Population-based surveillance of pediatric pneumonia: use of spatial analysis in an urban area of Central Brazil

Vigilância populacional de pneumonia em crianças: uso de análise espacial numa área urbana do Brasil Central

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Abstract

This study examined the spatial distribution of childhood community-acquired pneumonia detected through prospective surveillance in Goiânia, Brazil. Three spatial analysis techniques were applied to detect intra-urban geographic aggregation of pneumonia cases: Kernel method, nearest neighbor hierarchical technique, and spatial scan statistic. A total of 724 pneumonia cases confirmed by chest radiography were identified from May 2000 to August 2001. All cases were geocoded on a digital map. The annual pneumonia risk rate was estimated at 566 cases/100,000 children. Analysis using traditional descriptive epidemiology showed a mosaic distribution of pneumonia rates, while GIS methodologies showed a non-random pattern with hot spots of pneumonia. Cluster analysis by spatial scan statistic identified two high-risk areas for pneumonia occurrence, including one most likely cluster (RR = 2.1; p < 0.01) and one secondary cluster (RR = 1.3; p = 0.01). The data used for the study are in line with recent WHO-led efforts to improve and standardize pediatric pneumonia surveillance in developing countries and show how GIS and spatial analysis can be applied to discriminate target areas of pneumonia for public heath intervention.

Pneumonia; Child Health; Spatial Analysis

Introduction

Pneumonia is one of the most important causes of childhood morbidity and mortality, especially in developing regions [1,2]. Worldwide, pneumonia has not been included among reportable diseases in the official systems. Traditionally, for bacterial pneumonia, laboratory surveillance systems have provided invaluable reports focusing on serotype distribution and emergence of antimicrobial resistance of selected isolates among hospitalized pneumonia cases. There has been a great effort to systematically collect standardized laboratory data in both developed and developing countries, supported by international agencies [3,4,5,6]. Despite such relevant laboratory input, this type of surveillance based on results from sterile specimens taken from hospitalized children is prone to selection bias, since it tends to report the more severe cases, mostly with unfavorable evolution and underrepresenting the mild and moderate spectrum of disease occurrence. Another potential pitfall is the lack of a denominator, which compromises the external validity of the results and hinders estimation of the burden of pneumonia among children at the community level [7,8].

Recently, for epidemiological purposes, the World Health Organization (WHO) has standardized the radiological diagnosis of pneumonia in children to generate comparable data when assessing the impact of public health interventions in reducing the pneumonia burden [9]. Since 2000, a population-based network surveillance system has been built up, applying the WHO proposed methodology, using radiologically defined community-acquired pneumonia among children in a large city in Central Brazil. This approach aims to produce a baseline dataset anticipating the introduction of conjugate pneumococcal vaccine into the routine immunization schedule. In this innovative approach, it was possible to map the pneumonia cases taking advantage of the availability of a digital map and friendly software for spatial epidemiological analysis.

In the last decade, geographic information systems (GIS) have been increasingly applied as a tool for analysis and visualization of health-related spatial data, in epidemiological research, and to assist decision-making for disease intervention [10]. Experience has accumulated with spatial analysis of mortality data and reportable infections and chronic diseases for surveillance purposes [11,12,13]. Spatial analysis has been also extensively applied to several infectious diseases [14,15,16], but seldom incorporated into routine surveillance. Previously, we have used GIS to establish high-risk areas for endemic diseases and infant mortality and to assess the feasibility of a surveillance system based on spatially referenced epidemiological indicators to plan, implement, and evaluate the impact of public health interventions in an urban setting in Central Brazil [17,18,19]. In the present study, by using the pneumonia cases identified by the ongoing surveillance system in the city, and under the hypothesis that there was spatial distribution of children with community-acquired pneumonia, unlikely to occur by chance, we describe three different GIS-methodologies – Kernel estimation, nearest neighbor hierarchical technique, and spatial scan statistic – to detect intra-urban geographic clusters of community-acquired pneumonia among children under five years old, in order to address public health interventions.

Methods

Study area

The investigation was carried out in the Municipality of Goiânia, the capital of Goiás State, located in Central Brazil. Goiânia is a highly urbanized city with an area of 741 square kilometers (Figure 1). The municipality has a total population of 1,090,581, of whom 90,576 are children under 5 years old [20]. Infant mortality is estimated at 16 per 1,000 live births [21]. Regional health system attendance by children includes universal access and coverage, and inpatient treatment costs are reimbursed by the government system. The National Institute of Geography and Statistics, or National Census Bureau (IBGE) defines a census tract as the smallest geographic unit for which census and economic data are available. These units are pooled in 63 administrative divisions, called districts, which comprise unit areas of the municipality that are relatively homogenous with respect to population characteristics, economic status, and living conditions. IBGE and the Data Processing Division of the Municipality of Goiânia (COMDATA) rank the 63 urban districts by socioeconomic status, assuming twice the prevailing minimum wage (approximately US$ 108.00/month) for heads of families as the cutoff point for defining the poverty line. The city has a concentric pattern of income distribution, with high income converging in the inner districts. Four low socioeconomic pocket areas can be distinguished, located in the peripheral districts (Figure 1). The Northwest and Southwest areas correspond to recently expanded regions of the municipality, characterized by new settlement. The West and East areas congregate a more well-established migrant community [22].

Surveillance of community-acquired pneumonia

Data on incidence of community-acquired pediatric pneumonia were derived from an ongoing population-based surveillance of *Streptococcus pneumoniae* and *Haemophilus influenzae*, launched in May 2000. An active and passive surveillance network was set up with a multi-site approach to increase the case detection of pneumonia among children under five years of age, following the WHO Recommended Surveillance Standards [23]. At the outpatient level, children were seen by a pediatrician and those who met the WHO criteria for pneumonia were referred for chest radiographs. In our setting, chest radiographs are routinely taken at the pediatric outpatient clinic when pneumonia is suspected. According to the pediatrician's judgment, children who presented a radiograph compatible with bacterial pneumonia were referred to hospital regardless of disease severity. In Goiânia, all requests for hospital admission must be authorized by a "Call Center System" (Hospital Admissions Electronic Information System), located in the Municipal Health Secretariat, where the research team closely monitored and recorded the baseline data for all hospitalized pneumonia cases on a daily basis. This electronic system includes public, private, charitable, and university pediatric hospitals, accounting for nearly 90 percent of pneumonia admissions. For each hospitalization request the system assigns the patient to the hospitals according to availability of beds and proximity to the patient's residence. At the hospital level, all chest radiographs were photographed with a digital camera, and the files were sent to two independent radiologists previously trained according to the recent WHO guidelines for interpretation of radiological diagnosis of pediatric pneumonia [9]. In the case of each child with radiologically defined pneumonia, the respective household was visited to verify the address and conduct an interview. Median time between hospitalization and home visit was four days to minimize loss of information. The protocol was reviewed and approved by the regional and national research ethics committees. Written consent was obtained from parents or guardians of children.

Georeferencing cases

The digital map of Goiânia city, which displays blocks, streets, and lots (households) was used to locate the children's residence. Home addresses were manually geocoded to individual points by using the geographic information system software ArcView GIS 3.2 (Environmental Systems Research Institute, Inc., Redlands, United States), generating a map layer of the cases, which was incorporated into the district layer. Fortunately, the available digital map has recently been updated, incorporating data from the last population count [20]. For 19 (2.6%) addresses not available on the digital map, a global positioning system (GPS) was used to mark the household coordinates on site by the field team, to allow all diagnosed cases to be geocoded.

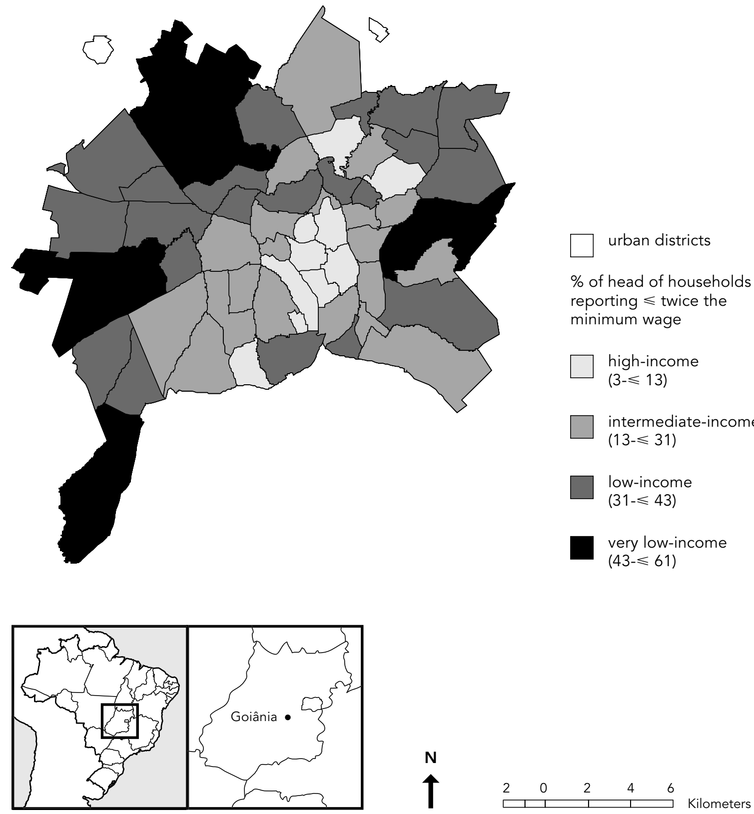
Spatial analysis

Exploratory data analysis was conducted to exclude districts with extremely low population values using SPSS software (Statistical Package for Social Science, v. 10.0.1). The population at risk used in analyses was obtained from the 2000 Census [20]. Out of a total of 63 districts, seven had less than 200 inhabitants, considered extremely low values by exploratory analysis, and were thus excluded from spatial area analysis. The descriptive statistics method was applied to estimate the annual pneumonia incidence rates per 100,000 children under 5 years of age by district with the respective 95 percent confidence interval (95%CI) [24]. A chloropleth map showing the pneumonia incidence rate per 100,000 using natural breaks was depicted to allow a traditional descriptive epidemiological approach and comparison with the spatial analysis results.

Exploratory spatial point pattern analysis was performed first, based on the coordinates of pneumonia cases at the household level using the extension Spatial Analyst 1.0 for ArcView software. The Kernel method was applied to assess the intensity of pneumonia cases over the municipality's area. This is an interpolating and smoothing technique appropriate for individual point location, which involves placing a symmetrical surface over each point using a bandwidth of the region of influence, without considering the underlying risk population [25]. In Kernel estimation a window is moved across the municipality area, and the intensity of pneumonia cases is computed with-

Figure 1

Socioeconomic status by districts according to head of family's income. Municipality of Goiânia, Goiás State, Brazil.



in this window. Cases within this window are weighted according to their distance from the center of the radius, the point at which intensity is being estimated. A smoothed surface was displayed as a continuously shaped map representing intensity levels of cases. The amount of smoothing was determined as a 3,000-meter radius, since this value gave an adequate representation of the pneumonia distribution in the municipality, minimizing biased smooth or spiky distribution patterns.

Since there are different scales to the clustering of points (different geographic levels), the hierarchical clustering technique was applied to identify these levels. Nearest neighbor hierarchical analysis (NNH) was performed to identify spatially close groups of cases, by using the CrimeStat® software (version 2.0) [26]. This approach compares the distance from each case to its closest neighbor with the nearest neighbor distance expected for a random distribution of points [27]. In this technique two or more pneumonia cases are first grouped (first-order clusters) on the basis of the nearest pneumonia neighbor. Then the pairs of cases are grouped into second-order clusters. This process is repeated until either all cases fall into a single cluster or else the grouping criteria fail. Thus, there is a hierarchy of clusters that can be used to visualize spots that deserve priority attention for implementing public health intervention. A one-tailed probability level of five percent was used, and each cluster was required to contain a minimum of five cases, since it represented a meaningful number of cases for accurately selecting hot-spot target areas. To identify the approximate cluster location, a standard deviation ellipse was calculated for each cluster. To allow an accurate display of the cluster from a regional view, two standard deviations were set to cover more than 99 percent of the cases in each cluster. First-level clusters included cases that were closer than the threshold and belonged to a group having at least five cases. Having established the first-order clusters, subsequent clusters were searched to produce a hierarchy of clusters.

Spatial cluster analysis was used to test whether the confirmed pneumonia cases were distributed randomly over space and, if not, to evaluate any identified spatial disease clusters for statistical significance. Spatial scan statistic [28], which is a cluster detection statistic, was applied to test the null hypothesis that the relative risk (RR) of pneumonia cases was the same between any districts, or groups of districts. To account for seasonality, clusters of pneumonia cases in the high-incidence season (winter: May-August) were tested compared to the overall period. The calculations were performed using the SaTScan® software, version 2.1.3 [29], designed specifically to implement the spatial scan statistic. The SaTScan® imposes a circular window on the map, which moves over the area and centers on the centroid of each district allowing the maximum cluster size to be set to any value less than or equal to 50% of the total population. The dataset was scanned for clusters in spatial dimensions ranging from 0 to 30% of the total population at risk, since this cluster size allowed districts with low occurrences of pneumonia cases outside the scanning window rather than districts with exceptionally high pneumonia occurrences inside the window [29]. For each window of varying position and size, the software tested the risk of pneumonia inside and outside the window, with the null hypothesis of equal risk. Under the null hypothesis of spatial randomness, the expected number of cases in each window is proportional to the combined population of districts whose centroid is inside the circle. In this way, the scan spatial statistic adjusts for uneven population distributions. The dataset was scanned for clusters with only high rates of pneumonia (equivalent to a one-sided statistical test). Clusters were identified by scanning window associated with the maximum likelihood, and a likelihood-ratio test statistic was calculated. The distribution of the likelihood-ratio and its corresponding p value were obtained by Monte Carlo simulations – randomly generating 999 replications of the dataset under the null hypothesis. For hypothesis testing, the test statistic was calculated for each random replication as well as for the study dataset (a total of 1,000 statistics), and if the latter was the most extreme 5% of all test statistics calculated, then the hypothesis test was significant at $p = 0.05$. Thus, the problem of performing multiple tests using many scanning windows was addressed and type-I error was restricted to 5%. The number of replications chosen was considered to provide moderate statistical power while minimizing computing time.

Results

Seven hundred twenty-four incident cases of pneumonia were confirmed by the independent radiologists through the active and passive surveillance system in Goiânia during a 16-month period (May 2000-August 2001). This period includes two high seasons (May-August) and one inter-season (September-April), respectively the dry and rainy seasons. An annual risk rate was estimated at 566 cases per 100,000 children under five years old. As shown in Table 1, the highest incidence rate of pneumonia (919.3 per 100,000) was detected in the very low-income region of the city, where 35.6 percent of the pneumonia cases were located. There was an increased linear trend in pneumonia incidence rate from high-income areas to very low-income areas.

The chloropleth map depicted in Figure 2 shows the pneumonia incidence rates per 100,000 children less than 5 years of age by district. 62.9% of total cases and 42.0% of the total population were located in the 21 districts where incidence rates were over 857 cases per 100,000. The spatial location of each pneumonia case geocoded to points is displayed in Figure 3. After applying the Kernel smoothing method, visual evidence of non-random spatial distribution of cases was shown, with the dark shading locating hot spots of pneumonia cases (Figure 4a). The numerical score of the legend on Figure 4a represents the absolute number of pneumonia cases per square kilometer. The nearest neighbor hierarchical technique was applied to the point distribution and identified 33 first-order clusters and 3 second-order clusters of pneumonia cases (Figure 4b). The second-order clusters ($p < 0.05$) (displayed as three major ellipses) covered larger neighborhoods, while first-order clusters covered smaller neighborhood areas.

Spatial cluster analysis is presented in Figure 4c. High-risk areas for pneumonia occurrence were identified by the spatial scan statistic, which searched for clusters using the maximal spatial cluster size of ≤ 30% of the total population. These cluster areas contained 28.7% of the area's total population, including one primary cluster and four secondary clusters. The most likely cluster (largest log-likelihood statistic) was geographically circumscribed in one district in the Northwest of the municipality (RR = 2.1, $p < 0.01$), with 69 observed cases, compared to an expected 33. The significant secondary cluster comprised eleven Northeast districts with 186 cases observed compared to 142 cases expected (RR = 1.3, $p = 0.01$). These two clusters represented 35.3% of all pneumonia cases. Table 2 presents the re-

Figure 2

Chloropleth map of pneumonia incidence rates per 100,000 children under 5 years old. Municipality of Goiânia, Goiás State, Brazil, May 2000 to August 2001.

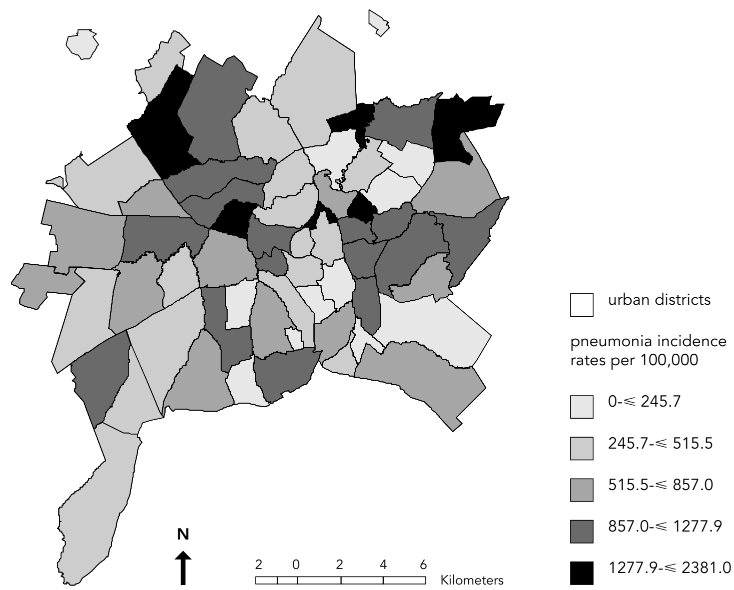


Table 1

Incidence rate of pneumonia cases among children under five years old according to income level in Goiânia, Brazil, May 2000 to August 2001.

Income areas	**No. districts**	**No. cases**	**Population (< 5 years)**	**Incidence rate x 100,000 (95% confidence interval)**	
High income	10	16	6,597	242.5	143.6-385.2
Middle income	20	237	31,686	748.0	657.5-847.4
Low income	21	213	24,229	879.1	767.3-1,002.7
Very low income	12	258	28,064	919.3	812.6-1,036.2
				χ^2 for trend = 23.1; $p < 0.001$	
Total	63	724	90,576	799.3	742.9-860.0

Figure 3

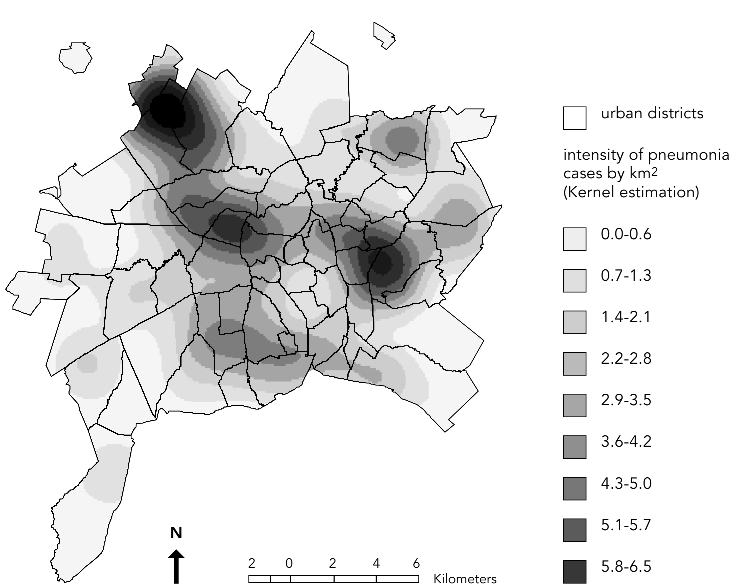
Point pattern distribution of 724 georeferenced pneumonia cases.
Municipality of Goiânia, Goiás State, Brazil, May 2000 to August 2001.

Figure 4

Spatial analysis of 724 georeferenced pneumonia cases.
Municipality of Goiânia, Goiás State, Brazil, May 2000 to August 2001.

Figure 4a

Hot-spot intensity of pneumonia cases by Kernel method.



spective incidence rate of pneumonia cases according to the identified clusters. Spatially significant clusters identified during the high-incidence season (May-August) were similar to those seen overall, with overlap of risk areas regardless of the seasonality (data not shown). This is not surprising, since most cases occurred during the peak season.

Discussion

In the current prospective surveillance we examined the spatial pattern of pediatric pneumonia using three spatial analysis models. The spatial statistics applied in this study clearly revealed a non-random distribution of pneumonia cases within the urban districts of the municipality. Taken together, the three GIS-methodologies generated comparable spatial patterns. While Kernel and nearest neighbor hierarchical techniques ignored the distribution of the population at risk, the spatial scan statistic adjusted for population distribution at the district level. Since Kernel estimation is an easy and fast visualizing exploratory method, it has mostly been employed to identify potential foci of outbreaks or events, which require prompt direct interventions to block spread of transmission [30,31,32].

Using a GIS and spatial statistics, we identified "pocket risk areas" of pneumonia among children within an urbanized developing area. The two-point spatial methods showed pneumonia agglomeration in strikingly analogous areas. The nearest neighbor method utilized an indicator of spatial association to determine which groups of neighboring cases were significantly related to each other. The spatial scan technique calculated a maximum likelihood ratio of pneumonia cases relative to the underlying population under five years old and identified the group of urban districts that rejected the null hypothesis of no clustering. Because each technique bases its cluster detection on its own criteria, different areas were selected by each method; however, the overlap of some districts indicates that the two analytical methods illustrate different elements of the same clusters. Consequently, these spatial analytical techniques are seen as corresponding and are best used in tandem rather than individually.

The analysis demonstrated that combining thorough surveillance information with spatial analysis techniques could increase understanding of the epidemiology of pneumonia. Thus, areas characterized by recently crowded settlements and low socioeconomic conditions were strongly associated with the risk of acquiring pneumonia. The association of low socioeconomic development with susceptibility to pneumonia has been well-documented by observational studies [33,34]. In our spatial approach, increased risk of pneumonia was seen in one district located in the impoverished Northwest area of the city, where spatial scan analysis identified the most likely significant cluster (RR = 2.1, p < 0.01). A secondary cluster was detected in the poor East area of the city (RR = 1.3, p = 0.01); nevertheless the population in this area is served by a better urban infrastructure as compared to the Northwest area. The nearest neighbor technique identified clusters in this same Northwest area of the municipality, but in a broader way, since this analysis uses points instead of areas as the spatial unit and is not affected by the districts' boundaries. These cluster areas are composed of urban districts with a concentration of disadvantaged environmental conditions, mainly lacking sanitation infrastructure. These neighborhoods originated from district-to-district population flows and newly arrived migrants from poor neighboring counties, as a consequence of the demographic shift towards the Northwest districts of the municipality.

The relationship between pneumonia and infant mortality has been traditionally reported in the literature, mostly in descriptive studies [35,36,37]. Increased risk of pneumonia mortality in children has been associated with malnutrition [38,39,40]. For instance, in Brazil, childhood pneumonia plays a substantial role in infant mortality, accounting for 10% of all infant deaths [40]. In a prior study on the spatial distribution of infant mortality conducted in Goiânia, a cluster of post-neonatal mortality was detected in the Northwest area of the municipality [19], overlapping a pneumonia cluster, revealed by both the nearest neighbor analysis and the spatial scan statistic in the present study. The link between increased mortality from pneumonia in children and the proportion of families below the poverty line has already been reported [41]. More recently Szwarcwald et al. [42] examined the association between the infant mortality rate and spatial pattern of poverty in Rio de Janeiro and showed that the spatial concentration of poverty was the best predictor of infant mortality.

By using a traditional epidemiological approach, as displayed in the chloropleth map, it was possible to ascertain areas of high pneumonia rates, over 857 per 100,000 children, comprising 63% of the total pneumonia cases. Although this amount of cases is almost twice the pneumonia cases detected when compared

Figure 4b

Three second-order neighborhood clusters (p < 0.05) by nearest neighbor hierarchical technique.

Figure 4c

Risk areas for pneumonia occurrence by spatial statistic with two significant clusters of high-risk areas (maximum cluster size < 30% total population): most likely cluster, RR = 2.1; p < 0.01; secondary cluster, RR = 1.3, p = 0.01.

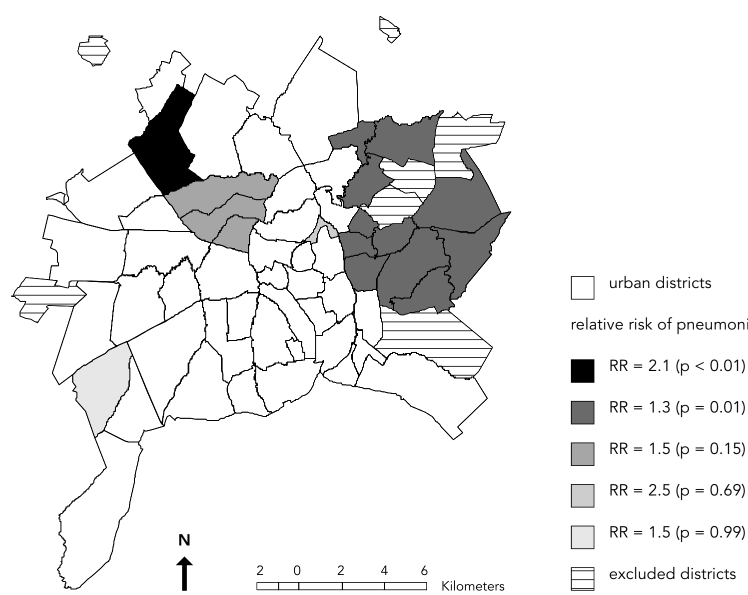


Table 2

Pneumonia case analysis in children under five years old in the municipality of Goiânia, Brazil, May 2000 to August 2001, using the spatial scan statistic.

Cluster type	No. districts	No. cases		Population (< 5 years)	Incidence rate x 100,000	RR[1]	P value[2]
		Observed	Expected				
Most likely	1	69	33	4,067	847.7	2.1	0.002
Secondary	11	186	142	17,433	533.1	1.3	0.014
Secondary	3	66	44	5,391	611.7	1.5	0.152
Secondary	1	9	3	443	1,015.1	2.5	0.687
Secondary	1	14	9	1,143	612.0	1.5	0.999

[1] Relative risk.
[2] 1,000 Monte Carlo simulations.

to the spatial cluster analysis (35%), the corresponding areas were scattered over the municipality, yielding a mosaic-like pattern. In contrast, the tools and spatially-based methods applied in this study were able to demarcate neighboring risk regions, optimizing priority target areas for educational and preventive measures. In this sense, they revealed what would not have been learned by simply mapping pneumonia incidence rates by district and plotting the location of individual cases. Although census tracts could theoretically be the finest level of geographic data resolution, this type of spatial unit would undoubtedly produce a large number of census tract groups with no occurrence of pneumonia and/or small populations, the latter leading to unstable incidence rates. In developing conurbation, funds spent on preventive programs might be better spent on neighborhoods where cost-effectiveness can be maximized. One of the advantages of using the district as the spatial unit of analysis lies in the coverage of a sizeable area; with a larger area to target, it might be more cost-efficient than a microenvironment.

It has been well-recognized that the usefulness of GIS for health information management depends on data quality [13]. Several methodological issues in the current prospective pneumonia surveillance system should be emphasized. Data came from a primary source in a parallel data collection process independent of the official or government source. The case definition of pneumonia based on chest radiography achieves high specificity in the pneumonia diagnosis. Chest radiography is a feasible exam routinely applied for pneumonia diagnosis in our setting. In the present study, the selection of community-acquired pneumonia comprising all degrees of severity avoided selection bias, allowing appropriate inferences to the whole target population of children up to two years of age. Thus, the results tend to reproduce the clinical scenario observed at the outpatient level. Moreover, the detection of population-based incident pneumonia cases made the cases more representative, leading to better external validity of the results. Therefore, the community pneumonia cases detected herein are as representative of a population-based approach as possible. We are aware that we may be missing presumably treated pneumonia cases. However, in our region, chest radiography is universally used to confirm suspected pneumonia.

In the present study we took advantage of the external validity of the pneumonia cases to explore the GIS combined with spatial statistics to areal monitoring. Great impact on both morbidity and mortality prevention may come from this GIS-based surveillance approach since it offers a useful tool in defining local areas for primary health care planning, supporting the control program to enhance pneumonia vaccine coverage. In our setting, health care delivery is organized using the administrative boundaries as the reference. Thus, the spatial scan statistic, which preserves these borders, becomes the best option to convince policy-makers that it is possible to optimize health care resources using spatial epidemiological data.

To our knowledge this is the first large-scale community surveillance of community pneumonia among children conducted in a well-defined geographic area of Brazil. The data used for the study are in line with recent WHO-led efforts to improve and standardize pediatric pneumonia surveillance in developing countries. Finally, the present study is a useful demonstration of how GIS and spatial analysis can be incorporated into routine infectious disease surveillance, orienting child care and vaccine implementation.

Resumo

Este estudo avaliou três técnicas de análise espacial para detectar aglomerados intra-urbanos de casos de pneumonias na infância: método de Kernel, técnica de hierarquia de vizinhos próximos e estatística espacial scan. Setecentos e vinte e quatro casos de pneumonia confirmados radiologicamente foram identificados entre maio de 2000 a agosto de 2001, por vigilância prospectiva implementada em Goiânia, Goiás, Brasil. Todos os casos foram georreferenciados em mapa digital. A incidência anual de pneumonia foi de 566 casos/100 mil crianças. Utilizando-se análise descritiva tradicional obteve-se uma distribuição em mosaico das taxas de incidência de pneumonia, enquanto a utilização de métodos espaciais mostrou uma distribuição não aleatória dos casos. A estatística espacial scan identificou duas áreas de alto risco para ocorrência de pneumonia, incluindo um aglomerado primário (RR = 2,1; p < 0,01) e um aglomerado secundário (RR = 1,3; p = 0,01). Os dados utilizados neste estudo mostram como o sistema de informação geográfica e a análise espacial podem ser aplicados para a identificação de áreas alvo de pneumonia para intervenções em saúde pública.

Pneumonia; Saúde Infantil; Análise Espacial

Contributors

A. L. S. S. Andrade, C. M. T. Martelli, and S. A. Silva participated in the study design, analysis, and interpretation of the data and drafting of the manuscript. R. M. Oliveira participated in the organization and coordination of the field work and analysis of the results. O. L. Morais Neto and J. B. Siqueira Jr. contributed to the data analysis and interpretation and conducted a critical review of the manuscript. L. K. Melo participated in the data processing and analysis. L. H. R. Vilela contributed to the analysis of the results and performed a critical review of the manuscript. J. L. Di Fábio participated in the study design, interpretation of the results, and critical review of the manuscript.

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