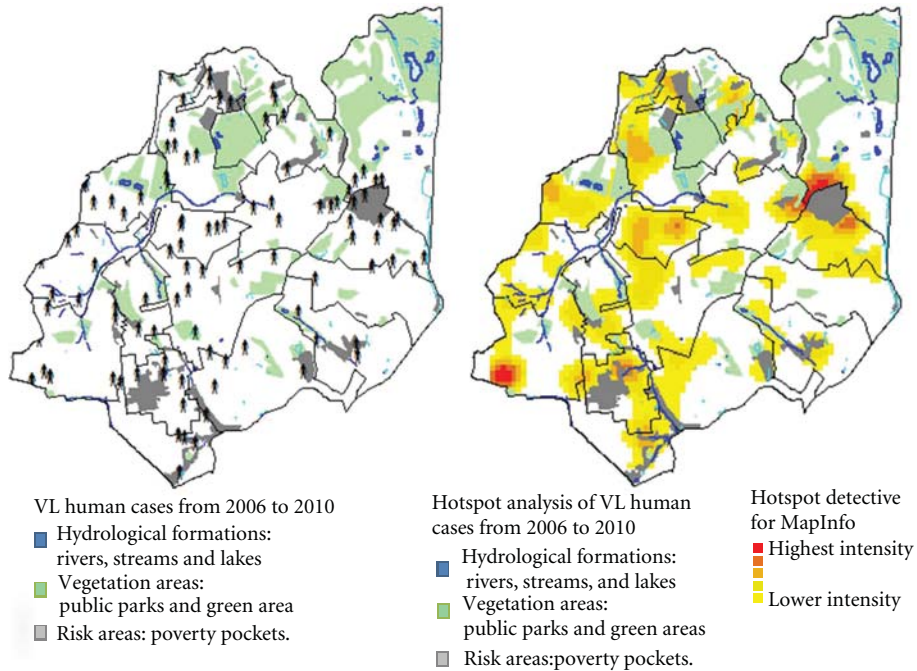


FIGURE 6: Map of VL human cases from 2006 to 2010 overlaying the maps of Vegetation (green areas and parks), Hydrographic (rivers, streams and lakes) and poor areas and social risk.

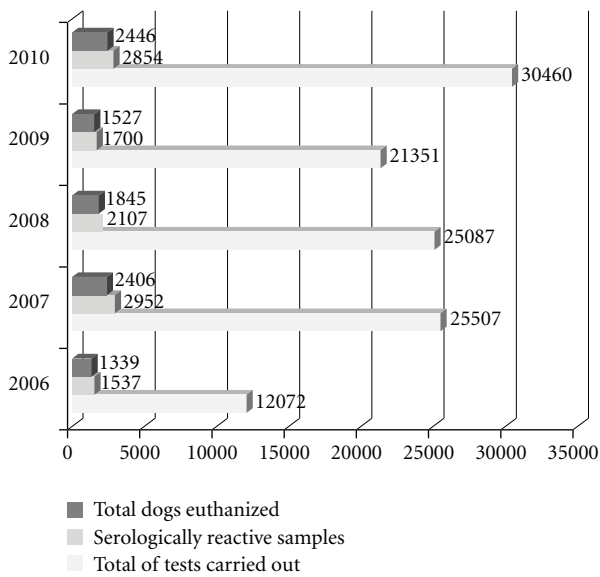


FIGURE 7: Total number of canine serological tests for *Leishmania* sp performed, total of positive samples, and total of dogs euthanized in SD Venda Nova from 2006 to 2010.

the insecticide spraying was programmed in broader and contiguous geographic areas.

Moreover, the DS prepared a project of Environmental Management and implemented with the Center for Zoonosis Control a projects of castration of dogs domiciled, both project following the criterion of priority areas.

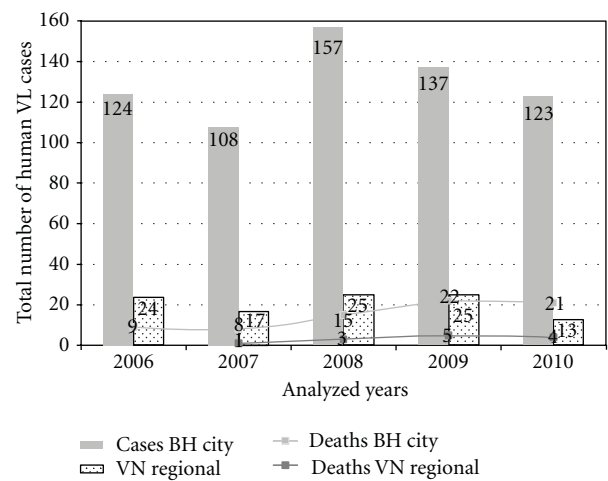


FIGURE 8: Cases and deaths of Visceral Leishmaniases in Belo Horizonte City and in the Venda Nova Sanitary District from 2006 to 2010.

4. Final Considerations

The process of territorial expansion and increasing the number of VL human cases in Belo Horizonte City ordered the municipal management to development and gradual implementation of an Operating Plan to reorganize the actions of disease control in the nine SD of the city [9].

The Plan for Intensification guaranteed the hiring of agents to control endemics diseases, purchase of personal

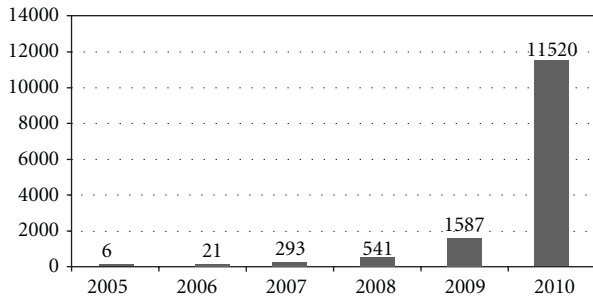


FIGURE 9: Cases of dengue fever in SD Venda Nova from 2005 to 2010.

protection equipment, laboratory equipment, sprayers, reform of the physical area, and increasing processing capability of the Laboratory for Zoonoses to 18,000 samples per month, the hiring of an expert to develop a specific information system for the program of leishmaniasis, production of information materials, staff training, among others [13]. Venda Nova SD could expand the capacity of organization and production of control activities to VL, encompassing aspects relating to technical and operational structures. This situation becomes clear when we analyze the significant increase in the number of samples collected in serosurveys and the tendency to regularize the number of buildings sprayed with insecticides (Figure 7).

The MSH ranked the areas of disease transmission, based on the cumulative incidence of human cases in each CA. Other specific indicators of CA are added such as rates of seropositive dogs, environmental conditions favorable for the transmission and vulnerability index to health risk. This categorization is fundamental for the planning, selection, and definition of measures to be undertaken in the different CA particularly the canine serosurveys and vector control.

Venda Nova SD is improving the process of developing the programs to control VL adding specific information on the analysis. These analyses of previous years measures made possible a better assessment of actions and their results after the implementation of the SCZOO. These analyses have been improved in 2010 with the training technical professionals in MapInfo software.

Rates of positivity to canine exams from 2006 to 2010 show that the canine infection is an expansion process on SD. VL is an emerging disease in BH mainly concentrated in underprivileged areas, with a number of factors which contribute to its occurrence [14]. However, as occurred to VL human cases in SD Venda Nova is clearly noticed that the occurrence of serologically positive dogs is spread out throughout all geographical area of SD, and areas of concentration of serologically positive dogs occur both in risk areas and outside them (Figure 3).

Despite the tendency of stabilization or decline in human cases in SD, is evident a necessity for additional entomological and environmental indicators to support the control measures. Several scientific works on leishmaniasis have been developed in BH, including those allowed characterize their sand flies fauna, there is no systematic measure that evaluated

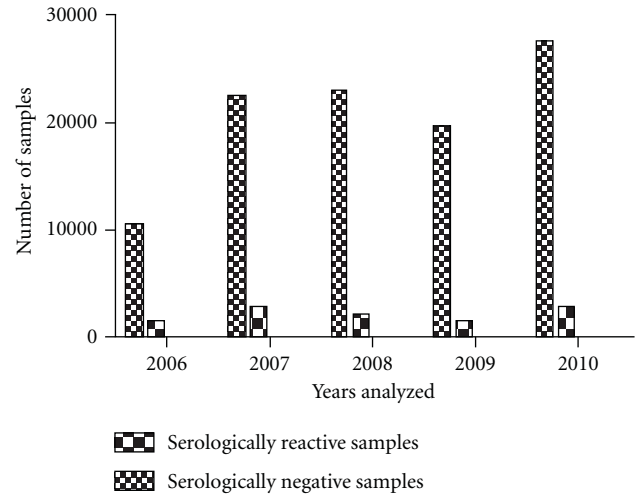


FIGURE 10: Chi-square testes to compare the proportions of serologically positive samples from 2006 to 2010. (There is no significant difference between 2007 and 2008 ($P : 0.0874$), the comparisons between the others pairs of years are significant different ($P < 0.0001$). The proportion in 2010 is significantly greater than in 2009 ($P < 0.0014$)—Z test for proportions).

the effectiveness of control measures on the vector population.

Studies on the phlebotomine fauna in BH demonstrated the presence of vector of *Leishmania* which are etiologic agents of VL and MCL [15, 16]. A study conducted from 2001 to 2003, covering all SD of BH City reported the presence of 15 species with the predominance of *Lutzomyia longipalpis*. Vector of *Leishmania braziliensis* as *Nyssomyia whitmani* and *Nyssomyia intermedia* has also been reported [16]. Other study conducted in Northeast SD from 2006 to 2007 records important vector species and high natural infection rates which indicate the strength of infection to *Leishmania infatum* in Northeast SD. Natural infection rates for *Leishmania infatum* were 14.3%, 3.80%, 19.0% for *Nyssomyia whitmani*, *Nyssomyia intermedia*, and *Lutzomyia longipalpis*, respectively [17].

Environmental surveillance, considering the use of vector attributes, should be focused on ecological, epidemiological, climatic, and social factors engaged in domestication and urbanization of insects [18]. However, even though the adaptability of sand fly vectors to anthropic-modified environments could be one of the factors related to the increase of leishmaniasis in BH, others no less important are related to new habits of population in occupation of the urban space, which eventually provide contact among human beings, vectors, and reservoirs [19–21].

Regarding this perspective, the environmental management strategy that is being implemented by SD Venda Nova could favor the control VL and other urban diseases. Several studies point out the need to modify the environment of the backyards of houses to control urban health problems. Another paper pointed that the use of spatial analysis tools for epidemiological surveillances indicate the need to correlate

the vector density with peridomestic environmental aspects [22].

SMH worked intensively to control LV. A total of 752,243 samples of canine blood were analyzed, 50451 seropositive dogs were euthanized, and 496,397 buildings were sprayed from 2006 to 2010 [9]. These data suggest that only the removal of seropositive dogs and spraying of buildings does not control the occurrence of infection in dogs by *Leishmania* sp. One example of this was described in the municipality of Araçatuba where 45% houses with a dog euthanized due to leishmaniasis replaced the dog in a period of one year [23].

A change of view is necessary on the occurrence of this disease in urban areas where the disease has characteristics yet unknown. Analyses of data distributed in the geographic spaces are increasingly appreciated in health management, it points useful information for the planning and evaluation of actions, based on the distribution of disease, location of health services, and environmental characteristics [24]. An example can be observed in the change of the programming from 2006 to 2010 and the programming to 2011 in SD Venda Nova, the access to information and the possibility to mapping the events can change the control strategies.

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Review Article

Distribution and Abundance of Phlebotominae, Vectors of Leishmaniasis, in Argentina: Spatial and Temporal Analysis at Different Scales

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Received 15 July 2011; Revised 27 September 2011; Accepted 2 October 2011

Academic Editor: Maria Goreti Rosa-Freitas

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The spatial-temporal analysis of the abundance of insects, vectors of tegumentary leishmaniasis (TL) and visceral leishmaniasis (VL), was performed in Argentina using spatial-temporal increasing scales. In the microscale (microfocal), the effect of the primary vegetation-crop interface in vector abundance was observed, and also how the shelters, food sources, and other environmental characteristics contribute to habitat microheterogeneity and so to a microheterogeneous vector distribution. In the mesoscale (locality or epidemic focus), the results from different foci of TL (rural and periurban) and VL (urban) suggested a metapopulation structure determined partially by quantifiable habitat variables that could explain the increase of risk associated to an increase of vector-human contact due to climatic or anthropogenic changes. In the macroscale (regional), captures of vectors and records of human cases allowed the construction of risk maps and predictive models of vector distribution. In conclusion, in order to obtain valid results transferrable to control programs from spatial studies, special attention should be paid in order to assure the consistency between the spatial scales of the hypotheses, data, and analytical tools of each experimental or descriptive design.

1. Introduction

The leishmaniasis comprise a set of clinical manifestations produced by different Trypanosomatidae parasites of the genus *Leishmania*, transmitted by the bite of the female of Phlebotominae, in America from the genus *Lutzomyia*. The three main clinical forms are visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (ML), the last two are called together tegumentary leishmaniasis (TL).

In Argentina were characterized four species of *Leishmania* from human cases: *Leishmania* (*Viannia*) *braziliensis*, *Le.* (*Leishmania*) *amazonensis*, *Le.* (*Viannia*) *guyanensis*, associated with cutaneous leishmaniasis, and *Le. infantum* associated to visceral leishmaniasis [1].

The association of the parasite *Leishmania* with its vector is generally “species specific,” with restrictive vector competence, although permissive vector species were also described [2, 3]. *Lutzomyia* is the largest genus of vectors present in America, with approximately 500 species, 40 of them are vectors of leishmaniasis. The classification adopted in this study was from Young and Duncan [4].

In Argentina, 28 species of Phlebotominae (23 species of *Lutzomyia*, 4 of *Brumptomyia*, and 1 species of *Oligodontomyia*) were recorded, distributed in 13 provinces, and from those were incriminated as vectors of TL caused by *Le. braziliensis*: *Lutzomyia neivai*, *Lu. whitmani*, *Lu. cortezzi* complex (*Lu. Cortezzi*-*Lu. sallesi*), *Lu. Migonei*, and *Lu. Pessoai*, the first three being reported with natural infection

of *Leishmania* or *Le. braziliensis*. The vector incriminated in VL caused by *Le. infantum* was *Lu. longipalpis* [1].

In Argentina, the reservoir of *Le. braziliensis* has not been identified yet, and no animal meets all the criteria required to be defined as such, but horses, cats, canids, and primates can become infected and have clinical manifestations [5, 6]. The dog, although highly susceptible, seems not likely to be a reservoir for transmission of *Le. braziliensis* to humans, given the low supply of parasites to the vector, although this topic has been thoroughly discussed without conclusive results yet [5, 7, 8]. By contrast, the dog was incriminated as the main reservoir of the VL in urban foci [1].

The knowledge of the mechanisms that modulates the incidence of vector-borne diseases due to changes in the environment can contribute to the planning of control strategies [1, 9, 10]. This approach is especially important for TL, as the distribution and abundance of vectors are usually the best indicators in space and time of the parasite transmission, while the analysis based on human cases could introduce errors and bias both in space and time by the asymptomatic unrecorded incidence and the dispersion of the data obtained by anamnesis, respectively [1, 10, 11]. The predictive risk maps in turn allow a proper allocation of resources and to prioritize vector control activities.

However, in this kind of spatial analysis, the outcome of any study will depend on the scale of the problem and the resolution of the data, so when remote sensing data are used, it is important to choose the correct sensor according to the objectives. This source of data is highlighted as in the field of ecoepidemiology, and the study of the impact of environmental changes on human health, the effects of land use, and land-cover changes is an increasing area of interest. In addition, these tools associated with the use of algorithms allow modeling and predictions based on different environmental variables like indicators [12–18].

Therefore, the research goal of this paper is to offer examples of applications of objective spatial-based tools, in order to assist in the selection and interpretation of the scales of analysis, describing different studies from diverse scenarios of transmission of leishmaniasis in Argentina.

2. Materials and Methods

In this work, spatial and temporal scales were used of increasing size and successively inclusive, and consequently, the results are presented in the different scales of analysis: microscale (microfocal), mesoscale (locality or epidemic focus), and macroscale (regional).

2.1. Study Area. The researches were carried out in an endemic area for TL or VL, and the sites corresponded to three major ecoregions: the Yungas subtropical forest, dry and humid Chaco, and Paranaense forest (Figure 1).

Traps were usually located in the “worst scenario,” an operational definition for the site within the study area, most likely to find phlebotomine due to habitat conditions. Thus, in the context of these designs for spatial analysis and environmental-driven changes in population abundance of

vectors, it has more biological significance than a spatial centroid [19, 20]. The specific place and habitat selected in each study depends on the specific objectives of each one (detailed in the results).

2.2. Sampling of Phlebotomine. In general, the insects were captured from afternoon to the following morning by light CDC traps (from 17.00 to 9.00 h) [21]. The sampling effort is detailed in subsequent items for each stage and area of study. In the laboratory, phlebotominae were separated from other insects and then were clarified and mounted for identification. The determination to species level according was made with the key of Young and Duncan [4], modified by Filho et al. [22] to *Lu. neivai*.

2.3. Microfocal Scale. Two studies are developed for microscale analysis: the first addressed the problem of “edge effect,” and it was aimed to define the dynamics and distribution of vectors of TL in an area soon to be deforested; the second work was related to the study of the distribution of risk associated with vector abundance and the characterization of “worst scenarios.”

2.3.1. Edge Effect. In the last two decades, it was speculated about the effect that deforestation has on vector-borne diseases. Thus, in order to evaluate whether “deforestation” influences the dynamics and distribution of TL vectors, an experimental sampling design in an area prone to be deforested was developed. The deforestation is associated to crop culture highly technified, with regular procedures along the year, and without human settlements, so the deforestation process-induced interface was assumed as the main anthropical-driven intervention in the area [23]. The study area was in northern Argentina, in the hyperendemic area of TL produced by *Le. braziliensis*, and corresponds to the ecoregion of the Yungas subtropical forest (Figure 1). The area had a surface of 1000 × 500 m, where three sets of 5 traps separated by 100 m were placed; two sides of the polygon were in contact with primary vegetation, and the other two sides were in contact with soybean crops (Figure 2). A total of 20 monthly samplings were made including all the seasons. For analysis, we used two secondary matrices, one of them with distance measurements (value in meters from the edge of each trap crop) and the other matrix with the meteorological variables (daily). Twelve meteorological variables were selected based on bibliography of studies made in the same area (minimum, maximum, and mean temperatures [°C]; total precipitation [mm]; mean relative humidity [%]; atmospheric pressure [mbar]; mean visibility [km]; mean wind speed [km/h]; maximum sustained wind speed [km/h]; total number of rainy days per month; total number of stormy days per month; total number of foggy days per month) and were provided by a meteorological station located 4.3 km from the study area, and the Oran Aero station located 25 km from there. To estimate the community of species and the influence of meteorological variables, nonmetric multidimensional scaling (NMDS) and Kendall’s correlation coefficients are used. The multivariate

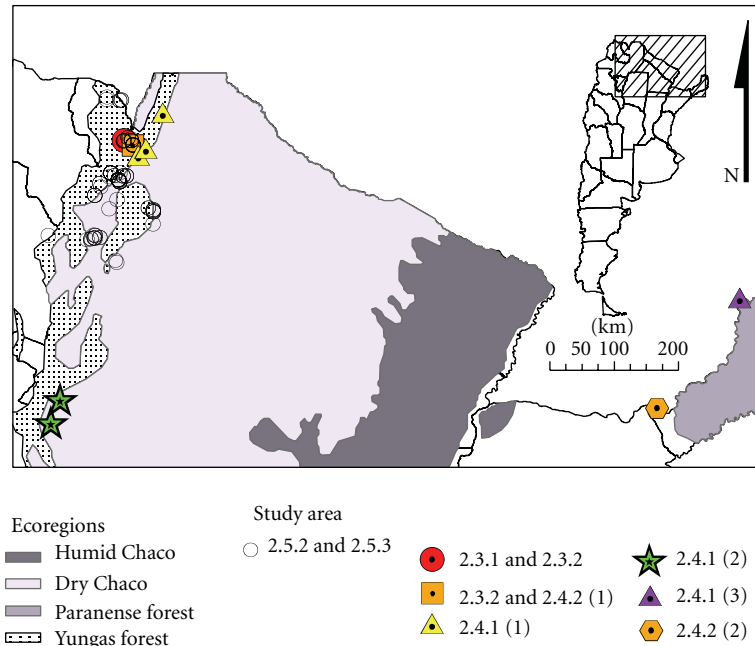


FIGURE 1: Areas of study in Argentina.

analyses were made using PC-ORD 5.0 [24] and univariate analyses [25]. All statistical tests were considered significant at $P < 0.05$.

2.3.2. Risk Distribution/Abundance/“Worst Scenario”. To assess the distribution of risk at microscale, traps were placed in periurban areas in all the possible shelters and sources of blood intake for the vectors in the *Le. braziliensis* in the TL hyperendemic area [26]. The study was performed in the city of Oran distant 22 km from the study area of point 1 (Figure 2). Oran is the most populous city in the area and has the highest incidence of TL in Argentina. The captures were performed during the season of transmission (five consecutive days) and in the other season of vector activity (four consecutive days).

The traps were placed on sites considered “worst scenario,” and five variables related to rainfall and temperature were considered (accumulated precipitation, maximum and minimum temperature, night sample without precipitation, and night with drizzles). Meteorological data were obtained from the National Weather Service and Oran Aero station. Test of independence was performed, in order to determine whether or not there are significant differences between the sampling sites based on the abundance of different species, proportion of females/males, and the percentage of pregnant females. Chi-square test was performed, taking the abundance of vector species or its females as an indicator of the distribution of the spatial probability of human-vector contact during interepidemic periods. All statistical tests were considered significant at $P < 0.05$.

Sex ratios (female: male) were computed only when the total capture had more than 15 individuals.

2.4. Focal Scale. The mesoscale section presents four works related to landscape changes caused by deforestation, fragmentation, and urbanization. The space-time pattern of phlebotomine is studied during the inter-epidemic period or during the period of increasing human cases of TL or VL in urbanized areas, associated with environmental variables. These spatial-based studies are focused on the vector abundance as indicator of risk due to vector-human effective contacts distributed at foci scale. To have a more appropriate approach to the actual transmission scenario, the infection rate and density of vectors should also be taken into account, but during inter-epidemic periods, the infection is usually very low, besides the fact that the trap-associated captures are representative of a very small area, so the data interpretation of density or infection rates could have microfocal-based biases at mesofocal scale.

2.4.1. Landscape Modification/Deforestation/Fragmentation. (1) The study area corresponds to three municipalities in the northern province of Salta (Pichanal, Embarcación, and Mosconi) (Figure 1) and corresponds to the ecoregions of Yungas and areas of transition to dry Chaco region [27]. The sites are categorized as primary forest, secondary forest, xeric woodlands, periurban, and rural areas. To know the space-temporal pattern of Phlebotominae during a TL inter-epidemic period, captures were made in different environments [28]. The distribution of phlebotomine abundance between the categories of the environment was tested (chi-square).

Three captures were made in each site between October and November. Time series analysis was performed additionally, with the data from one rural and one periurban site in each municipality (the one with more phlebotominae),

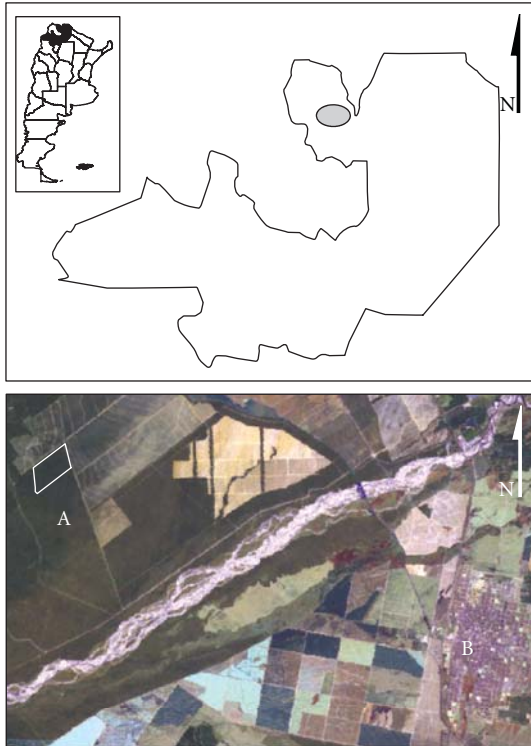


FIGURE 2: Study area. Landsat 5TM satellite image (231/076, composition bands 752, 18/09/2006), provided by National Commission on Space Activities (CONAE).

once a week (year 1), or once every two weeks (year 2) for a 130-week period beginning in October. The meteorological variables (rainfall, mean temperature) were obtained from Cargill SA and station Oran Aero. All statistical tests were considered significant at $P < 0.01$.

The abundance of phlebotomine was estimated using the William's geometric mean. Time series statistics were computed by the SYSTAT software package [29]. Autoregressive integrated moving average (ARIMA) models and multivariate general linear stepwise models ($P = 0.15$ including covariance) were tested [29, 30].

(2) After an increase of reported cases of TL in Alberdi, Tucumán province, the distribution of phlebotomine and cases was studied in a cross-sectional survey [31]. The study focus was made in the city of Alberdi and surroundings (Figure 1). The collections were made at 10 sites with previous reported cases; the traps were placed overnight on two consecutive nights. The sites were classified according to its potential habitats for phlebotomine in an empirical scale from 1 to 4, which was obtained by adding the following dichotomous attributes (presence 1, absence 0): (a) river or stream at a distance < 200 m; (b) land of about 5 m, surface shaded $> 70\%$; (c) patches of trees of at least 15 m wide and 5 m in length or a dense patch of sugar cane of 2 m diameter; (d) dwelling or rest place for domestic animals (pigs, goats, horses, and chickens) or a site with minimal disturbance of the vegetation of 50 m^2 . Differences between categories were

evaluated with Fisher's exact (with smaller samples) and Chi-square tests. All statistical tests were considered significant at $P < 0.05$.

Meteorological data were obtained from the National Weather Service, Argentina Air Force, and a station from Tucumán capital. The sequential satellite images (Landsat 7 TM) were obtained from the National Commission on Space Activities (CONAE). The case records were obtained from the Hospital of Concepcion and the Provincial Health System of the province of Tucumán (SIPROSA).

During the year following the outbreak described previously, it was reported again the increase of cases of TL in the two nearest (northern) departments of the department of Alberdi, Simoca, and Monteros, in the province of Tucumán [32]. The captures of phlebotomine were made in 29 sites with antecedent of recent cases of TL in the two above-mentioned departments (Figure 1). The environments were characterized as residual vegetation and periurban-vegetated peridomestic habitats related to TL cases. The differences between sites were tested with the same statistical tests used in the previous cross-sectional study. The case records were obtained from the Hospital of Concepcion and the SIPROSA.

(3) The work was realized in a recently deforested area in Iguazú, province of Misiones (Figure 1), where farms of approximately 100×400 m were made on the edge of patches of remnant primary or secondary forest. There, the traps were placed in homes and animal pens of three farms from June 2006 to March 2008, with an average periodicity of 15.6 days [33]. The mean abundance (Wilcoxon test) and the composition of the community (test of independence) were compared in homes and in the pens. To study the association between the abundance obtained in each environment in each sample, a Spearman correlation analysis was performed on the average of the three farms and for each farm individually. The association (Spearman) between the abundance of the most abundant species and environmental variables (minimum temperature, daily average, and maximum daily rainfall) was studied. This analysis was done on the abundance obtained at the day of sampling and applying a delay up to 10 samples (156 days). All statistical tests were considered significant at $P < 0.05$.

2.4.2. Urbanization. (1) In the study area, the community had a perception of urban transmission although the biological antecedents (Section 2.4.1 (1)) suggested a different pattern, an usual misperception also observed in other foci [34]. In order to solve this contradiction, captures were performed to assess the relative abundance of vectors in different habitats, as an indicator of the spatial distribution of the probability of human-vector contact during the inter-epidemic period in the urban surrounding [26]. *Lutzomyia* captures were made in the city of Oran (Figure 1) (previously described in point 2) and its surroundings during the two stations of highest activity of phlebotomine (Fall and Spring) in 12 sites identified as "worst scenario" [19, 20]. The variables selected were accumulated precipitation, maximum and minimum temperature, night sample without precipitation, and night with drizzles, obtained from the National

Weather Service and Oran Aero stations. To determine differences between the sampling sites, chi-square tests were performed based on the abundance of species, proportion of females/males, and the percentage of pregnant females. All statistical tests were considered significant at $P < 0.05$.

(2) In order to study the spatial distribution of *Lu. longipalpis* in the city of Posadas-Garupá in northeastern Argentina (Figure 1), just after the first autochthonous case of VL was reported, the city was divided into areas of 400×400 m [35]. Each trap was placed in a peridomestic habitat, with the criteria for “worst scenario” in each area [19, 20]. To study the spatial dependence of the abundance of *Lu. longipalpis*, a semivariogram using the spherical function was performed. The abundance of *Lu. longipalpis* was interpolated for the whole study area from the sampling points, using a common *Krigging* procedure [36–39].

A total of 35 environmental variables were selected based on vector biology and variables associated with the risk of VL reported in the literature [40–45] for the 305 sampled homes, and its possible role was evaluated as indicator of the abundance of *Lu. longipalpis*. The environmental variables were obtained based on mapping (tree coverage and water courses), census variables (demographic and socioeconomic level indicators, with resolution of census tract, INDEC 2001) and surveyed at the point of sampling (presence of chickens). A chi-square test was performed for the presence of chickens in houses with and without phlebotomine: (1) considering all dwellings with at least one sand fly captured and (2) considering only those households with medium and high abundance (>30 sand flies). In order to analyze the association of *Lu. longipalpis* with environmental variables, multiple regression analysis, stepwise forward [46] using generalized linear models (GLMs), was performed [47, 48]. All statistical tests were considered significant at $P < 0.05$.

Samplings were repeated in 2009 in order to compare the spatial distribution of the vector between 2007 and 2009. The methodology used for spatial dependence and interpolated abundance of *Lu. longipalpis* was the same as described in the paragraph above. The total surface occupied by areas of medium and low abundance between years (>60 individuals and <30 – <60 individuals, resp.) was computed. Although the environmental analysis methodology for 2007 and 2009 samples was the same, the environmental variables in the last year were surveyed at microscale level (35 environmental variables for each sampled house) [49].

2.5. Regional Scale. The macroscale research about the distribution of phlebotomines in areas of endemic transmission was performed developing qualitative risk maps and models of potential distributions.

2.5.1. Distribution of Phlebotominae in Endemic Area/Prevalence of Different Species. To develop phlebotomine species presence/absence and relative abundance maps, all published and unpublished records obtained by researchers of the Research Network of Leishmaniasis in Argentina (REDILA) [50], in which the authors are members, were used. The epidemic outbreaks reported in Argentina since 1985 were

incorporated on the ecoregions maps according to the place and year of the main focus. Table 1 shows the records of Phlebotominae present until today in Argentina [10]. *Lutzomyia longipalpis* was recorded since 2004 in the province of Formosa [51].

2.5.2. Qualitative Risk Maps. A risk map was developed from captures made in the provinces of Jujuy and Salta (Figure 1) [56]. To assess differences in species diversity and their abundance were used Fisher’s exact (sample sizes small) and chi-square tests. All statistical tests were considered significant at $P < 0.05$.

Categories were defined with different risks of transmission, based on the abundance and diversity of phlebotomine captured.

2.5.3. Maps of Potential Distribution. In the endemic TL area, in northwestern Argentina, the potential distributions of *Lu. neivai* and *Lu. migonei* [56] were modeled from previous records. The analysis of ecological niche modeling was performed with the algorithm of maximum entropy distribution, MaxEnt (maximum entropy modelling system) program. The study included 19 bioclimatic variables (<http://www.worldclim.org/>) with a resolution of 30 arc-seconds, and each cell is in fact a square of approximately 1 kilometer side ($0.93 \times 0.93 = 0.86$ km²). The same resolution was used for the digital elevation model (Shuttle Radar Topographic Mission-SRTM, <http://glcf.umd.edu/data/srtm/>). Jackknife tests were conducted to see which variables are more influential in building models. The model was evaluated with the values of the ROC (receiver operating characteristic) and validated the models with the Kappa index. At the country level, it was modeled on a preliminary distribution of the vector incriminated in the transmission of *Le. infantum*-VL and *Lu. longipalpis*.

3. Results and Discussion

3.1. Microfocal Scale

3.1.1. Edge Effect. The impact of human intervention in populations of vectors of *Leishmania* in a scenario of recent deforestation [23] was observed. This hypothesis of increased risk of transmission associated with deforestation was raised also for malaria, Chagas’ disease, and other pathologies [57, 58]. *Lutzomyia neivai* had been previously incriminated as the vector in the area of study (TL) and was the most abundant species [59]. The species abundance was associated most frequently with precipitation, and therefore, with humidity, and presence/absence of rain during the sampling dates. Although some association with maximum temperatures was found, this factor would not by itself be able to explain the changes in abundance (Table 2).

The Kendall correlation analysis showed a significant negative strong association between the distance from each trap to the edge of the crop and axis 1 (Kendall Tau = 0.48, $N = 15$, $P < 0.01$). Thus, the traps that were located

TABLE 1: Species of Phlebotominae registered by provinces captured by the researchers from the Research Network of Leishmaniasis in Argentina (REDILA), Argentina.

| N | Species | Provinces | | | | | | | | | | | | |
|----|-----------------------------|---------------------------|-----------|-------|---------|------------|---------|-------|----------|-----------|-------|---------------------|----------|---------|
| | | Buenos Aires | Catamarca | Chaco | Córdoba | Corrientes | Formosa | Jujuy | Misiones | Río Negro | Salta | Santiago del Estero | Santa Fe | Tucumán |
| 1 | <i>Lu. alphabeticata</i> | | | | | | | | | | | | | |
| 2 | <i>Lu. aurauensis</i> | | | | | | | X* | | | | | | |
| 3 | <i>Lu. bianchigalataiae</i> | | | | | | | X | | | | | | |
| 4 | <i>Lu. cortelezii</i> | X* | | X | | X | X | X | | X | X | X | | X |
| 5 | <i>Lu. evandroi</i> | | | | | | | X | | | | | | |
| 6 | <i>Lu. fisheri</i> | | | | | X | | | | | | | | |
| 7 | <i>Lu. lanei</i> | | | | | | | X | | | | | | |
| 8 | <i>Lu. longipalpis</i> | | | | | X | | X | | | | | | |
| 9 | <i>Lu. migonei</i> | | X | X | X | X | X | X | | X | X | X | | X |
| 10 | <i>Lu. misionensis</i> | | | | | X | | X | | | | | | |
| 11 | <i>Lu. monticola</i> | | | | | X | | X | | | | | | |
| 12 | <i>Lu. neivai</i> | | X | X | | X | X | X | | X | X | X | | X |
| 13 | <i>Lu. oswaldoi</i> | | | | | | | X | | | | | | |
| 14 | <i>Lu. pascalei</i> | | | | | | | X | | | | | | |
| 15 | <i>Lu. peresi</i> | | | X | | | | | | | | | | |
| 16 | <i>Lu. pessoai</i> | | | X | | X | | X | | | | | | |
| 17 | <i>Lu. punctigeniculata</i> | | | | | | | | | X | X | | | |
| 18 | <i>Lu. quinquefer</i> | | | X | | | | X | | X | X | | | |
| 19 | <i>Lu. sallesi</i> | | | X | | X | | X | | | | | | |
| 20 | <i>Lu. shannoni</i> | | | X | | | | | X | | | | | X |
| 21 | <i>Lu. sordellii</i> | | | X | | X | | | | | | | | |
| 22 | <i>Lu. torensis</i> | | | X | | | | | | | | | | |
| 23 | <i>Lu. withmani</i> | | | | | X | | X | | | | | | X |
| | | <i>Brumptomyia</i> | | | | | | | | | | | | |
| 24 | <i>Br. avellari</i> | | | X | | X | | X | | | | | | |
| 25 | <i>Br. brumpti</i> | | | X | | | | X | | | | | | |
| 26 | <i>Br. guimaresi</i> | | | | | X | | X | | X | | | | |
| 27 | <i>Br. pintoi</i> | | | | | | | X | | | X | | | X |
| | | <i>Oligodontomyia</i> sp. | | | | | | | | | | | | |
| 28 | [52] | | | | | | | | | | | | X | |

* Historical references, previous to 1960 [53–55].

TABLE 2: Coefficients of Kendall correlations between the values of dates on the NMDS axes based on species composition and meteorological variables ($n = 40$).

| Variable | Axis | Kendall Tau |
|----------------------|------|-------------|
| Maximum temperature | 1 | 0.281** |
| | 2 | 0.309*** |
| Minimum temperature | 1 | 0.045 |
| | 2 | 0.198 |
| Precipitation | 1 | -0.377*** |
| | 2 | -0.124 |
| Atmospheric pressure | 1 | -0.081 |
| | 2 | -0.191 |
| Relative humidity | 1 | -0.256** |
| | 2 | -0.113 |
| Mean visibility | 1 | 0.242* |
| | 2 | 0.321*** |
| Mean wind speed | 1 | -0.109 |
| | 2 | 0.060 |
| Maximum wind speed | 1 | 0.025 |
| | 2 | 0.060 |
| Rainy day | 1 | -0.280** |
| | 2 | 0.035 |
| Foggy day | 1 | -0.086 |
| | 2 | -0.052 |

Note: * $P < 0.05$, ** $P > 0.01$, and *** $P > 0.001$.

on the edge of primary vegetation-crop cultures captured a significantly greater number of vectors (mainly *Lu. neivai*) than the traps placed in less disturbed areas. Further, it was found that small modifications of the landscape (deforestation logging) produce an immediate increase in abundance of phlebotomine. The increase in vector abundance occurred at the interface regardless of the presence of human settlements close to the primary vegetation patch, perhaps due to accumulation of potential food sources (granivorous, synanthropic rodents, and richness of niches in the ecotone). The supply of food can make even that occasional wild reservoirs visit peridomestic environment, as observed with sloths [60]. But this effect is in turn magnified when the man is installed with its pets close to the interface [61] then generating a source of permanent shelter and food for the phlebotomine concentrate on the interface.

3.1.2. Risk Distribution/Abundance/“Worst Scenario”. In this microfocal study 22 km from the area studied in Section 1, in the periurban-ruralized border of Oran city, significant differences were observed in phlebotomine abundance according to the distance between the capture site and the nearest patch of dense vegetation (extradomestic), and between the trap and the distance to the site where an eventual food source or shelter for vector resting was concentrated. The range of simultaneous captures varies from 1 to 3000 *Lu. neivai* individuals between sites 50 m apart from each other (Table 3). In addition to the spatial variation, the variation between days showed that temporary

shelters, located between source populations of vectors and the food source on the track of the odor plume of attraction, can hold numerous phlebotomine if animals occasionally overnight there or the weather conditions are adverse to the blood-seeking flight of *Lu. neivai*. Further, the abundance of sand flies appears to be more sensitive to the variable climate in peridomestic habitats than in patches of secondary vegetation. Thus, the heterogeneity of vector abundance in time and space on a small scale should be taken into account in assessing the representativeness of sampling monitoring according to the habitat microheterogeneity [62].

3.2. Focal Scale

3.2.1. Landscape Modification/Deforestation/Fragmentation.

(1) In the hyperendemic area of northwestern Argentina, the results of the longitudinal capture (130 weeks, 45,000 phlebotomines) during the inter-epidemic period just after the first recorded TL outbreak suggested a metapopulation structure dynamics of phlebotomine, by a time series analysis, which found that populations in the patch of vegetation near the homes had a significant autocorrelation every 5-6 weeks (adult-adult cycle). In longer periods (double), the extradomestic population had cross-correlation with the peridomestic population, so periodic settlements of local extinguishable populations (peridomestic) from source populations (extradomestic) were proposed. The vector *Lu. neivai* [59] showed a positive association with rainfall in previous years, due to the generation of new eventual breeding sites and also showed an association with temperature and relative humidity at 20 weeks (increased metabolic activity) (Figure 3). Thus, outbreaks can be caused by unusual rainy periods (ENSO), followed by years of moderate rainfall and temperature [63].

(2) The cases of TL in the area of JB Alberdi were clustered in time and space during outbreaks. The main transmission period of TL took place during the first half of the fall. The distribution by age and sex of the cases suggested peridomestic transmission. The spatial distribution of cases showed a strong association of risk with the gallery forest of the Marapa River. The abundance of *Lu. neivai* increased consistently with the scale of habitat type, and so was also consistent with metapopulation dynamics structure (Figure 4). Besides, positive association was observed a between the pattern of rainfall and occurrence of cases, this pattern also coincided with the volume of water observed in sequential images of a nearby dam and detected a minor contribution of the band in the green, which is associated with change in land use or less plant growth (Figure 5). Further, in a close dam area (potential source population), a significant decrease in the population of the colony of insectivore's bats, blood supply to the phlebotomine was produced, so this could have affected the dispersion of vectors.

In the captures performed in Simoca and Monteros departments, the most abundant species were *Lu. neivai* (57%) and *Lu. migonei* (42%) (Table 4). *Lutzomyia migonei* is primarily zoophilic and could act as a “hinge” between the zoonotic cycle and the human cases or maintain the

TABLE 3: *Lutzomyia neivai* (female/male) captured by night and site, Oran, Salta, Argentina.

| Sites | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Total <i>Lutzomyianeivai</i> | F : M | GF% | Total <i>Lx</i> |
|-------|-----------|--------|-------|---------|-------|------------------------------|-------|-------------------|-----------------|
| A1 | 1901/1084 | 87/54 | 1/0 | 6/10 | 74/18 | 2069/1166 | 1.8 | 0.9 ^a | 6/2 |
| A2 | 232/237 | 32/31 | 0/0 | 1/2 | 6/3 | 271/273 | 1.0 | 3.0 ^b | 1/0 |
| A3 | 293/146 | 81/157 | 0/0 | 7/4 | 33/12 | 414/319 | 1.3 | 2.7 ^b | 10/12 |
| A4 | 164/113 | 35/53 | 0/0 | 4/2 | 12/8 | 215/176 | 1.2 | 5.6 ^c | 5/6 |
| A5 | 23/16 | 0/1 | 0/0 | 1/1 | 1/2 | 25/20 | 1.2 | 12.0 ^d | 1/2 |
| A6 | 35/39 | 5/4 | 0/0 | 0 | 2/0 | 42/43 | 1.0 | 0 ^e | 2/3 |
| A7 | 13/6 | 2/2 | 0/0 | 0 | 0/1 | 15/9 | 1.7 | 13.3 ^d | 1/1 |
| A8 | 2/2 | 0 | 0/0 | 0 | 0 | 2/2 | na | na | 0/0 |
| A9 | 1/1 | 3/0 | 0/0 | 1/1 | 1/1 | 6/3 | na | na | 0/0 |
| A10 | 25/38 | 22/27 | 0/0 | 1/2 | 33/12 | 81/79 | 1.0 | 17.3 ^f | 1/0 |
| Rain | 0.0* | 0.2** | 3.0* | 1.0* | 0* | | | | |
| MT | 26.5 | 20.8 | 12.6 | 15.6 | 18.0 | | | | |
| mT | 17.3 | 18.8 | 11.7 | 10.7 | 12.0 | | | | |
| B1 | 250/240 | 55/46 | — | 296/186 | | 601/472 | 1.3 | 12.3 | 5/6 |
| B2 | 238/66 | 36/23 | 38/29 | 154/85 | | 466/203 | 2.3 | 13.1 | 7/8 |
| B3 | — | — | — | 2/0 | | 2/0 | na | na | 1/0 |
| B4 | — | 3/13 | — | 2/0 | | 5/13 | na | na | 0/0 |
| B5 | — | — | — | 1/0 | | 1/0 | na | na | 1/0 |
| rf | 0.0 | 0.0 | 0.0 | 0.0 | | | | | |
| MT | 34.0 | 26.7 | 29.5 | 35.6 | | | | | |
| mT | 16.1 | 10.0 | 10.4 | 16.9 | | | | | |

A1–A10: sites surrounding the pig pen (season of transmission, Autumn); B1–B5: sites surrounding the banana trees patch (season of vector activity, Spring); F : M: *Lu. neivai* female/male ratio; GF%: *Lu. neivai* proportion of gravid females; rf: cumulative rainfall (mm); MT: maximal temperature (°C); mT: minimal temperature (°C); na: less than 15 individuals; a, b, c, d, e, f: each letter differed significantly from the other with a $P < 0.05$; *: without rain during the night; **: drizzles during the night.

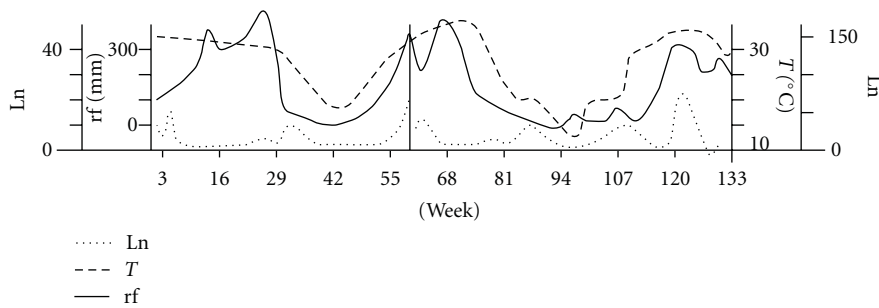
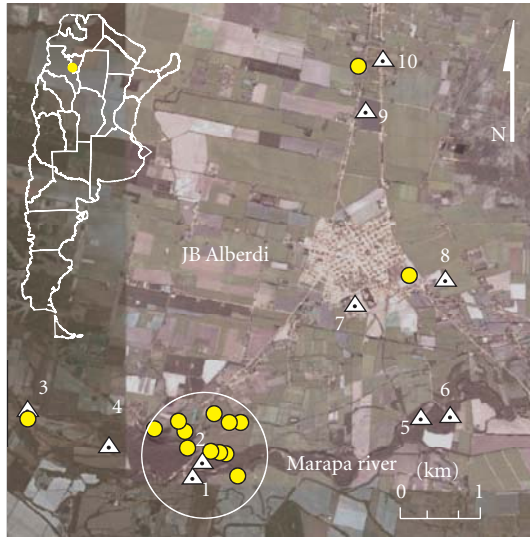


FIGURE 3: “Lowess” smoothed series *Lutzomyia neivai* captured in Salta, Argentina. Weekly (left, year 1) or every other week (right, year 2) collections. Rainfall (rf) and mean temperature (T) records during the same period.

parasite circulating in the interepidemic zoonotic cycle [64]. The foci generated during 2004 north to JB Alberdi focus showed similar characteristics in relation to the cases and the composition of Phlebotominae populations more than the mentioned JB Alberdi outbreak described in the previous paragraph, but with a distribution of vectors in space, almost exclusively peridomestic, and a broader temporal and spatial profile of cases.

(3) In the northeastern country border, close to the Iguazu waterfalls, in a recent deforested area, more than 20.000 Phlebotominae belonging to 17 species were captured associated to the TL focus. *Lutzomyia whitmani* and *Lu. migonei* represent almost the total of the captures in both

environments, and *Lu. whitmani* was the dominant species followed by *Lu. migonei*. The abundance of phlebotomine was higher in pigsties than in houses ($P < 0.0001$, 91.26 % in pigsties). *Lutzomyia whitmani* was also the dominant species (up to 98.9%) in many foci of TL in Brazil, where it was suggested to be in the process of adapting to modified environments, present in domestic (endophilic and endophagic) and peridomestic habitats, and feeding on humans, domestic and synanthropic animals [65–71]. This species was present in pigsties and houses during all the seasons, although higher abundances were observed during the warm months. The mean abundance recorded in pigsties and houses was positively associated between these sites for



- △ CDC traps
● TL cases

FIGURE 4: Study area with location of cases and the sites of capture Phlebotominae, Alberdi, Tucumán. The white circle (2 km) encloses the clustered cases and the most abundant sites with phlebotomine. Image taken from Google Earth, version 5.1.3533.1731 <http://earth.google.com/>.

Lu. Whitmani, as it was already described [67, 72]. The species is anthropophilic but also an opportunistic feeder, and so the pigsties and hen houses could provide blood, shelter, and breeding places for this vector [73–75]. In both environments, the abundances of *Lu. whitmani* and *Lu. migonei* were associated positively ($P < 0.05$ in both cases) with all the temperature variables when no delay was applied in the analysis, and this may be related to the increase of metabolism of adults. In general, this association reaches a maximum value at 31 or 47 days previous to the captures (increase of metabolism in all the stages), and it was associated positively with the daily accumulated precipitation at 31 days previous to the sampling session, but this association become in significant when the effect of the temperature variables was removed ($P > 0.05$). The precipitation may be related with the humidity of the soil and the survival of the larvae, as the lag is consistent with the phlebotomine larval period [76, 77]. However, probably precipitations are not a limiting factor in this study area, where the climate is subtropical without dry season [78], and the rains are relatively frequent along the year.

3.2.2. Urbanization. (1) The captures made around and within Oran city, in the hyperendemic TL area, showed that *Lu. neivai* was the most abundant species. In Autumn, the most important site in relation with the abundance was associated with animal dwellings with vegetation in the periurban, south-east corner border of the city, followed in abundance by a place with a smaller animal dwelling in the east edge, and two sites with secondary vegetation

TABLE 4: Phlebotominae discriminated by species and habitat (H), Simoca-Monteros, Tucumán, Argentina.

| H | T+/TT | <i>Lutzomyia neivai</i> N° (%) | <i>Lutzomyia migonei</i> N° (%) | <i>Lutzomyia cortelezzii</i> N° (%) | Total |
|----|-------|--------------------------------|---------------------------------|-------------------------------------|-------|
| T | 18/29 | 201 (57,1) | 147 (41,8) | 4 (1,1) | 352 |
| RV | 3/6 | 11 (73,3) | 4 (26,7) | — | 15 |
| PU | 15/23 | 190 (56,4) | 143 (42,4) | 4 (1,2) | 337 |

T: total per site; RV: residual vegetation; PU: periurban.

and an artificial water reservoir in the southern border of the city. In Spring, the site with highest capture rates of *Lu. neivai* was placed on the border of a neighborhood recently settled, again showing an association between the density of vegetation and the abundance of phlebotomine. Thus, there was a distribution of vectors clustered in “hot spots” outside the city, with very few individuals present in only one of five sites within the city. Therefore, as it was previously reported for *Lu. intermedia* in Rio de Janeiro [79], this distribution suggests that the risk of effective human-parasite-vector contact in urban environments during inter-epidemic periods is still associated with patches of periurban vegetation despite the urban residence cases and the perception of urban transmission by the population, even the health system agents.

(2) After the first autochthonous reported case of human VL in Argentina in Posadas city area, *Lu. longipalpis* was found in the whole Posadas city and the contiguous Garupá village (Figure 1). A total of 2,428 individuals (male/female: 3.5) were captured in 42% of sites sampled (Figure 6(a)). The mean abundance was 8.29 individuals/trap (SD 39.84, range 0:498). In addition, 8 *Lu. cortelezzii*, 3 *Lu. neivai*, and 1 *Lu. whitmani* were captured. *Lutzomyia longipalpis* showed spatial autocorrelation of 590 meters (nugget: 21 mts; maximum variance: 1893) between sites. These parameters showed no change in semivariograms to four different directions, showing isotropy. *Lutzomyia longipalpis* showed an increase in abundance towards the center of the city of Posadas, with six areas of highest abundance (>60 individuals) and other points of mean abundance (30 to 60 individuals) (Figure 6(b)). The best generalized linear model found to explain the abundance of *Lu. longipalpis* included as explanatory variables, the percentage of households with economic deprivation and materials (negative) the percentage of land area covered by trees and shrubs, and percentage of households without electricity (positive). The model explained 31.24% of the total deviance, with 293 degrees of freedom. There was a marginal association between the presences of *Lu. longipalpis* and chickens ($P = 0.07$, odd ratio: 1.53, IC₉₅: 95:0.97 : 2.44). However, when considering only positive houses with medium or high abundance (>30 individuals) and the rest as negative, this association was significant ($P = 0.02$, odds ratio: 3.26, IC₉₅: 1.217 : 8.77).

The spatial autocorrelation found in the same study area for 2009 was 688 m, a distance similar to that found in 2007. The pattern of areas of medium and highest abundance in a matrix of low abundance was also preserved. This result

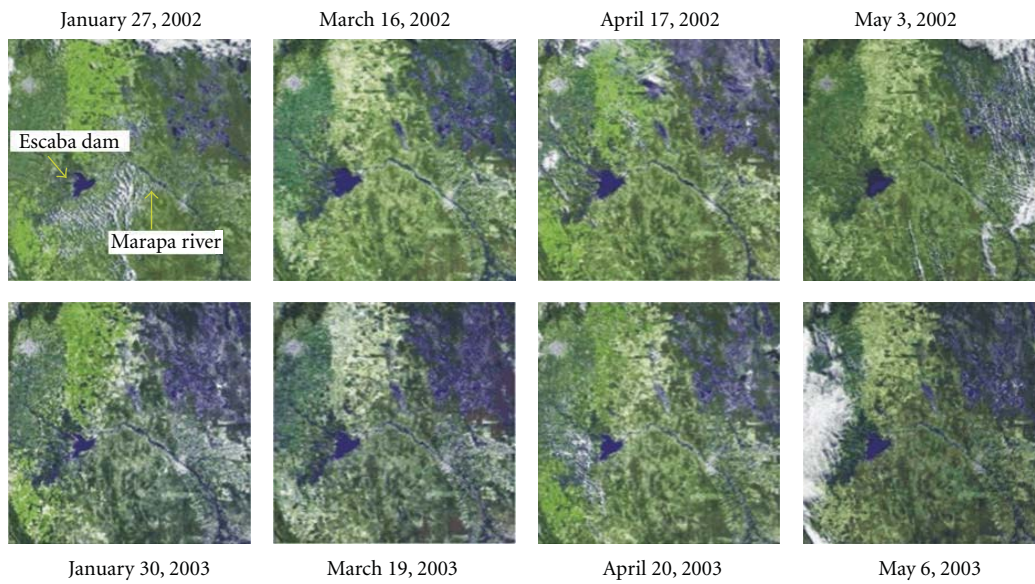


FIGURE 5: Sequential satellite images from January to March, 2002 and 2003, Alberdi, Tucumán. S103 LANDSAT 7 TM 230/79: 26° 29' 23' S, 65° 19' 48' W, 26° 46' 12' S, 65° 25' 11' W, 28° 06' 36' S, 65° 45' 00' W, 26° 23' 23' S, 65° 48' 36' W; provided by CONAE.

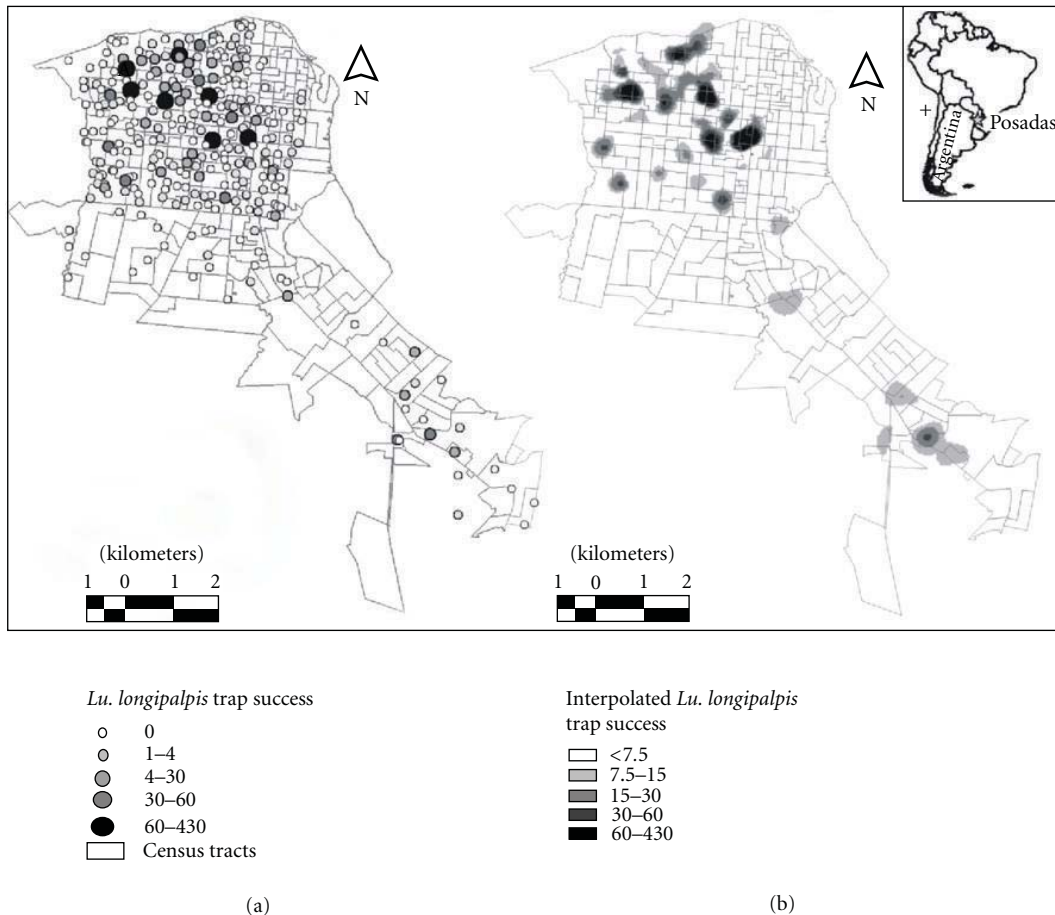


FIGURE 6: Spatial distribution of *Lu. longipalpis* in Posadas and Garupá. (a) Captures success and (b) predicted abundance interpolated by common *Krigging* procedure.

confirms the observations made previously in 2007, when the existence of microsites for the establishment of the vector in the urban environment was recorded, consistently with the heterogeneity provided by the urban landscape. This pattern of clustering of areas with high risk of transmission of VL was observed also in Brazil, based on human and canine cases of VL [80, 81]. The mean of captures was three times higher than those obtained in 2007 although the percentage of houses with *Lu. longipalpis* decreased from 2007 to 2009. Regarding the spatial pattern observed in 2009, from the areas of high and medium abundance obtained in 2007, three increased their size and only four of them maintained their position. The area occupied by patches of intermediate and high abundance increased by 6 orders of magnitude from 2007 to 2009. The environmental variables that best explained the abundance of *Lu. longipalpis* to microscale level were the proportion of area occupied by trees and shrubs within a ratio of 100 m from the sampling point, the presence of accumulated junk, cement in peridomicile, and lemon trees. The presence of cement in the peridomicile would indicate that the houses are in the urban area of the city and not in the rural periphery. The proportion of trees and shrubs in a radius of 100 m and the presence of lemon trees and junk in the peridomicile would indicate particular features for insect breeding success as shadow and available organic matter in soil and also exclude the downtown area [82].

3.3. Regional Scale

3.3.1. Distribution of Phlebotominae in the Endemic Area/Prevalence of Different Species. The first outbreak of TL in Argentina took place in the northwest of Argentina between 1985 and 1987; from this year until the 90s, there were other outbreaks in the ecoregion of the Yungas. Between 1990 and 1994 besides the outbreaks in the Yungas, the dry Chaco region began to report outbreaks. In the period 1995–1999, the humid Chaco and Paranaense region (east) were added to the outbreak records. Finally, from 2000 up to now, human TL cases clustered in time and space in 9 out of the historic 10 endemic provinces are reported periodically as small outbreaks (Figure 7(a)) [1, 10, 50, 83]. Currently, 28 species of phlebotomine were recorded distributed in 13 provinces (Figure 7(b)), and they show a variation in the prevalence (relative abundance) through the ecoregions, consistent with the different epidemiological scenarios.

- (i) Northwest: no transmission and with *Brumptomyia* sp., zoophilic dominant species.
- (ii) Yungas forest, humid Chaco, Paranaense región, and gallery forests of larger rivers of the dry Chaco, with suburban/rural outbreaks and *Lu. neivai* dominant, associated with *Lu. migonei*.
- (iii) Dry Chaco: with sporadic transmission or clustered by contact with “hot spots” and *Lu. migonei* dominant.

Northern border of Paranaense forest: with outbreaks in deforestations and *Lu. whitmani* as dominant species, this region is the richest in biodiversity.

3.3.2. Qualitative Risk Maps. The risk categories (Figure 8) differed significantly from each other:

- (i) no risk/unknown risk (R1): captures without phlebotomine or with *Brumptomyia* spp. (zoophilic species);
- (ii) sporadic transmission risk (R2): with *Lu. cortelezzii/Lu. migonei/Lu. quinquefer* (not incriminated as vector), wild environments, and cases of TL not much frequent;
- (iii) potential transmission risk/epidemic transmission risk (R3): sites with *Lu. neivai* (captures with ≤ 30 phlebotomines/trap) with environmental change, current presence of isolated cases of TL, and sites with *Lu. neivai* (30–3781 phlebotomines captures/trap) + *Lu. migonei* and current presence of clustered cases of TL.

The category R1 was restricted to the area of influence of Baritú National Park in the north of Argentina, the category R2 was recorded in the Chaco region, and finally, the category R3 with potential or actual epidemic risk was mainly located in the Yungas forest, basin of the San Francisco-Bermejo Rivers [56].

The last category happens in some areas with low human intervention of the Yungas forest, but mainly in areas heavily modified by man, with constant sources of food for phlebotomine (humans and pets). These categories were consistent with previous data of captures in 14 provinces (>100,000 phlebotomine) [1].

3.3.3. Maps of Potential Distribution. The analysis of ecological niche modeling for *Lu. neivai* and *Lu. migonei* resulted in an accuracy close to 1 (perfect [84]) of their geographical distribution. The variable that predicts more effectively the distribution of the data was “the driest month precipitation.” The rainfall influences the reproduction and breeding of this vectors, as it was observed in other studies using different time scales [28, 31, 32, 85], and constituted a significant variance in the dynamics of these insects. The validation of the prediction was more accurate for *Lu. neivai* with a high percentage of sites correctly predicted and a Kappa index with a major agreement with reality [86]. The same analytical approach was used for *Lu. longipalpis* captures of 2009–2010 and 2010–2011, showing that the risk of potential spread of the species is southward along the Uruguay River, on the Argentina-Brazil-Uruguay border (unpublished data).

4. Conclusions

The microscale analysis of the distribution of vectors of TL in northwestern Argentina allowed to verify the edge effect, with an increase of abundance at the interface between primary vegetation and crop, thus indicating one of the

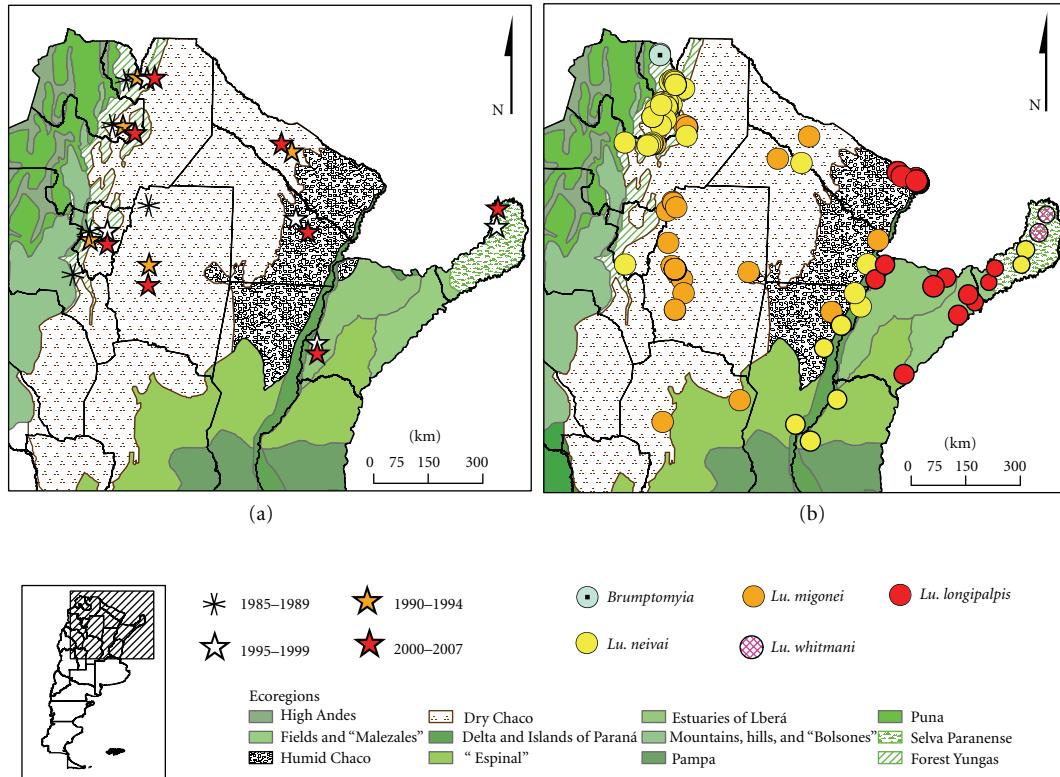


FIGURE 7: Leishmaniasis in Argentina by ecoregions. Distribution of outbreaks by site and period (a) and distribution of Phlebotominae dominant by different ecoregions with standardized captures (b). Source of Phlebotominae: captures of the Research Network of Leishmaniasis in Argentina (REDILA).

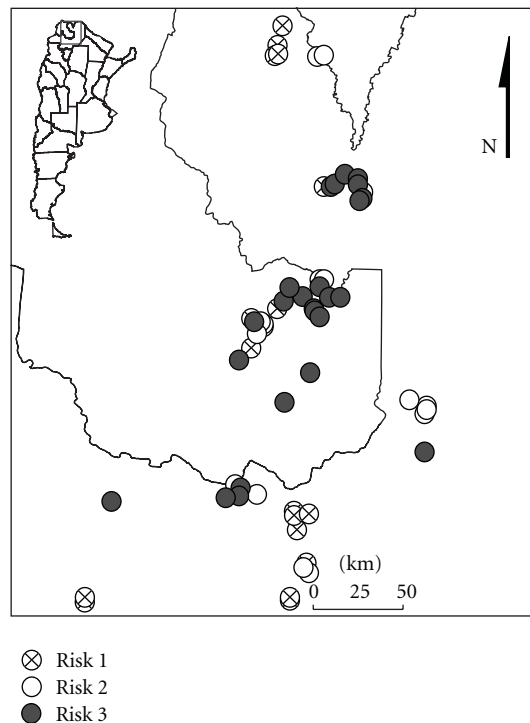


FIGURE 8: Risk categories by diversity and abundance of Phlebotominae captured in Jujuy and Salta provinces, Argentina. Image taken by Google Earth, version 5.1.3533.1731 <http://earth.google.com/>.

mechanisms by which deforestation could increase the risk of human-vector contact, and therefore the risk of transmission of the parasite. Already in the anthropic environment, the distribution and spatial orientation of food sources, physical refuges, and edge with primary vegetation determines the heterogeneous distribution of vectors just in the order of meters. This phenomenon at microscale could explain differences of risk in the nearest and apparently similar scenarios.

These results also provide evidence to the strategies based on environmental management as an alternative to intervention with insecticides, and also relativize the generalization of data from isolated captures (rates of infection, source of food, abundance, etc.).

The highest exposure or dependence of phlebotomine to weather conditions in the peridomestic area that in the adjacent natural environments suggests source populations of extradomestic vector, thus the monitoring at domicile/peridomicile reflects better the risk few meters around the capture site, while the monitoring on the edge of primary/secondary vegetation reflects the seasonal pattern of the population.

Based on these results, new questions were asked for the focal scale, whether the cross-correlations among extradomicile-peridomicile, the clustered distribution in space like “hot spots,” and the clustering in time depending on current and previous weather conditions were consistent with a metapopulation structure.

According to this metapopulation hypothesis, the source populations of vectors at sites with favorable environmental conditions for feeding and breeding would have the capability to colonize peridomestic sink populations. The landscape changes, the deforestation, fragmentation, and animal management change also the probability of recolonization and stability of the peridomestic populations, concentrating or diluting the vectors, parasites, or reservoirs, by modifications of its source or sink populations.

In this mesoscale, the climate variables were associated with the success of capture of vectors during the trapping and in previous periods to sampling, according to the modulation of adult activities (food search, attraction to light traps), but also the whole population size in the medium to long-term regulations (metabolic rate, life span, soil conditions for larval stage, etc.). These phenomena were observed for *Lu. neivai* in northwest foci and for *Lu. whitmani* in the northeast focus of Argentina, both with *Lu. migonei* as a possible “hinge” between the zoonotic/anthropo-zoonotic cycle, and with different trends to colonize human environments, according to the history of the focus and the human intervention on the landscape. However, despite this trend toward the domestic habitat, for the species listed, the alleged “urbanization” of the transmission of the etiologic agent of TL in Argentina, from the spatial and temporal study perspective, is still associated with patches of periurban vegetation, in the peridomestic-vegetation ecotone, despite the urban residence of human cases.

On the other hand, *Lu. longipalpis* is concentrated in hot spots or areas of high and medium abundance, embedded in a matrix of low abundance or absence of vectors within

the urban environment. The variables associated with these “hot spots,” by its urban feature are related to attributes of human activities (raising chickens, trees, housing conditions, and landscape of the surrounding public space). This heterogeneous pattern of abundance was maintained between years, despite the increase in human cases, with areas of highest abundance which grew in size, changed position, or generated *de novo*. These changes also suggest the possibility of a metapopulation structure, which changes for or against the reproductive success of the vectors. Further, a “hot spot” could change its size or be extinguished, acting alternatively as a source or sink population.

The results on the mesoscale support intervention strategies focused in time and space, with higher impact and cost-effectiveness than broader or regular actions against the population of vectors. The mesoscale data also show the difficulty to propose generalized recommendations such as zoo prophylaxis, since the existence of a concentration of food sources close to the man and the consequent increase in vector populations may decrease or increase the risk of human-vector contact. These contradictory outcomes could depend on differences in animal management (colonization of vectors in poultry and pens), the distance, orientation, and size of the source of blood in relation to the site of sleeping of humans, and their habits (housing characteristics, visiting hours and degree of contact with domestic animals, and synanthropic animal attraction sources).

Based in turn on results in the scale of focus, and the ability of environmental and climatic variables to predict the distribution of vectors, the hypotheses on a regional scale by developing of models based on spatial analysis were raised.

Maps of historical distribution of outbreaks and of records of vectors were generated; besides, the scenarios of transmission and phlebotomine species present by ecoregion were characterized. The overlap of scenarios and possible vectors allowed in turn develops a qualitative risk map for Argentina. And finally, from the captures data the potential distribution of the main vectors of TL and VL was modeled, defining the climatic variables that best explain the current distribution, and hence the potential distribution if these conditions change.

These studies allow a better allocation of resources, characterization of scenarios, and defining the appropriate actions in relation to human cases and reservoir (dogs-VL), according to the appearance in the area of epidemic transmission, sporadic cases, or in an area without vectors. Similarly, they allow us to anticipate by projection areas of eventual risk in space and time due to human intervention and settlement, instability, and local or global climate change.

In conclusion, spatial and temporal analysis of the distribution and abundance of TL and VL vectors in Argentina, discriminated by scales, can generate information that contributes to design strategies for prevention and control of leishmaniasis. It should be noted that the scales from microfocal to regional, although they are inclusive to each other in increasing order, require questions, resolution, data quality, and different analytical tools to support the conclusions appropriate to each scale.

Acknowledgments

The authors thank the members of the Research Network of Leishmaniasis in Argentina for their permanent collaboration: Soraya Acardi, Denise Fuenzalida, Ignacio Gould, Javier Liotta, Mariana Manteca, Mariela Martínez, Andrea Mastrángelo, Matías Parras, Juan Rosa, Cristina Remondegui, Soledad Santini, Enrique Szelag, and Lucrecia Villarquide. We appreciate the operative participation of different actors on national, provincial, municipal, and local levels in the capture of insects, as well as the residents that permit the field work. These studies were partially supported by CONICET and subsidies from FOCANLIS (ANLIS, Dr. Carlos G. Malbrán), PFIP-MINCYT, and ANPCyT.

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Review Article

Use of Indicator Kriging to Investigate Schistosomiasis in Minas Gerais State, Brazil

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Received 15 July 2011; Accepted 20 September 2011

Academic Editor: Claudia Codeço

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Geographic Information Systems (GISs) are composed of useful tools to map and to model the spatial distribution of events that have geographic importance as schistosomiasis. This paper is a review of the use of the indicator kriging, implemented on the Georeferenced Information Processing System (SPRING) to make inferences about the prevalence of schistosomiasis and the presence of the species of *Biomphalaria*, intermediate hosts of *Schistosoma mansoni*, in areas without this information, in the Minas Gerais State, Brazil. The results were two maps. The first one was a map of *Biomphalaria* species, and the second was a new map of estimated prevalence of schistosomiasis. The obtained results showed that the indicator kriging can be used to better allocate resources for study and control of schistosomiasis in areas with transmission or the possibility of disease transmission.

1. Introduction

Schistosomiasis *mansoni* is an endemic disease, typical of developing countries [1, 2]. In Brazil, the schistosomiasis is caused by the etiological agent *Schistosoma mansoni*, whose intermediate host is species of mollusk of the *Biomphalaria* genus.

The *S. mansoni* was introduced in Brazil by the African slavery trade during the sixteenth century [3]. The Brazilian port of Salvador and Recife received most of the slaves [4], originated from endemic regions. In the early eighteenth

century, there was a large migration of slave labor due to the decline of sugar production in the Northeast of Brazil and the discovery of gold and diamond in the Minas Gerais State. It is estimated that one fifth of the population at that time moved to Minas Gerais [5], using the “ways of São Francisco” [6] as the main access route. Probably, in these early migrants also came to schistosomiasis.

In Minas Gerais, there are seven species of *Biomphalaria* genus: *B. glabrata*, *B. straminea*, *B. tenagophila*, *B. peregrina*, *B. schrami*, *B. intermedia*, and *B. occidentalis* [7–9]. Come in these, only *B. glabrata*, *B. tenagophila*, and *B. straminea*

have been found naturally infected by *S. mansoni* [10, 11]. *B. glabrata* is of great epidemiologic importance, due to its extensive geographic distribution, high infection indices, and effectiveness in the schistosomiasis transmission. Moreover, its distribution is almost always associated with disease occurrence [12]. *B. tenagophila* was found naturally infected by *S. mansoni* in state of Minas Gerais, and it is responsible for the focus maintenance in the city of Itajubá [13]. *B. straminea*, although had not been found infected in state of Minas Gerais, was considered responsible for Paracatu's focus [14].

They are commonly found in a wide of habitats, both natural (streams, creeks, ponds, swamps) and artificial (irrigation ditches, small dams), particularly in shallow and slow running waters (less than 30 cm/s), where the substratum can be the muddy or rocky bed and with floating or rooted vegetation, pH between 6 and 8, NaCl content below 3 by 1000, and mean temperature between 20 and 25 degrees C [15–17].

The study of the habitat of these mollusks, as well as their behavior in relation to the climate, results in valuable information when the goal is the disease transmission control [18].

Environmental and socioeconomic factors may influence the spatial distribution of schistosomiasis. Under these circumstances, the Geographic Information System (GIS) can be applied to characterize, to better understand the interconnection of these factors, and to provide a more complete picture of disease transmission [19]. GIS allows a complex analysis of a large number of information and displays the results of this analysis in graphical maps. These techniques have become important tools for the design and implementation of control programs [20], enabling a better distribution of state resources to allow a direction more suitable for disease control [21–23]. Among these tools, we can cite the indicator kriging, which allows to data spatialization aiming at map generation. It also gives information about inference uncertainties that can be used as quality restrictions of the classification process.

This study is a review of the use the indicator kriging of the Georeferenced Information Processing System (SPRING) to make inferences about the presence of the species of *Biomphalaria* (*B. glabrata*, *B. tenagophila*, and/or *B. straminea*), intermediate hosts of *Schistosoma mansoni*. Also, using numerical indicator kriging, a new map of estimated prevalence of schistosomiasis, in areas without information in the Minas Gerais State, Brazil, is presented.

2. Methodology

Kriging may be defined as a technique of statistical inference, which allows the estimation of values and the uncertainties associated with the attribute during the spatialization of a sample property [24].

To achieve the objectives, two approaches have been considered: categorical and numerical indicator kriging. The categorical indicator kriging was based on the information of the mollusks species, and the numerical indicator kriging used data from the prevalence of schistosomiasis.

The procedure for adjustment of the semivariogram is not straightforward and automatic, but interactive, because the interpreter does the first adjustment and checks the adequacy of the theoretical model [25]. After the models fitted for each class (categorical) or quartile (numerical), the indicator kriging was applied to obtain an approximation of the conditional distribution function of random variables.

The numerical indicator kriging was conducted in the entire state using the schistosomiasis prevalence data (lower quantile, median and upper quantile) from 999 localities.

The categorical indicator kriging was performed in each of the fifteen river basins (Buranhém, Doce, Grande, Itabapoana, Itanhém, Itapemirim, Jequitinhonha, Jucuruçu, Mucuri, Paraíba do Sul, Paranaíba, Pardo, Piracicaba/Jaguari, São Francisco and, São Mateus) using the mollusk data. The mollusk attributes (class of species and localization) were distributed along the drainage network of 15 River Basins, according to the methodology used by Guimarães et al. [22]. The classes used for this study were defined as *B. glabrata*, *B. tenagophila*, *B. straminea*, *B. glabrata* + *B. tenagophila*, *B. glabrata* + *B. straminea*, *B. tenagophila* + *B. straminea*, *B. glabrata* + *B. tenagophila* + *B. straminea*, and without *Biomphalaria*. The class without *Biomphalaria* includes information about the nonoccurrence of *Biomphalaria* species or information about non-transmitter species in Brazil, such as *B. peregrina*, *B. schrammi*, *B. intermedia*, and *B. occidentalis*.

The indicator kriging was done in the software SPRING [26]. In Appendix A is described the geostatistical modeling used in the indicator kriging.

2.1. Data Set. Schistosomiasis prevalence values (P_v) were obtained from the Brazilian Schistosomiasis Control Program (PCE) through the Annual Reports of the Secretary of Public Health Surveillance (SVS) and the Secretary of Health in the State of Minas Gerais (SESMG). The PCE in Minas Gerais had its beginning in 1986, and since 2000 has been under the coordination of the SESMG in collaboration with Municipal Health Systems. The PCE prevalence information is available for municipalities and localities [18]. The Kato Katz technique is the methodology used to determine prevalence, examining one slide per person. The spatial distribution of the schistosomiasis prevalence is presented in Figure 1(a), for the 255 municipalities and for the 999 localities used in this study.

Data on the distribution of *Biomphalaria* mollusks were provided by the Laboratory of Helminthiasis and Medical Malacology of the René Rachou Research Center (CPqRR/Fiocruz-MG). Mollusks were collected in breeding places from different municipalities in Minas Gerais at different periods, using scoops and tweezers, and then packed to be transported to the laboratory [27]. Specific identification was performed according to the morphology of the shells, reproductive system, and renal ridge of the mollusks [28–33], and also by low stringency polymerase chain reaction and restriction fragment length polymorphism [34]. The spatial distribution of the *Biomphalaria* species data are presented in Figure 1(b).

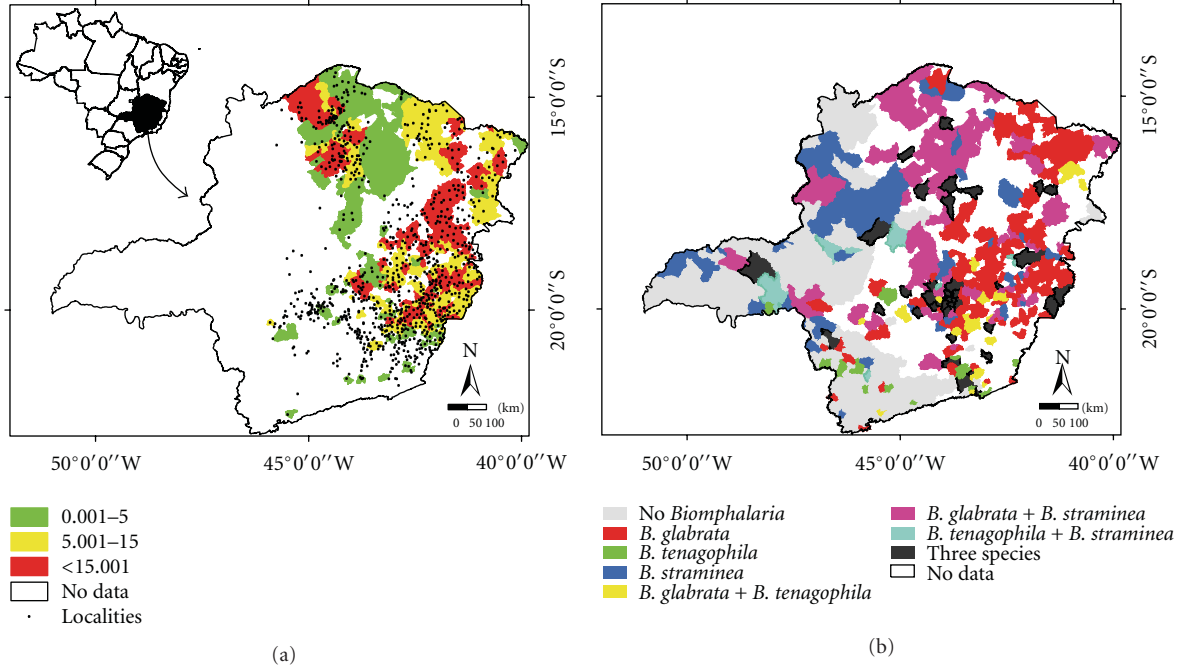


FIGURE 1: (a) Distribution of the schistosomiasis prevalence on the municipalities (%) 0.001–5.000 (green), 5.001–15.000 (yellow), and above 15.001 (red), and localities (black points); (b) distribution of the *Biomphalaria* species in Minas Gerais.

2.2. *Test of the Hypothesis for Differences between PCE and Indicator Kriging Estimated Prevalences.* To assess whether the estimates made by kriging methods were close to those obtained by PCE, initially estimates were made by municipalities, by averaging the estimated prevalence for all the grid points belonging to that municipality.

A regression line was then adjusted, using the prevalence provided by the PCE as dependent variable and the prevalence provided by kriging as independent variable, that is,

$$P_{PCE} = \beta_0 + \beta_1 P_K, \quad (1)$$

where P_{PCE} and P_K are the PCE and kriging prevalences, respectively.

A hypothesis test was then performed to determine, with a 95% confidence level, whether the intercept was zero and the slope parameter was equal to 1:

$$\begin{aligned} H_0 : \beta_0 = 0, \quad \beta_1 = 1, \\ H_1 : \beta_0 \neq 0 \quad \text{or} \quad \beta_1 \neq 1. \end{aligned} \quad (2)$$

If the null hypothesis is accepted, it can be concluded that, in average, the kriging estimates are equal to PCE prevalences at a 95% confidence level.

3. Results and Discussions

The indicator kriging procedure, based on the fitted semi-variograms, was applied using the sample data presented in Figure 1, to generate a regular grid of 250 meters of resolution (x, y) over the Minas Gerais State.

The following results were obtained to achieve the objectives.

3.1. *Using Categorical Data (Thematic).* The resulting map of the species distribution generated by applying the mode estimator (Appendix A.2.—(A.6)) is presented in Figure 2(a).

Figure 2(b) presents a map of the uncertainties associated with the classification, computed by using (A.7) of Appendix A.2. The map of uncertainties shows that the higher uncertainties are concentrated among class transition areas.

The methodology was validated using a sampling procedure. The fieldwork was conducted in the São Francisco and Paraíba do Sul River Basins where no information existed about the presence of the mollusks. More details about the results for the São Francisco and Paraíba do Sul River Basins can be found in Guimarães et al. [22] and Guimarães [18] and Tibiriçá et al. [9].

The research of mollusks was accomplished in five municipalities in the São Francisco River Basin (SFRB) and in nine municipalities in the Paraíba do Sul River Basin (PSRB). The mollusks collected were sent to the analysis of the species in the Laboratory of Helminthiasis and Medical Malacology of the René Rachou Research Center (CPqRR/Fiocruz-MG). Also, the mollusks collected in the PSRB were identified at the Parasitology Laboratory in the Federal University of Juiz de Fora (UFJF) and the Entomology Laboratory of the GRS/JF, Secretary of Health in the State of Minas Gerais (SESMG). Collection and identification of the mollusks were performed according to the methodology described in Section 2.1.

Figures 2(c) and 2(d) show the historical *Biomphalaria* species (Figure 1(b)) at the surveyed municipalities of São Francisco and Paraíba do Sul River Basins, respectively.

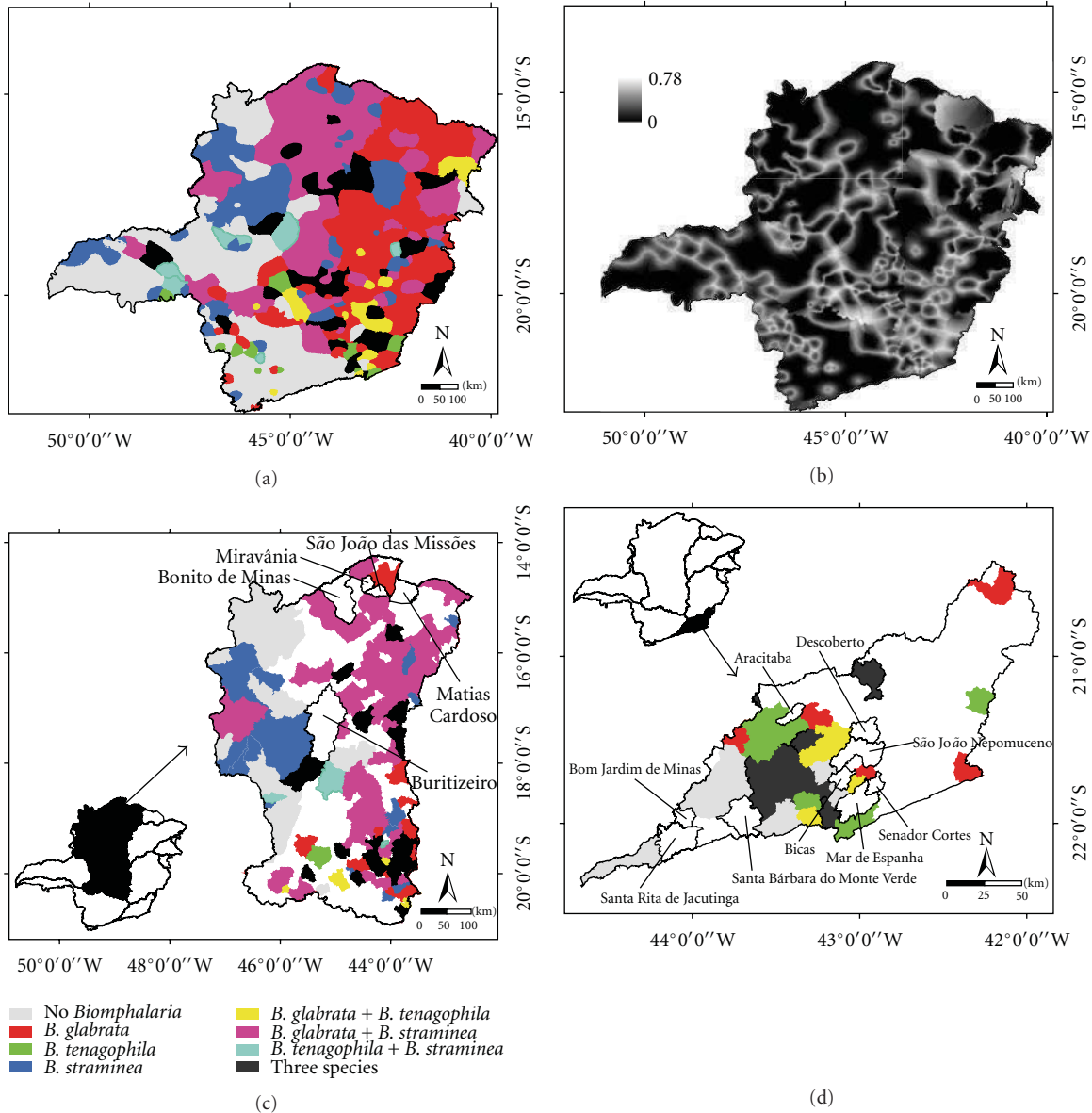


FIGURE 2: (a) Map of the estimated species distribution, (b) map of the uncertainties, (c) historical *Biomphalaria* species in São Francisco River Basin, and in (d) Paraíba do Sul River Basin. (Source: [18, 22]).

Table 1 presents the estimated and found species, as well as the value of the uncertainty mean for searched municipality, and the collection points, where these species had been found.

To explain the differences in the two basins, some considerations should be made. Figure 2 shows the spatial distribution of *Biomphalaria* species according to historical data. We can observe from this figure that the SFRB (Figure 2(c)) has a better spatial distribution of species surveyed than the PSRB (Figure 2(d)).

The municipalities surveyed in the SFRB had 100% accuracy with at least one specie estimated, but this value for the PSRB was 66.67%.

About 50% of the municipalities of SFRB have historical information about the *Biomphalaria* species in one of eight

classes (*B. glabrata*, *B. tenagophila*, *B. straminea*, *B. glabrata* + *B. tenagophila*, *B. glabrata* + *B. straminea*, *B. tenagophila* + *B. straminea*, *B. glabrata* + *B. tenagophila* + *B. straminea*, and without *Biomphalaria*). However, for PSRB, this information is only 32% of the municipalities and one of five classes (*B. glabrata*, *B. tenagophila*, *B. glabrata* + *B. tenagophila*, *B. glabrata* + *B. tenagophila* + *B. straminea*, and without *Biomphalaria*).

As kriging is affected by the amount and spatial distribution of input data, this may explain the differences in the two basins.

This fact is also reflected in the uncertainties of the estimates. Comparing the values presented in Table 1, the SFRB had an overall uncertainty mean of 0.232, and the PSRB had an overall uncertainty mean of 0.332. Therefore,

TABLE 1: List of surveyed municipalities and their attributes found in the San Francisco and Paraíba do Sul River Basins (Source: [9, 18, 22]).

| River basin | Municipalities | Estimated species | Uncertainty mean | Found species |
|----------------|------------------------------|--|------------------|---|
| São Francisco | Bonito de Minas | <i>B. straminea</i> , <i>B. glabrata</i> | 0.002 | <i>B. straminea</i> , <i>B. glabrata</i> |
| | Buritizeiro | <i>B. straminea</i> , <i>B. glabrata</i> | 0.250 | <i>B. straminea</i> |
| | Matias Cardoso | <i>B. straminea</i> , <i>B. glabrata</i> | 0.290 | <i>B. straminea</i> |
| | Miravânia | <i>B. straminea</i> , <i>B. glabrata</i> | 0.358 | <i>B. straminea</i> |
| | São João das Missões | <i>B. straminea</i> , <i>B. glabrata</i> | 0.279 | <i>B. straminea</i> |
| Paraíba do Sul | Aracitaba | <i>B. glabrata</i> , <i>B. tenagophila</i> | 0.281 | Other ^a (Bp) |
| | Bicas | <i>B. glabrata</i> , <i>B. tenagophila</i> | 0.419 | <i>B. glabrata</i> , <i>B. tenagophila</i> |
| | Bom Jardim de Minas | Without <i>Biomphalaria</i> | 0 | Without <i>Biomphalaria</i> |
| | Descoberto | <i>B. glabrata</i> , <i>B. tenagophila</i> , <i>B. straminea</i> | 0.487 | <i>B. glabrata</i> , <i>B. tenagophila</i> |
| | Mar de Espanha | <i>B. glabrata</i> , <i>B. tenagophila</i> , <i>B. straminea</i> | 0.525 | <i>B. tenagophila</i> , Other (Bp,Bo) |
| | Santa Bárbara do Monte Verde | <i>B. glabrata</i> , <i>B. tenagophila</i> , <i>B. straminea</i> | 0.302 | Other (Bp) |
| | Santa Rita de Jacutinga | Without <i>Biomphalaria</i> | 0 | <i>B. tenagophila</i> , Other (Bp) |
| | São João Nepomuceno | <i>B. glabrata</i> , <i>B. tenagophila</i> | 0.438 | <i>B. glabrata</i> , <i>B. tenagophila</i> , Other (Bp) |
| | Senador Cortes | <i>B. glabrata</i> , <i>B. tenagophila</i> | 0.535 | Other (Bp) |

^a Other: Class without *Biomphalaria*, species found in this class is not the transmitter of schistosomiasis; Bp: *B. peregrina*; Bo: *B. occidentalis*.

the overall uncertainty for PSRB is 43.1% greater than for SFRB.

3.2. *Using Numerical Data.* Figure 3 shows the spatial distribution of schistosomiasis-estimated prevalence by kriging (Figure 3(a)), map of the uncertainties (Figure 3(b)), estimated prevalence by kriging by type of classes (Figure 3(c)), and in Figure 3(d) the mean estimated prevalence by kriging for the 255 municipalities where the PCE prevalence information is available.

The PCE prevalence values (Figure 1(a)) and the respective kriging estimates (Figure 3(d)) were plotted together. Figure 4 shows the scatter plot as well as the regression line. The hypothesis test (A.12) was performed, and the null hypothesis was accepted, indicating that there is no significant difference between the PCE prevalence and the kriging prevalence means, with a significance level of 0.05.

Table 2 presents the comparison between the prevalence of PCE and the prevalence estimated by kriging by type of classes: low (prevalence among 0.001 to 5), medium (5.001 to 15), and high prevalence (above 15).

TABLE 2: Comparison between PCE and kriging prevalences for the 255 municipalities.

| | | PCE | | | Total |
|---------|--------|-----|--------|------|-------|
| | | Low | Medium | High | |
| Kriging | Low | 37 | 2 | 0 | 39 |
| | Medium | 28 | 62 | 32 | 122 |
| | High | 5 | 33 | 56 | 94 |
| | Total | 70 | 97 | 88 | 255 |

From Table 2, it can be noted that

- (i) 60.8% of the municipalities are estimated in the same class as they belong;
- (ii) 37.2% of the municipalities had the prevalence estimated in the adjacent class, that is, from low to medium class, from medium to high class, from high to medium class, or from medium to high class;
- (iii) less than 2% of the municipalities had the low prevalence estimated a high class;

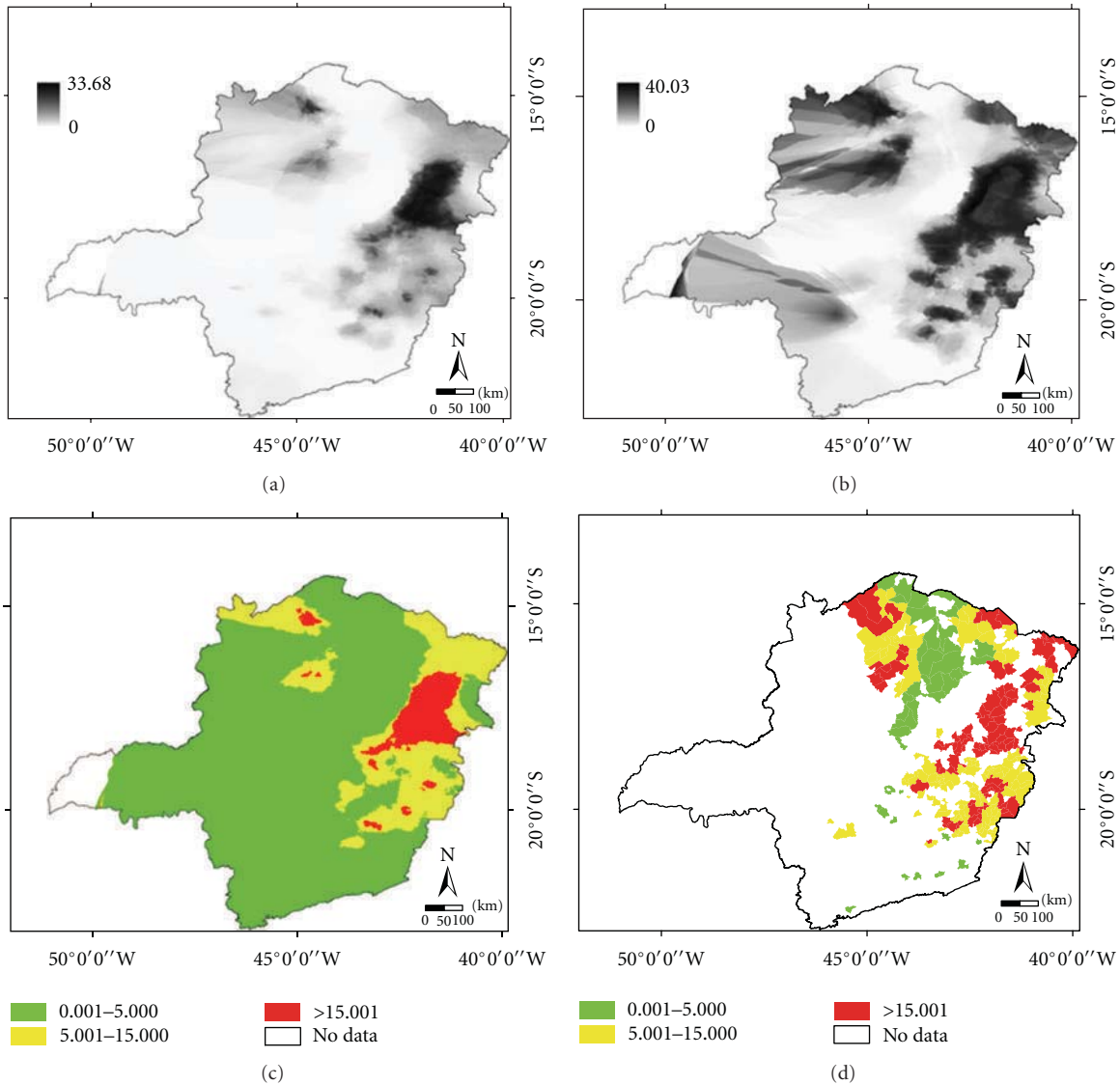


FIGURE 3: (a) Estimated prevalence by kriging, (b) map of the uncertainties, (c) estimated prevalence by kriging by type of classes, (d) mean estimated prevalence by kriging for 255 municipalities.

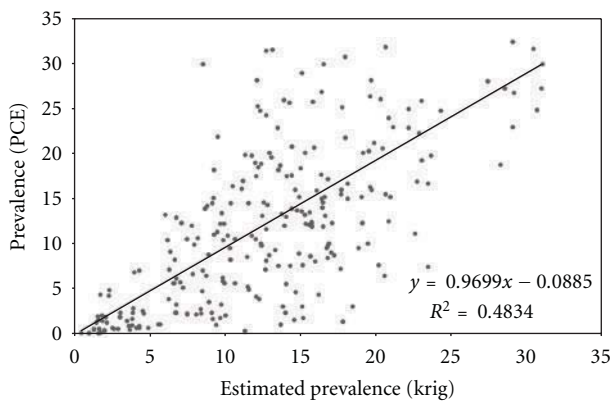


FIGURE 4: PCE- and kriging-estimated prevalences for 255 municipalities.

(iv) when the estimated class is not in the same class of the PCE, the kriging has a trend of about 25.9% to overestimate and about 13.3% to underestimate the prevalence values.

4. Conclusions and Future Work

Indicator kriging showed to be a rather robust tool since its results presented a very good agreement with the field findings. This tool allowed to determine and to delimit, respectively, the distribution of the *Biomphalaria* species and the areas of risk (map of uncertainty of the *Biomphalaria* species).

Kriging is an auxiliary useful tool to guide the fieldwork, indicating the places with higher probability of occurrence of the considered species, with particular attention to those

species that are more important for disease transmission. The results of this tool can be used to better allocate the always limited resources for distribution studies and the development of strategies for mollusk control.

Some important issues, related to the nature and precision of the *Biomphalaria* species data, need to be considered when looking at the results: the data were obtained from historical records (most occurring before the broad usage of GPS equipment), and the information is given in a municipality level basis. Because of this, an assumption was made that the species found in the municipalities are uniformly distributed inside the municipality drainage network. The authors believe, however, that other type of distribution hypothesis would not greatly affect the results.

To improve the accuracy of an estimate using kriging, it would be necessary to obtain data with better location and spatial distribution of the information collected in the fieldwork.

Also, the kriging proved to be a suitable tool, and their results showed a good agreement with the PCE data.

This technique can be used to estimate the schistosomiasis prevalence in the municipalities of Minas Gerais where the prevalence is not determined by the PCE. The results of this tool can be used to better allocate resources for studies in areas with medium and high prevalence.

The entire methodology of this study used free software allowing the playback of the methodology in other states of Brazil where there is no information about the type of *Biomphalaria* and/or schistosomiasis prevalence at no cost.

Conditioned to appropriate funds existence, an extensive malacological survey is recommended for better evaluation of the methodology and also GPS utilization in all future fieldworks.

It is also recommended to obtain data on the schistosomiasis prevalence in western Minas Gerais (nonendemic region). Thus, one can obtain a better estimate of prevalence at the state level and not only in the endemic area.

Appendix

A.

A.1. Indicator Kriging. A spatial attribute inside a region A of the earth surface can be modeled, from a geostatistical point of view, as a random function [35, 36]. For each spatial position $\mathbf{u} \in A$, the attribute value of a spatial data is modeled as a random variable (RV) $Z(\mathbf{u})$, which can assume different values with an associated probability of occurrence. In the n sample positions \mathbf{u}_α , $\alpha = 1, 2, \dots, n$, the values $Z(\mathbf{u})$ are considered deterministic, that is, they can be considered RV's with probability 100% of occurrence. The distribution function of $Z(\mathbf{u})$, conditioned to n sample points, can be defined by Deutsch and Journel [36]

$$F(\mathbf{u}; z | (n)) = \text{Prob}\{Z(\mathbf{u}) \leq z | (n)\}$$

for a numerical attribute,

$$F(\mathbf{u}; z | (n)) = \text{Prob}\{Z(\mathbf{u}) = z | (n)\}$$

for a categorical attribute.

(A.1)

The function $F(\mathbf{u}; z | (n))$ models the uncertainty about the values of $z(\mathbf{u})$, in nonsampled positions \mathbf{u} , conditioned to the n samples. This function can be evaluated from the inference procedure-denominated indicator kriging.

The indicator codification of the RV $Z(\mathbf{u})$, for a cutoff value $z = z_k$, generates the indicator RV $I(\mathbf{u}; z_k)$ using the following nonlinear mapping function:

$$I(\mathbf{u}; z_k) = \begin{cases} 1 & \text{if } Z(\mathbf{u}) \leq z_k \\ 0 & \text{if } Z(\mathbf{u}) > z_k \end{cases} \quad \text{for a numerical attribute,}$$

$$I(\mathbf{u}; z_k) = \begin{cases} 1 & \text{if } Z(\mathbf{u}) = z_k \\ 0 & \text{if } Z(\mathbf{u}) \neq z_k \end{cases} \quad \text{for a categorical attribute.}$$

(A.2)

The cutoff values, z_k , $k = 1, 2, \dots, K$, are defined depending on the number of samples. It is necessary that the amount of codified samples with value 1 are enough for defining, with success, a variography model for each cutoff value [37].

The conditional expectation of the indicator RV $I(\mathbf{u}; z_k)$ is computed by

$$\begin{aligned} E\{I(\mathbf{u}; z_k | (n))\} &= 1 \cdot \text{Prob}\{I(\mathbf{u}; z_k) = 1 | (n)\} \\ &\quad + 0 \cdot \text{Prob}\{I(\mathbf{u}; z_k) = 0 | (n)\} \\ &= 1 \cdot \text{Prob}\{I(\mathbf{u}; z_k) = 1 | (n)\} \\ &= F^*(\mathbf{u}; z_k | (n)). \end{aligned} \quad \text{(A.3)}$$

This estimation, made through ordinary kriging over the indicator values, yields a least-square estimate for the distribution function at the cutoff value z_k [36]. A set of $F^*(\mathbf{u}; z_k | (n))$ estimates, obtained for different k cutoff values, can lead to an approximation of the conditional distribution function (cdf) of $Z(\mathbf{u})$.

The expression of the ordinary indicator kriging estimator is given by:

$$F_O^*(\mathbf{u}; z_k | (n)) = \sum_{\alpha=1}^{n(\mathbf{u})} \lambda_{O\alpha}(\mathbf{u}; z_k) i(\mathbf{u}_\alpha; z_k). \quad \text{(A.4)}$$

The weights $\lambda_{O\alpha}(\mathbf{u}; z_k)$ are obtained by solving the following system of equations:

$$\sum_{\beta=1}^{n(\mathbf{u})} \lambda_{O\beta}(\mathbf{u}; z_k) C_1(h_{\alpha\beta}; z_k) + \phi(\mathbf{u}; z_k) = C_1(h_{\alpha\alpha}; z_k)$$

$$\forall \alpha = 1, 2, \dots, n(\mathbf{u}), \quad \text{(A.5)}$$

$$\sum_{\beta=1}^{n(\mathbf{u})} \lambda_{O\beta}(\mathbf{u}; z_k) = 1,$$

where $\phi(\mathbf{u}; z_k)$ is a Lagrange parameter, $h_{\alpha\beta}$ is the vector defined by the positions \mathbf{u}_α and \mathbf{u}_β , h_α is the vector defined by the positions \mathbf{u}_α and \mathbf{u} , $C_1(h_\alpha; z_k)$ is the autocovariance defined by $h_{\alpha\beta}$, and $C_1(h_\alpha; z_k)$ is the covariance defined by h_α . The autocovariance is determined by the theoretical variography model defined by the set I when $z = z_k$.

The indicator kriging, simple or ordinary, gives, for each cutoff value k , an estimator which is also the better least square estimator of the conditional expectation of the RV $I(\mathbf{u}; z_k)$. Using this property, it is possible to compute estimates of the cdf values of $Z(\mathbf{u})$ for several values of z_k , belonging to the $Z(\mathbf{u})$ domain. The set of the estimated values of the cdf's of $Z(\mathbf{u})$, for the cutoff values, is considered a discrete approximation of the real cdf of $Z(\mathbf{u})$. The greater the number of cutoff values, the better the approximation.

The indicator kriging is nonparametric. It does not consider any type of a prior distribution for the random variable. Instead, it permits the construction of a discrete approximation of the cdf of $Z(\mathbf{u})$. The values of the discrete probabilities can be directly used to estimate the values that characterize the distribution, such as average, variance, mode, and quantiles.

A.2. Estimation and Uncertainty Evaluation for Categorical (Thematic) Data. The optimal estimation $z^*(\mathbf{u})$, for a categorical attribute represented by K classes c_k , $k = 1, \dots, K$, can be defined as

$$z^*(\mathbf{u}) = c_k \text{ iff } p_k(\mathbf{u}) > p_i(\mathbf{u}) \quad \forall i, k = 1, \dots, K, i \neq k, \quad (\text{A.6})$$

where $p_k(\mathbf{u}) = F^*(\mathbf{u}; z_k | (n))$, when $z_k = c_k$, is the estimated probability of the class k at the location \mathbf{u} . This estimator is known as the mode estimator since it considers the highest probability, and the classifier that assigns classes at each position \mathbf{u} using the mode estimator is known as mode classifier.

The species classification probability for each position, inside the region of interest, is obtained with the values for indication (0 or 1) of the neighboring known samples (nearer) of \mathbf{u} . This probability is estimated by using ordinary kriging, where the weighted value of each neighboring sample of \mathbf{u} is calculated considering the variability model defined by the semivariograms for each class.

Usually, inferences about local uncertainties for categorical attributes, $\text{Unc}(\mathbf{u})$, are made using the complement of the mode probability, which is defined as

$$\text{Unc}(\mathbf{u}) = 1 - \text{Max}_{k=1}^K [p_k(\mathbf{u})]. \quad (\text{A.7})$$

The mode estimator, as defined in (A.4), has the advantage of classifying all locations \mathbf{u} inside the region of interest. An alternate methodology consists in considering the uncertainties as restrictions to the classification process.

In this case, only locations with an uncertainty below a predefined threshold (U_{max}) are classified, that is

$$z^*(\mathbf{u}) = \begin{cases} c_k \text{ iff } (p_k(\mathbf{u}) > p_i(\mathbf{u}) \wedge \text{Unc}(\mathbf{u}) < U_{\text{max}}) \\ \forall i, k = 1, \dots, K, i \neq k, \\ \phi \text{ otherwise,} \end{cases} \quad (\text{A.8})$$

where $z^*(\mathbf{u}) = \phi$ means that the value was not estimated or the location was not classified.

A.3. Estimation and Uncertainty Evaluation for Numerical Data. For numerical attributes are estimated univariate cumulative probabilities $p_k(\mathbf{u})$, $k = 1, \dots, K$, of the cutoff values k . These probabilities are used to infer the value and uncertainty in the RV location \mathbf{u} not sampled. The estimated value of the numerical RV may be the mean or median of the distribution. The uncertainty $\text{Unc}(\mathbf{u})$ can be determined by the variance $\sigma^2 = E\{(Z(\mathbf{u}) - E\{Z(\mathbf{u})\})^2\}$, and this variance can be used to define confidence intervals of type

$$\text{Prob}\{Z(\mathbf{u}) \in [z^*(\mathbf{u}) \pm 2\sigma(\mathbf{u})]\} \cong 0.95 \quad (\text{A.9})$$

when the variable presents a symmetry level that allows to assume the normality hypothesis.

For distributions highly asymmetrical, a more robust measure is the interquartile range defined as the difference between the highest and lowest quartile:

$$Q = z_{0.75}^*(\mathbf{u}) - z_{0.25}^*(\mathbf{u}), \quad \text{where } z_p^* = F^{*-1}(\mathbf{u}, p | (n)). \quad (\text{A.10})$$

From the inferred cdf, $F^*(\mathbf{u}; z | (n))$, it is possible to derive various probability intervals such as the 95% interval $[q_{0.025}; q_{0.975}]$, such that,

$$\text{Prob}\{Z(\mathbf{u}) \in [q_{0.025} - q_{0.975}] | (n)\} = 0,95 \quad (\text{A.11})$$

with $q_{0.025}$ and $q_{0.975}$ being the quantiles 0.025 and 0.975, respectively, of cdf. Attribute values referring to the quantiles are estimated from the fit function and of the cutoff values used in the indicator kriging.

Also, uncertainties can be estimated for ranges of attribute values. The probability of a value $Z(\mathbf{u})$ be within an interval $(a, b]$ is computed as the difference between the values of cdf for the b and a thresholds:

$$\text{Prob}\{Z(\mathbf{u}) \in (a, b] | (n)\} = F(\mathbf{u}, b | (n)) - F(\mathbf{u}, a | (n)). \quad (\text{A.12})$$

After model fittings, indicator kriging procedures were applied to obtain an approximation of the conditional distribution function of the random variables. Based on the estimated function, maps of mollusk spatial distributions along with the corresponding uncertainties for the entire state and also map of estimated prevalence of schistosomiasis were built.

Acknowledgments

This study was supported by CNPq (Grant nos. 300679/2011-4, 384571/2010-7, 302966/2009-9, and 308253/2008-6).

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