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Spatial and temporal epidemiology of *Mycobacterium leprae* infection among leprosy patients and household contacts of an endemic region in Southeast Brazil

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ABSTRACT

Background: Leprosy is a chronic infectious disease that remains a public health problem in low- and middle-income countries. Household contacts of leprosy patients (HHCs) have increased risk of developing disease and are important links in the chain of transmission of *Mycobacterium leprae*. Based on epidemiological and operational factors, the global elimination strategy depends on the geographic stratification of endemic areas to intensify control activities. The purpose of the study was to integrate epidemiological indicators and serology into the spatial and temporal analysis of *M. leprae* infection, in order to understanding of the dynamics of transmission, essential information for the control of leprosy.
Methodology: Using location-based technologies and epidemiological data obtained from leprosy cases (N = 371) and HHCs (N = 53), during a 11 year period (2004–2014), we explored the spatial and temporal distribution of diagnosed cases: stratified according their disease manifestation; and of subclinical infection among HHCs: determined by serology (anti-PGL-I ELISA and anti-NDO-LID rapid lateral-flow test); in order to assess the distribution pattern of the disease and the areas of greatest risk of illness, in a highly endemic municipality (Ituiutaba, MG) in the southeast region of Brazil.
Results: Seropositivity among HHCs was: 17% (9/53) for anti-PGL-I ELISA; and 42% for the NDO-LID rapid lateral-flow test. Forty-nine percent of the contacts were seropositive to at least one of the immunological tests.
Discussion: We observed substantial spatial heterogeneity of cases throughout the urban perimeter. Even so, four main clusters of patients and three main clusters of subclinical infection were identified.
Conclusions: Spatio-temporal epidemiology associated to serological assessment can identify high-risk areas imbedded within the overall epidemic municipality, to prioritize active search of new cases as well support prevention strategies in these locations of greater disease burden and transmission. Such techniques should become increasingly useful and important in future action planning of health interventions, as decisions must be made to effectively allocate limited resources.

1. Introduction

Leprosy is a chronic infectious disease caused by an obligate intracellular pathogen, *Mycobacterium leprae*. Disease progression is slow and insidious, if left untreated, can lead to lifelong physical, psychological and social disabilities. The bacillus is highly infectious; in hyperendemic settings almost half the population may present asymptomatic infection (Barreto et al., 2012; Lima et al., 2015), but only a minority of about 10%, will progress to overt disease since in most people the immune response is able to naturally eliminate *M. leprae* infection (Araujo et al., 2015; Goulart et al., 2015).

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The World Health Organization (WHO) recommend that leprosy should be classified into two main groups: paucibacillary (PB) patients, those with less than five lesions and a good cell mediated immune response (CMI), having few or no detectable bacilli in their skin; and multibacillary (MB) patients, those with more than 5 lesions containing a high bacillary load due to poor CMI resulting in unrestricted growth of the bacilli (WHO, 2012).

Numerous studies have shown that those individuals living with an untreated person with leprosy, called household contacts (HHCs), have the highest risk for later developing clinical disease (Araujo et al., 2015; Bakker et al., 2006; Douglas et al., 2004; Fine et al., 1997; Goulart et al., 2008; Moet et al., 2004, 2006; Reis et al., 2014; Sales et al., 2011; van Beers et al., 1999). The close contact with a MB case prior to their diagnosis and seropositivity to *M. leprae*-specific antigens, have been consistently associated with increased risk of developing disease among HHCs of leprosy patients (Araujo et al., 2015; Bakker et al., 2006; Douglas et al., 2004; Fine et al., 1997; Goulart et al., 2008; Moet et al., 2004, 2006; Sales et al., 2011; van Beers et al., 1999).

Tests to detect the humoral response against the *M. leprae*-specific phenolic glycolipid antigen (PGL-I) have been widely used for decades as an indicator of infection, to determine infection rates in the general population, and to assess the titer for purposes of correctly assigning the multidrug treatment regimen for PB or MB cases. Increasing anti-PGL-I titers correlate well with higher bacillary loads at the lepromatous end of the spectrum (Cho et al., 2001), while they are low or absent in PB disease. Not only is PGL-I highly antigenic, but it is involved in receptor mediated invasion of Schwann cells of the nerve (Ng et al., 2000) and downregulation of the inflammatory immune response, which enables the persistence of *M. leprae* in the organism (Spencer and Brennan, 2011).

Recently, a rapid quantitative serological test for the detection of *M. leprae* infection has been developed based on the detection of IgM antibodies against NDO (natural disaccharide synthetic antigen portion of PGL-I) and IgG antibodies against a multiepitope chimeric fusion protein: leprosy IDRI (Infectious Disease Research Institute) diagnostic 1 (LID-1) (Duthie et al., 2014a). The combination of these two antigens in a single test (NDO-LID) has been reported as: a potential tool to be used as a trigger for physical examination or increased monitoring of particular individuals in order to provide early leprosy diagnosis (Qiong-Hua et al., 2013); as a diagnostic test for MB leprosy (Duthie et al., 2014b); and as a better test for the serological assessment of the immunological status of general population (Fabri et al., 2015).

Epidemiological indicators are mainstays for determining the operational aspects of leprosy control programs and developing sound surveillance systems (WHO, 2000). The key indicators are: overall new case detection rate (number of new cases detected per 100,000 population); new case detection rate among children under 15 years old (new cases/100,000 < 15 yrs.); and detection rate of new cases diagnosed with grade 2 disability (G2D), the highest grade of leprosy disability, which can include destruction of the nerves of the hands, feet and eyes leading to muscle weakness or complete loss of movement or function, bone resorption of fingers and toes, ulcers, paralysis, amputations and blindness (WHO, 2000).

The application of geographic information systems (GIS) and spatial analysis has been used to identify the distribution of leprosy at national, regional and local levels (Barreto et al., 2015, 2014; Paschoal et al., 2013). These modern analytical tools are used to monitor epidemiological indicators over time, to identify risk factors and clusters of high endemicity, as well, to indicate where the resources should be targeted (Queiroz et al., 2010), which is particularly important for areas of limited funds.

This study utilized current epidemiological indicators and laboratory tests, along with the spatio-temporal analysis, to characterize the tendency of leprosy endemy, as means to investigate the distribution of new cases and of subclinical infection; and additionally support control strategies at the area of the study for the interruption of *M. leprae* chain of transmission.

2. Materials and methods

2.1. Study area

The study was undertaken at the municipality of Ituiutaba, in the state of Minas Gerais (MG), located in southeast Brazil, with estimated population of 103,330 inhabitants of year 2015. The city of Ituiutaba is considered of very high endemicity for leprosy (annual new case detection rate of 25/100,000). The classification of endemicity according to the case detection rate per 100,000 population was established as: low (<1), medium (1–10), high (10–19), very high (20–39), and hyperendemic (>40). According data from the Municipal Secretary of Health, during 2004–2014, 371 new cases were notified and treated as residents. The geographic localization of the MG state in the map of Brazil, the localization of the municipality in the map of the MG state, and the map of the urban area in the municipality region, are presented in Fig. 1.

2.2. Data management and analysis

The spatial distribution pattern of leprosy cases in Ituiutaba was determined by combining information from the National Notifiable Diseases Information System (SINAN—http://dtr2004.saude.gov.br/sinanweb), the Brazilian Institute of Geography and Statistics (IBGE—http://www.ibge.gov.br), and by mapping in the field. Supplementary data were obtained from the Municipal Secretary of Planning of Ituiutaba. The residences of people affected by leprosy in the urban area, reported during 2004–2014, were georeferenced with a handheld GPS (Global Positional Systems) device (Garmin 62s, Olathe, KS, USA) to produce detailed maps of the leprosy distribution. Using GIS (ArcGIS 9.2 and QGis 2.8.7- ESRI, Redlands, CA, USA), we drew point pattern maps, calculated the number of cases and the annual case detection rate per urban census tract and stratified per neighborhood areas. The census tracts are defined by the Brazilian Institute of Geography and Statistics (IBGE) as the territorial unit for census operations. A census tract is a partition of a municipality that is typically defined by easily identifiable boundaries, including natural features, as well as, features such as buildings, major roads, and land use.

2.3. Study subjects

The research protocol was approved by the institutional review board at the Federal University of Uberlandia (#1.127.833) and was conducted in accordance with the guidelines of the Declaration of Helsinki. After a brief discussion of the study's risks, benefits, and confidentiality, all participants agreed to take part in this study with written informed consent. For individuals under 18 years old, informed consent was obtained from legal guardians.

A total of 371 leprosy patients were diagnosed and reported in the municipality of Ituiutaba, MG, Brazil, during the 2004–2014 period (data of Municipal Secretary of Health). From this total, the epidemiological indicators were calculated. However, we were able to obtain information about the disease manifestation and addresses of 303 cases. Therefore, 303 cases were georeferenced, since 68 excluded cases did not have complete data, either for their address, or the disease classification, or they lived in rural areas. The variables analyzed were the patient's sex, age, address, neighborhood, geographical coordinates, information about the operational classification according to WHO (PB or MB), and the clinical manifestation according the Madrid classification, which is officially adopted by the Brazilian Ministry of Health. Madrid classification

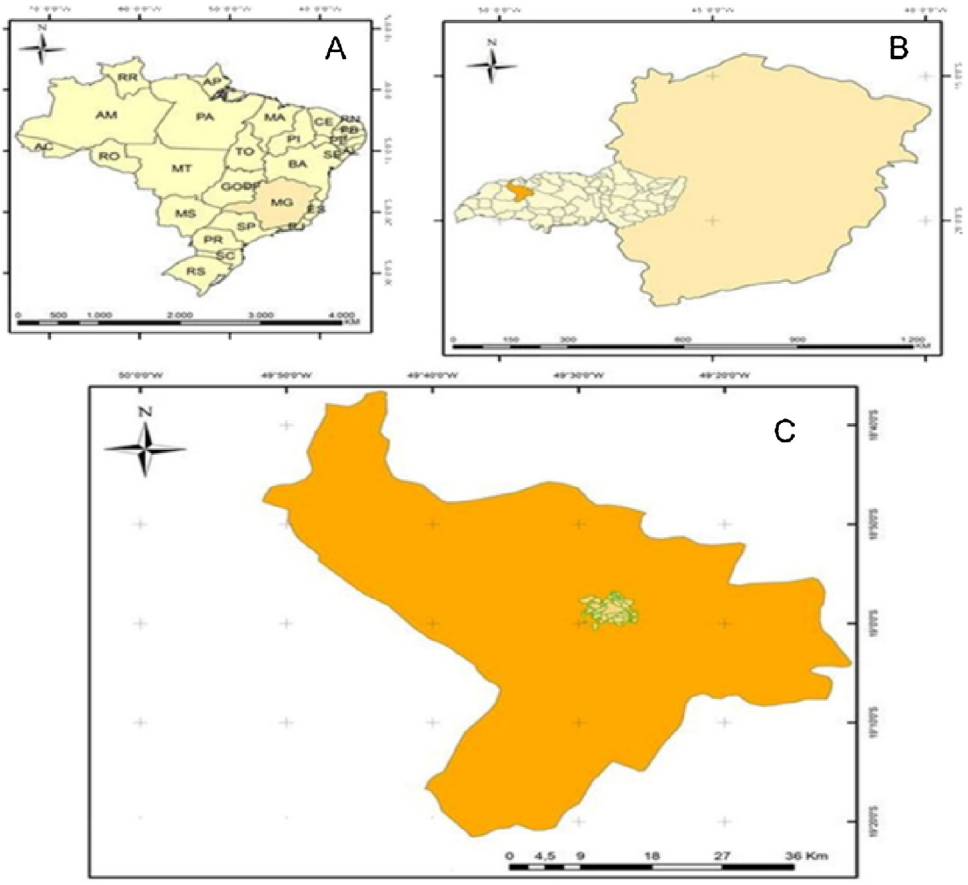

Fig. 1. (A) Geographic localization of the Minas Gerais state (MG) in Brazil map; (B) the localization of the Ituiutaba municipality in the MG state map; and (C) the urban area within the Ituiutaba municipality map.

stratifies the patients into: Indeterminate (I), Tuberculoid (T), Borderline (B), and Lepromatous (L).

Were also included in the study healthy HHCs (N = 53) of the new cases notified during the last 2 years of the study. The household contact was defined as a person who lives or has lived in the same dwelling as a leprosy patient in the last 5 years prior to the patient diagnosis. All HHCs were initially examined in a dermato-neurological clinical examination by specialized physicians experienced in leprosy, and did not present any signs or symptoms of the disease. HHCs' results corresponded to the time point of diagnosis of their index cases. For the purpose of data analysis, the HHCs were stratified according to the clinical (I, T, B, L) and operational (PB, MB) classifications of their index cases. The subclinical infection in HHCs was defined as a positive result in one of the *M. leprae*-specific serological assays, without any clinical sign or symptom of disease.

2.4. Serological assays

Peripheral blood samples were collected through venipuncture in order to perform the serological assays. An enzyme-linked immunosorbent assay (ELISA) to detect IgM antibodies against PGL-I was performed as previously described (Lobato et al., 2011). To moderate inter and intra variations between anti-PGL-I assays, absorbance results measured at 492 nm were converted into an ELISA index (EI), in which the optical density (OD) of the sample was divided by the OD of the cut-off (EI = ODsample/ODcut-off), with 1.1 as positivity threshold. The NDO-LID rapid lateral flow test to detect IgG/IgM antibodies against *M. leprae* antigens was performed according to manufacturer's instructions (OrangeLife, Rio de Janeiro, Brazil). The rapid test involved the addition of undiluted serum (10 μl) and running buffer (2–3 drops; ~100 μl) into the sample well, followed by readings of the immunochromatographic line development in the detection window after 10 min. Validation of the results required the visualization of a colored control line. A positive result was defined by the staining of both the control line and the test line; faint or no staining was considered a negative result. Visual readings were performed by a minimum of two independent readers.

2.5. Spatial and statistical analysis

Spatial scan statistic was applied to identify clusters of leprosy, as reported previously (Barreto et al., 2014). All HHCs also had their residential addresses georeferenced with the GPS device to analyze their spatial correlation with reported leprosy cases. Kernel density estimation was used for the generation of density maps per square kilometers (cases/km^2) which measured the spatial dependence of variables results per unit area.

To investigate the endemic trend of leprosy disease throughout the 11 year period of the study, annual time series of scatter plots were developed, and linear regression was applied for the analysis of coefficients over the years (Pearson correlation). Multiple comparison tests between proportions were used to compare whether there were statistical differences between the clinical and epidemiological variables. The Kappa test was calculated to measure the agreement between categorical results for the serological assays.

Non spatial statistical analyses were performed with software BioEstat version 5.3 (Mamiraua Institute for Sustainable

Development, Belem, PA, Brazil). The threshold for statistical significance was set at the alpha level of 5%.

3. Results

Of the 371 cases diagnosed from 2004 to 2014, 53% were female (195/371), 3% were children <15 years old, and 5% were found to be with G2D disability (Table 1). Using the WHO operational classification, 57.4% were MB (213/371) while 42.6% were PB (158/371), while using the Madrid system for clinical classification, the borderline form was the most prevalent, comprising 47.2% of the total (175/371) (Table 1).

In reference to the epidemiological indicators during the 11 year period (2004–2014), the yearly new cases detection rate ranged from: 39.1 to 25.3 per 100,000 population, remaining within the very high endemic parameter (20–39/100,000), with an overall new cases detection rate of 29.3/100,000 ($P = .0025$). The annual new cases detection rate among the population under 15 years old, varied from 18.5/100,000 to none, leaving from the hyperendemic to the low endemic parameter. Was observed that in the last year of this study, were not detected new cases among individuals <15 yrs. The trend line observed throughout the years has shown a statistically significant decrease in the detection rate of cases ($P = .0014$). As for the leprosy disability grading system, the proportion of G2D cases varied from 5% to 8%, respectively in years 2004 and 2014, reaching the highest proportion of 9.8% in 2005. It also featured a downward trend line, although not statistically significant ($P = .1122$). When dealing with small numbers changes in the numbers detected can lead to a large percent increase or decrease from year to year. Thereby, caution should be used when interpreting these trends.

The population density map demonstrated the spatial distribution of the population within the city urban area and were identified neighborhoods that had higher population density (Natal, Jerônimo Mendonça, Alcides Junqueira, Jardim do Rosário, and Progresso), consisting of 5600–11,800 inhabitants per square kilometers (Fig. 2A).

The spatial distribution of the leprosy cases in urban areas per census sectors of the city of Ituiutaba has demonstrated to be heterogeneous (Fig. 2B); in accordance to the highly endemic official parameters. Hyperendemic areas were mainly identified in the south and northeast regions of the municipality (Fig. 2C).

The population density per census sector (2A), the cases per census sector (2B) and the location of the 371 cases (PB and MB) (2D), show that cases are distributed throughout the urban area. On the map (2C) outlying areas that appear as hyperendemic (in red) are actually large areas with less population and cases vary from 1 to 3, which may present a type of data masking related to the mapping of small areas. The so-called "small numbers problem", needs to be considered in studies in small areas and/or populations (Wakefield and Elliott, 1999). However, in order to obtain a deeper understanding of infection and transmission "hot-spots", and to identify high-risk areas and shifts in disease clustering, we have used an approach of density surface map to show the case density per radius, as shown in Fig. 3.

Through the visualization of the overall density estimation map of leprosy cases (Fig. 3A) and the Kernel estimation per km^2 (Fig. 3B), was possible to observe the main areas where the clusters of disease (cases) were located, at neighborhoods of higher population density (Natal, Jerônimo Mendonça, Alcides Junqueira, Jardim do Rosário, and Progresso).

During the years of 2013 and 2014, were reported 41 cases of leprosy that resided within the city limits of the city of Ituiutaba, and were examined 53 contacts of these cases during the same period. It was possible to carry out dermatological and neurological examination with collection of samples for the serology in all 53 contacts. Of these, 17% (9/53) were seropositive for IgM anti-PGL-I ELISA assay, and 45.28% (24/53) were seropositive for IgG/IgM NDO-rapid lateral-flow test (Table 2). There was a statistically significant difference between the serological tests ($P = .0033$). The agreement between the tests was assessed and the kappa coefficient was 0.24 ($P = .0158$).

Among the contacts, 49.1% (26/53) were positive to at least one of the immunological tests. These were georeferenced in the digital grid map of the city of Ituiutaba, indicating the spatial distribution of the subclinical infection (Fig. 4A).

In order to show possible clusters of infection, the Kernel density estimation was applied, indicating two major clusters of contacts with subclinical infection in the census sectors corresponding to the neighborhoods of "Novo Mundo" and "Alcides Junqueira". Three other clusters with lower density of subclinical infection were visualized, corresponding to the neighborhoods of "Natal", "Centro", and "Alvorada" (Fig. 4B).

4. Discussion

This study applied GIS and spatial analysis together with serological assays for the characterization of the geographic distribution of new cases of leprosy and the localization of subclinical infection clusters, in the municipality of Ituiutaba in the southeast region of Brazil.

Our findings demonstrated that the new cases detected during the course of 11 years were heterogeneously distributed throughout the city area, in accordance with the epidemiologic indicators, and corroborating with other studies that employed spatial geographic analysis in settings highly endemic for leprosy (Montenegro et al., 2004; Sampaio et al., 2012). Different detection rates were observed within different neighborhood sectors, varying between low and hyperendemic, following the official parameters for the annual detection rate. The spatial analysis can indicate priority areas for the local control programs.

Analyzing the spatial disposition of these cases into the census tract, the kernel density estimation indicated large differences in the number of cases between distinct areas. The highest case densities overlap the census tracts with high population densities, observing a significant relationship between disease detection and population densities, as previously reported in other studies of spatial distribution of leprosy cases (Barreto et al., 2015; Barreto et al., 2014; Montenegro et al., 2004; Sampaio et al., 2012).

Our findings also revealed that the disposition of cases was predominant in neighborhoods with lower socioeconomic indicators. Indication that urban composition factors, such as: overcrowding, social inequality, no access to health services or precarious sanitation; continue as underlying characteristics that impact the incidence of leprosy, ratifying that poverty increase the likelihood of transmission of *M. leprae* (Bakker et al., 2004; Kerr-Pontes et al., 2004).

Once determined where most detected cases were concentrated, and where most subclinical infections were detected among contacts, it was possible to overlap spatial geographic density maps, mining both the detection rates and the serological results. Unsurprisingly, the regions corresponded, indicating that these are areas of active transmission of *M. leprae* (Barreto et al., 2015; Barreto et al., 2014); therefore, of upmost importance to the action planning of control strategies.

During the field incursions was identified that the area of the current neighborhood of "Natal", during the decades of 70s and 80s, was located a leprosarium (hospital that abrigated and treated leprosy patients), and at that time the patients and their families migrated to that area, and there they lived under the segregation,

Table 1
Frequency of variables results for the new cases of leprosy patients evaluated in the study.

Leprosy Patients (N = 371)			
Variables	N	%	p-value
Operational classification			
Paucibacillary	213	42.5	$P < .0001$
Multibacillary	158	57.4	
Sex			
Female	195	52.5	$P = .1735$
Male	176	47.4	
Age groups			
Under 15 years [a]	11	3	$P < .01$ [c,d]
15 to 34 years [b]	67	18	$P < .01$ [c]
35 to 59 years [c]	177	47.7	$P < .01$ [b]
60 years and above [d]	116	31.2	$P < .01$ [c]
Clinical classification			
Indeterminate [a]	53	14.2	$P < .01$ [c]
Tuberculoid [b]	111	30	$P > .05$
Borderline [c]	169	45.5	$P < .01$ [a,d]
Lepromatous [d]	38	10.2	$p < .01$ [c]
Leprosy disability grading system			
Grade 0 (no visible deformity or damage) [a]	254	68.4	$P < .01$ [c]
Grade 1 (anesthesia, but no deformity) [b]	98	26.4	$P < .05$ [c]
Grade 2 (deformity or damage present) [c]	19	5	$P < .05$ [a,b]

Note: p-value presented by the multiple comparison between results (ANOVA with post-hoc Tukey, phi coefficient), where statistical significance between the different variables ($P < .05$) are identified per letters [a], [b], [c], [d].

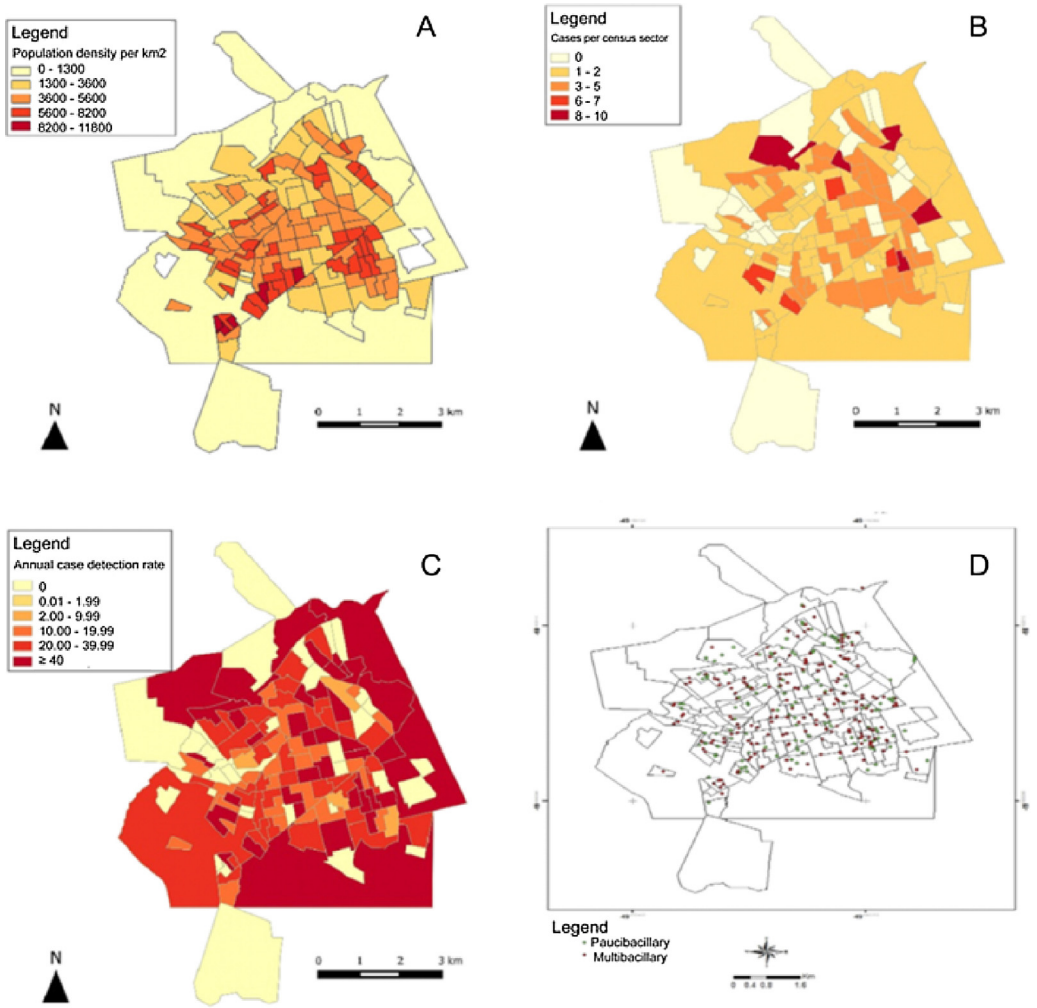

Fig. 2. Spatial distribution of leprosy cases in the city of Ituiutaba, MG (2004–2014). (A) Population density per square kilometers according census sectors. (B) Absolute number of new cases according census sectors. (C) New cases detection coefficient per 100,000 population according census sectors (level of endemicity, low to hyperendemic, according to official parameters). (D) Spatial distribution of leprosy cases according WHO operational classification [paucibacillary (PB) or multibacillary (MB)].

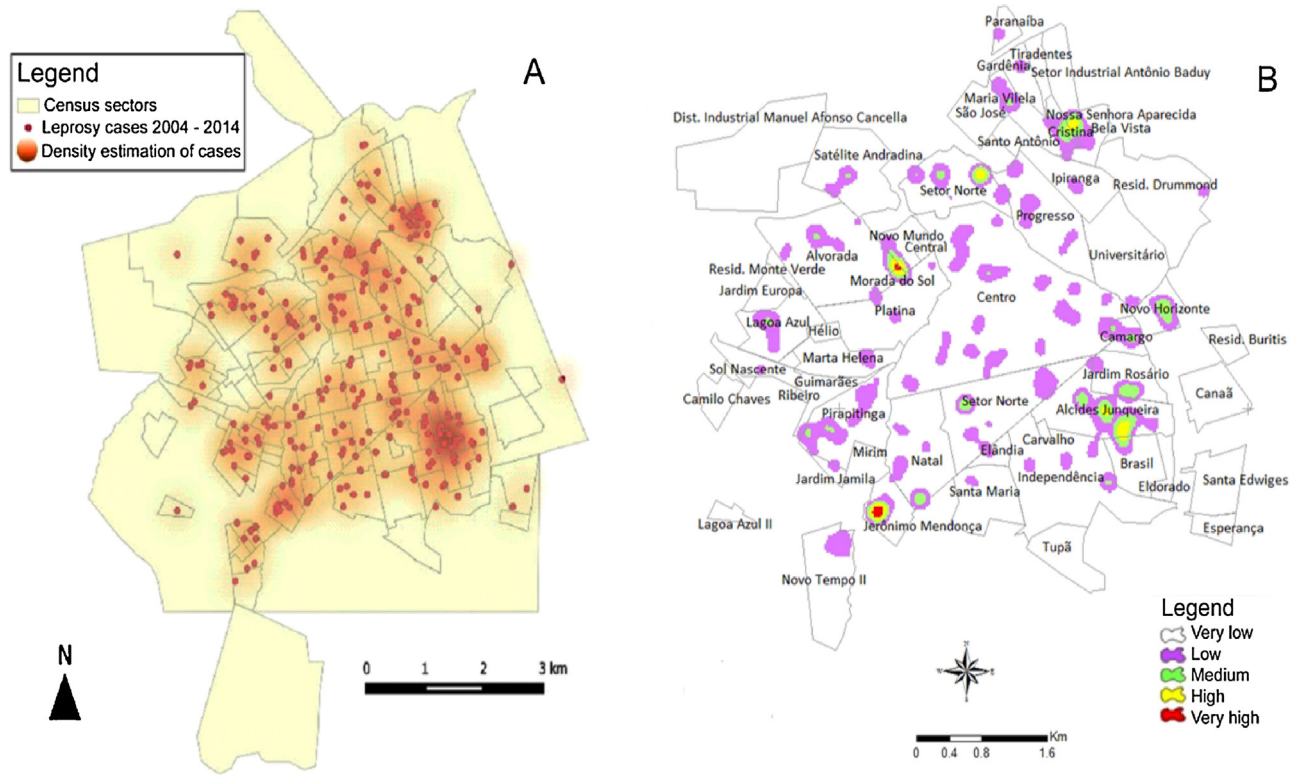

Fig. 3. Distribution of leprosy cases in the city of Ituiutaba, MG (2004–2014). (A) Overall distribution of cases from 2004 to 2014 by census sectors. (B) Density of cases per km^2 (Kernel) according neighborhoods.

Table 2
Frequency of variables results for the household contacts of leprosy patients evaluated in the study.

Household contacts of leprosy patients (N = 53)			
Variables	N	%	p-value
Seropositivity to assays			
anti-PGL-I ELISA	9	17	*P* = .0033
Rapid lateral-flow LID-NDO test	24	42.3	
Sex			
Female	30	56.6	*P* = .1729
Male	23	43.3	
Operational classification of the index case			
PB	15	23.3	*P* < .0001
MB	38	71.7	
Clinical classification of the index case			
Indeterminate [(a)]	18	34	*P* < .01 [(d)]
Tuberculoid [(b)]	12	22.6	*P* > .05
Borderline [(c)]	17	32.1	*P* < .01 [(d)]
Lepromatous [(d)]	6	11.3	*P* < .01 [(a,c)]

Note: p-value presented by the multiple comparison between results (ANOVA with post-hoc Tukey, phi coefficient), where statistical significance between the different variables ($P < .05$) are identified per letters [(a), (b), (c), (d)].

stigma and prejudice of the society. Once drugs became available to successfully treat the disease, neighborhoods or towns grew around these former leprosaria and the families living there were integrated into the population. The result is that leprosy prevalence still occurs at much higher rates around these former leprosaria throughout Brazil. This is an unfortunate legacy of former governments and health authorities who created these sites to segregate leprosy patients. As expected, the neighborhood of "Natal" was within a cluster for subclinical infection.

The epidemiological investigation of contacts demonstrated 17% seropositivity for IgM anti-PGL-I, and 45.3% seropositivity for the NDO-LID rapid lateral-flow test, which detects IgG/IgM antibodies, revealing recent and late exposure among the contacts, similar percentages to what has been shown in other study with both antigens (Fabri et al., 2015). The identification of a seropositive group of individuals with subclinical infection in a population is important not only because they are at risk of developing MB leprosy (Douglas et al., 2004; Duthie et al., 2014b; Goulart et al., 2008) (manifestation with great dissemination of bacilli); but also because they may act as asymptomatic carriers, facilitating the transmission of *M. leprae* to susceptible individuals (Araujo et al., 2012). The seropositivity to at least one of the tests among half the population of healthy HHCs (26/53) demonstrates the great extent of occult infections, as well the intense circulation of bacilli in this

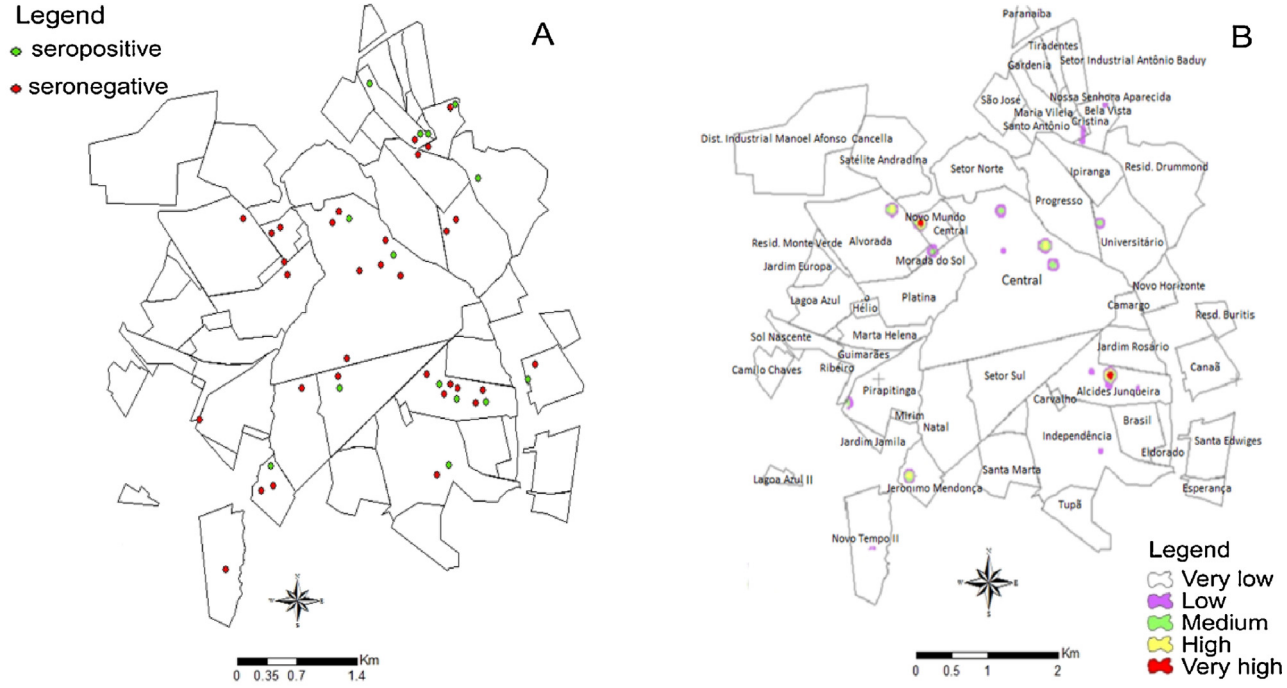

Fig. 4. Distribution of contacts of patients in the city of Ituiutaba, MG (2013–2014). (A) Distribution of contacts and serology results according census sectors. (B) Density of contacts with subclinical infection (seropositive to anti-PGL-I ELISA and NDO-LID lateral-flow test) per Km^2 (Kernel) according neighborhoods.

highly endemic area, which may be sustaining the bacillary burden, in spite of the control actions (Araujo et al., 2012).

Serological tests can be very useful, but their sensitivity is variable and more difficult to assess because of the exposure, infection, and early stage disease unclearness. Furthermore, specificity is likely to be inconsistent in different population groups and to be low in hyperendemic settings. Although some studies document a decline of antibody titer after effective treatment, a clear cut-off value has yet to be defined, in order to avoid inclusion of false positive subjects. Other condition of interest is seronegative PB cases, despite disease diagnosis.

It should be noted that we must never assume that in the regions where clusters were not identified there is no risk of disease or subclinical infection. The dynamic of the transmission of leprosy still poorly understood, as the rates may be influenced by the absence of adequate healthcare and surveillance in these specific areas.

The spatial analysis can be decisive in the planning of control measures, particularly in regions where the resources are limited, as in the case of leprosy endemic countries. Modern methodologies must be compared in different settings in order to improve its applicability to leprosy research. The precise factors to explain the distribution of leprosy are still to be revealed. Nevertheless, this study has shed light on the use of the spatio-temporal analysis together with social aspects and laboratory methodologies to support control strategies. Our group will further explore this spatio-temporal analysis, extending the territory and the assays involved in this research.

Competing interests

None declared.

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Ethical approval

Research protocol was approved by an independent ethics committee at the Federal University of Uberlandia (#1.127.833). All participants were informed about the research, voluntarily agreed to take part in this study without any financial incentive and signed consent forms.

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