A GEOSPATIAL ANALYSIS OF DENGUE FEVER IN CAMBODIA FROM 2002 TO 2012

by

Lori Phillips

BS, University of South Carolina, 2005

Submitted to the Graduate Faculty of

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Master of Public Health

University of Pittsburgh

2014

UNIVERSITY OF PITTSBURGH

Graduate School of Public Health

This thesis was presented

by

Lori Phillips

It was defended on

April 7, 2014

and approved by

Thesis Committee Chair: Ravi Sharma, PhD, Assistant Professor, Behavioral and Community Health Sciences, University of Pittsburgh

Willem Van Panhuis, MD, PhD, Assistant Professor, Epidemiology, Graduate School of Public Health, University of Pittsburgh

Joanne Russell, MPPM, Assistant Professor, Behavioral and Community Health Sciences, Director Center for Global Health, Graduate School of Public Health, University of Pittsburgh

Ravi Sharma. PhD

A GEOSPATIAL ANALYSIS OF DENGUE FEVER IN CAMBODIA FROM 2002 TO 2012

Lori Phillips, MPH

University of Pittsburgh, 2014

ABSTRACT

Dengue fever has reemerged as a serious public health issue worldwide. The disease is endemic in more than 100 countries, with an estimated 50-100 million cases annually. It is considered endemic to Cambodia and, despite multiple control programs, the country has 10,000-40,000 hospitalized cases per year for children less than 15 years of age. In order to have control approaches that are more effective, the patterns of dengue cases in Cambodia need to be further explored. This study seeks to clarify the patterns of dengue by performing a geospatial analysis of 11 years of national surveillance data, from 2002 to 2012. Various exploratory geospatial statistical tools were used to calculate the local indicators of spatial autocorrelation and the local Gi* statistic analysis to determine significant hot and cold spots of dengue fever at the district level of Cambodia. Results found that in most years between 2002 and 2012, the two urban centers of Cambodia, Phnom Penh and Siem Reap, were significant hot spots for dengue fever. This suggests that prevention and control programs should be targeted to these two areas specifically. However, these clusters corresponded with five of the seven sentinel surveillance sites used in the country. This analysis could be only a representative of more sensitive surveillance. Expanding sentinel surveillance activities to other provinces could reveal a clearer picture of dengue fever in Cambodia.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

I would like to thank Dr. Huy Rekol and Dr. Ngan Chanta of the National Dengue Control Program (NDCP) of the Cambodian Ministry of Health of Cambodia for his time and supervision for allowing me to work with the NDCP. Without their help and willingness to share knowledge, this thesis would not have been possible. I would also like thank my committee members, Dr. Sharma, Dr. Van Panhuis and Joanne Russell for their support and guidance throughout my time at the University of Pittsburgh.

1.0 INTRODUCTION

Vector-borne infectious diseases account for about 17% of the global burden of all infectious diseases[1]. These diseases include: malaria, yellow fever, West Nile virus, dengue fever, plus many more. Despite the control efforts for mosquito-borne illnesses, malaria, West Nile virus and dengue fever have reemerged as serious public health issues in tropical and sub-tropical countries. Dengue fever is one the fastest growing vector-borne diseases, with a drastic increase of cases in the last two decades [1]. The disease is endemic in more than 100 countries, with an estimated 50-100 million cases annually [2]. Dengue is considered endemic to Cambodia and, despite multiple control programs, the country still has 10,000-40,000 hospitalized cases per year for children less than 15 years of age [3]. In order to have control and prevention approaches that are more effective, the patterns of dengue cases in Cambodia should be explored. This study examines eleven years of dengue fever surveillance data from Cambodia for incidence and spatial-temporal patterns. These patterns should reveal where the most, and least, dengue cases occur in Cambodia and assist in targeting control and prevention activities.

2.0 BACKGROUND

2.1 DENGUE FEVER

Dengue fever is a mosquito-borne viral infection that has affected tropical and sub-tropical countries since the 18th and 19th centuries [4]. The primary mosquito vector is the female *Aedes aegypti*, which passes the virus to humans. The *Aedes aegypti* breed mostly in man-made water containers, such as large jars that catch rain water. The *Ae. aegypti* is distinct in that it feeds during the day, peak time being early in the morning and just before dusk. They also can bite multiple people during feeding time [5].

There are four different serotypes of the dengue virus (DENV), DENV-1, DENV-2, DENV-3 and DENV-4 [5]. If a person is infected and recovers from one serotype, they would have lifelong immunity against that one serotype. There is some partial immunity to the other serotypes, but this is only temporary. Infection with multiple serotypes or consecutive infection with a different serotype can increase the risk of developing more severe dengue [5].

The case fatality rate of dengue varies by region and country, but is usually around 1% or less, though some areas have rates as high as 5% [5, 6]. Dengue causes severe flu-like symptoms, including a high sustained fever, headache, severe joint pain and rash. These symptoms can last between two and seven days, after the four to ten day incubation period from the bite of an infected mosquito [7]. A person can be infected with the virus but present with no symptoms.

The United States Centers for Disease Control and Prevention (CDC) reports that, “as many as one half of all dengue infected individuals are asymptomatic…” [8]. Dengue hemorrhagic fever (DHF) is the more severe, and sometimes fatal, form of dengue. DHF causes plasma leaking, fluid accumulation and respiratory distress. Symptoms include vomiting, bleeding gums, fatigue and severe abdominal pain. Immediate medical care is necessary for DHF cases. There is currently no cure or vaccine for any serotype of dengue fever. If caught early, fluid replacement is most effective [5].

2.1.1 DISEASE BURDEN

More than 100 tropical countries have endemic dengue infections and DHF has been reported in more than 60 of these countries. It is considered endemic in the Americas, Southeast Asia, Western Pacific, Africa and Eastern Mediterranean. Of these, the Americas, Southeast Asia and Western Pacific carry the heaviest burden [2]. In the past 20 years, global reports of DHF have increased fivefold [4].

According to the World Health Organization [5] data, the average number of dengue fever/dengue hemorrhagic fever cases per year has risen from 908 between 1950 and 1959 to nearly 515,000 between 1990 and 1999. It is estimated that the real numbers are closer to 50 million cases a year, causing 24,000 deaths [9].

There are an estimated 2.5 billion people in the world at risk for dengue and about 70% of that number live in Asia Pacific countries [10]. This places a large burden on the region. In Southeast Asia dengue has been shown to have a significant economic and disease burden for the area. Shepard et al. conducted an analysis of the economic and disease burden of dengue in twelve Southeast Asian countries [11]. The study found an annual cost per capita of $1.65 and a disease burden of 372 Disability Adjusted Life Years (DALYs) per million persons. This rate is higher than Japanese encephalitis, upper respiratory infections and hepatitis B [11]. Clearly, dengue fever is a growing public health issue.

2.1.2 RESURGENCE OF DENGUE FEVER

The resurgence of dengue fever in the last 20 years could be attributable to increases in the geographic distribution of the *Ae. agyepti* mosquito and in the rate and geographic range of virus transmission [2]. Rapid global population growth and unplanned urbanization have resulted in substandard housing, inadequate water supply and waste management systems, which provide the vector with additional breeding grounds. An increase in air travel has allowed different strains, serotypes and genotypes to be introduced into other regions [2].

2.1.3 SURVEILLANCE

Dengue fever surveillance is key to having effective prevention and control program, however in most endemic countries, the surveillance systems are lacking. Estimates from many countries have been found to be consistently lower than what sample studies have shown. In 1996, the Laos surveillance system reported 2563 DHF cases, while the WHO reported 8197 cases [9]. In Indonesia, an evaluation found that only 31% of hospitalized DHF/Dengue Shock Syndrome (DSS) cases were actually reported to the appropriate authorities [9]. The WHO, in 2004, estimated there were 0.5 million DHF cases, but if underreporting studies are taken into account, the global incidence rate could have actually been around 1.5 million cases of DHF [9]. Most of these surveillance systems are passive in nature, meaning hospitals or health centers are responsible for reporting dengue cases through the government system. Guha-Sapir and Schimmer suggest implementing more sentinel, or active, surveillance to provide more accurate estimates of disease burden, as well as sampling of the private sector [9].

As of February 2013, there were five dengue vaccine candidates in different clinical phases [12]. This includes one in Phase III and two in Phase II testing. The most promising is a live attenuated chimeric tetravalent vaccine developed by Sanofi Pasteur, which underwent Phase IIb testing in Thailand and results were published in 2012 [13]. Results of this study found the vaccine was safe, with little to no side-effects and had an efficacy of 30.2% [13]. As a safe and efficacious vaccine is completed, surveillance will play a significant role in determining where to distribute the vaccine.

2.1.4 PATTERNS OF DISEASE TRANSMISSION

There are several factors that can affect the transmission of dengue fever. These include environmental, particularly climate change, human and vector behaviors, virulence of the four strains and immunity of human hosts [14]. Liebman et al. conducted a spatial dimensions study of dengue transmission in Iquitos, Peru from 1999 to 2003 and found that seroprevalance of the previously circulating DENV serotype can be a predictor of transmission risk for a different infecting serotype. The study also found that regardless of serotype, human movement is an important factor in defining the spatial dimensions of DENV transmission, as well as mosquito population [15]. Due to the fact that human movement and vector population can contribute to the spread of the dengue virus, spatial patterns of dengue are important to examine. Through examination of these transmission patterns, officials could identify where surveillance and interventions could be targeted to prevent or reduce further outbreaks [15].

The relationship between dengue fever in urban and rural areas is not completely understood. The virus has most commonly been thought of as an urban disease with cases originating in urban centers and spreading into the rural areas of a country [9]. However, recent studies have shown varying sources of dengue epidemics, including an increase of dengue in rural areas of Thailand and India [16-18]. In 1998, Thailand had a higher incident rate in rural areas compared to urban areas [16]. An increase in dengue fever among rural populations has also been seen in Central and South America [19]. Urbanization and increased transportation could be a potential reason for this spread of dengue to rural areas [20].

2.1.5 PREVENTION, CONTROL AND OUTBREAK RESPONSE

Until there is a vaccine available for prevention of dengue fever, vector control is the primary way to reduce dengue transmission [6]. This has proven difficult, as most endemic countries are considered low-income and lack the financial resources to sustain control programs. Guzman suggests the following principles must be present for effective dengue control: political will, improvement of public health infrastructure and vector control programs, inter-sector coordination, active community participation and reinforcement of health legislation [2]. Of these, she notes it is essential that the community accepts responsibility in dengue control [2].

Early detection and case management are two other methods for dengue prevention and control [9]. These are particularly important in decreasing DHF/DSS mortality rates. However, in many endemic countries patients must travel long distances to reach a health facility, and most times that facility is not equipped with a laboratory to confirm the dengue diagnosis. Overall strengthening of health systems is needed to improve both early detection and case management of dengue [9].

Thailand's patterns of dengue are cyclic in nature, meaning epidemics occur every few years, in this case every four to five years [21]. Barbazan, Yoksan and Gonzalez completed a study in 2001 that described and forecasted epidemics of dengue in Thailand [21]. The authors suggested that early detection of epidemics through a local case detection network would be ideal; however they recognized the financial and human resource burden this could entail. In addition, they recommended that the efficiency of control programs would be improved by defining the highest risk areas within provinces, which can be located through spatial analysis [21].

2.2 CAMBODIA

Cambodia is a country in Southeast Asia with a population near 15 million in 2012 [22]. It is bordered by Thailand to the west, Laos to the north, Vietnam to the east and the Gulf of Thailand. The country is characterized as agricultural and tropical, with a distinct monsoon season from May to October. Geographically, Cambodia contains 24 provinces, 185 districts, 362 communes and over 13,000 villages. Phnom Penh is the capital city, where about 20% of the population resides [23].

The overall health of Cambodia has been improving in the past decade as the country has developed. Infant mortality has dropped a third from 2005 to 2010, the under-five mortality rate also dropped significantly during the same time period [24]. The life expectancy at birth has increased to nearly 71 years in 2012 from 66 in 2004 [22]. The fertility rate has steadily declined from 3.4 births per woman in 2005 to about 2.6 births in 2013, though the rate is slightly higher in rural areas compared to urban [23, 24].

According to the 2010 Global Burden of Disease Study, Cambodia ranked third for health-adjusted life expectancy and fifth for age-standardized death rate per 100,000 when compared to similar countries. Compared to 1990, where the top three causes of mortality in terms of Years of Life Lost (YLL) were lower respiratory infections, diarrheal diseases and malaria, in 2010 the top three causes of YLL were lower respiratory infections, ischemic heart disease and cerebrovascular disease [25].

2.2.1 DENGUE FEVER IN CAMBODIA

In Cambodia, according to the 2010 Global Burden of Disease Study, dengue fever rose from the 29th cause of YLL for those under five, to 22nd from 1990 to 2010. It also rose for those between five and fourteen years of age in the same time period, from 19th to 13th in overall causes of YLL [26].

In Cambodia, the national data has shown high incidences in both rural and urban areas. A study of cases between 2006 and 2008 in Kampong Cham province of Cambodia that used community-based active surveillance, found that rural areas were typically affected by dengue to the same degree as urban areas [14]. Possible factors that could explain the high urban and rural rates include, rapid human growth and the lack of improved sanitation leaving more man-made water containers for vector breeding [14]. Vong et al. also cited that the majority of the rural villages in the study were located near a national roadway, which had frequent and regular traffic from the capital, which could allow dengue introduction from infected individuals passing through the area [14].

Most dengue control programs in Cambodia are focused on vector control. Larvacide campaigns are typically done twice a year, between April and July and between August and October. The NDCP targets water storage containers in the more populated areas and urban centers. They also provide education to the public through various education campaigns, including commercials that are aired countrywide [27].

Abate distribution is a common preventive measure taken in Cambodia as well. An ethnographic study of two villages in Kampong Cham Province was conducted in 2007 to describe the public knowledge of the vector, what practices were used to reduce breeding sites and if the distribution of Abate was successful [28]. During the time of the study, Abate was only used during the rainy season, despite water containers testing positive for larvae in the dry season. The study found that various unused water containers contained twice the number of larvae as used water storage containers, but these were not targeted for Abate distribution. The authors recommended that the reliance on Abate should be reevaluated, particularly its distribution to more affected water storage containers [28].

Khun and Manderson also completed a process evaluation of community and school-based health education for dengue control in rural Cambodia [29]. Dengue control is taught in primary schools, health centers and by the NDCP in Cambodia. The evaluation of this education found that there is no routine evaluation of the educational programs, messages can be confusing and health staff and teachers do not have the training, time or opportunity to deliver the education [29]. While the authors found that school children and their parents were familiar with the *Aedes agyepti* mosquito and the environmental factors that contribute to dengue, this knowledge was rarely translated to reducing to the risk of infection [29].

2.2.2 DENGUE SURVEILLANCE IN CAMBODIA

The initial surveillance system of Cambodia was passive, meaning hospitals and health centers were responsible for reporting cases of dengue to the Ministry of Health. This type of surveillance in Cambodia began in 1980. The National Dengue Control Program (NDCP) was created in 1996 and has been responsible for dengue fever surveillance in the country ever since [30]. The passive system was supplemented in 2001, when the NDCP implemented an active sentinel surveillance system to try to capture more dengue cases. This system started with three public hospitals and three private hospitals in four provinces. There are now seven hospitals in the sentinel system, representing four provinces. The NDCP actively records the number of dengue fever cases from these particular hospitals on a weekly basis [31].

It is important to discuss the clinical case definition used in reporting dengue fever cases in Cambodia. Since 2002, cases have been based on the World Health Organization definition. The WHO definition is as follows:

> Probable case-an acute febrile illness with two or more of the following: headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations, or leukopenia and supportive serology or occurrence at the same location and time as other confirmed cases of dengue fever.
>
> Laboratory confirmed by: isolation of dengue virus, detection of dengue virus RNA in serum or tissues and detection of specific dengue virus antigen; through serum samples by haemagglutination-inhibition, complement fixation, neutralization test, IgM-capture enzyme-linked immunosorbent assay or indirect IgG ELISA.
>
> Reportable case-any probable or confirmed case should be reported [32].

According to the Standard Operating Procedure manual of the NDCP, the definition to be used by hospitals and health centers in suspected dengue cases is: An acute febrile illness of 2-7 days duration with two or more of the following manifestations: headache, retro-orbital pain, muscle and joint pain, rash, hemorrhagic manifestations (positive tourniquet test, petechiae, etc.). All the suspected dengue cases (all ages and both gender) must be referred to a referral hospital for medical observation and monitoring [30].

As a person can be infected with dengue, but present with no symptoms, virological and serological testing is needed to confirm an actual dengue fever infection. Lab testing is also the only way to determine the virus serotype. However, due to limited resources in Cambodia, serological testing is only completed at five of the sentinel surveillance sites [31]. Additionally, both the active and passive surveillance systems only collect hospitalized cases of dengue. This fact along with the lack of lab confirmations could potentially be underestimating the burden of dengue fever in Cambodia.

A capture-recapture analysis was done between 2006 and 2008 in Cambodia [3]. The study used community-based active surveillance in Kampong Cham Province, the largest province, to determine dengue under-recognition of the national surveillance system. Results of this study found an incidence of dengue between 13.4 and 57.8 per 1,000 person-seasons (persons infected during dengue season, not year round) while the national surveillance data reported an incidence between 1.1 and 5.7 per 1,000 person-seasons for the same time period. Due to the national surveillance system only reporting on hospitalized cases, the NDCP is likely missing a large proportion of less severe dengue fever [3]. Currently, there are several dengue fever vaccines being developed and put through clinical trials, as a vaccine becomes available, surveillance will become a vital factor in its distribution [12].

3.0 METHODS

District level data was provided by the National Dengue Control Program of the Ministry of Health in Cambodia and was deemed exempt from human subjects research by the University of Pittsburgh Institutional Review Board.

3.1 DATA

Dengue fever cases between 2002 and 2012 were reported both passively and actively on a weekly basis to the NDCP using the WHO case definition discussed earlier. This study used the aggregate number of cases per district per month for analysis. Population data for years 2002 through 2012 was also provided by the NDCP, which are national census numbers. Population figures were made available for provinces and districts of each year.

Yearly provincial incidence was calculated by dividing the number of annual cases by the total population and then multiplied by 100,000. This was also done for the provincial and district levels. Monthly incidence was determined similarly at the provincial and district level. Histograms of incidence by month were created to visualize yearly trends of dengue fever at the two separate administrative levels.

3.2 GEOSPATIAL ANALYSIS

Multiple geographic information systems were used to analyze and visualize the data. GeoDa and Crime Analytics for Space-Time, both of which are open-source software programs available from the GeoDa Center for Geospatial Analysis and Computation from the University of Arizona [33]. These programs, along with ArcGIS, were used to map clusters and perform spatial-temporal analysis for this study. Country, Provincial and District shapefiles were obtained from the Global Administrative Areas website [34]. Shapefiles were downloaded for Cambodia for all administrative layers.

Spatial-temporal analysis was completed using CAST for years 2002 to 2012 at the district level. Gi* cluster analysis was done to show if there was clustering of high or low values of dengue fever around one location. The null hypothesis is there is no clustering of high or low values around location *i*, the test statistic is close to zero and the alternate hypothesis is there is clustering of high or low values around location *i*; a significant positive value implies a clustering of high values and a significant negative value indicates a clustering of low values. The formula is shown below, where x_{jt} are the values of a variable observed at location *j* and time *t*. These values are multiplied by the spatial weights set, w_{ij}, which denotes the locations to include in the analysis and how to weight them. A Queen weight was used in this analysis, which means all districts sharing any boundary point are considered neighbors. The sum of these observed values is subtracted from the expected value, the sample mean, multiplied by the sum of the weights. Finally, this difference is divided by the standard deviation. The Gi* statistic is a Z-score. The significance of each Gi* value is done using a Monte Carlo randomization of the dataset. A large, positive Z score means there is clustering of high values, or a hot spot, and a small, negative Z score means clustering of low values, or a cold spot [35].

$$G_{i,t}^{*} = \frac{\sum_{j} w_{ij,t}\, x_{j,t} - W_{i,t}^{*} \bar{x}_{t}}{s_{t} \sqrt{\frac{(n_{t} S_{1i,t}^{*}) - W_{i,t}^{*2}}{n_{t} - 1}}}$$

Local Indicators of Spatial Autocorrelation (LISA) identifies the association between an observation and its neighbors by using the local Moran's I statistic and classifies the observations, as shown in Table 1. Neighbors were defined as the districts that shared any boundary with the district being analyzed. The average of the local Moran statistic is proportional to the Global Moran's I value. Local Moran's I formula is below; w_{ij} is the spatial weight between i and j, n is the number of observations, x_i is an attribute for feature i, X bar is the mean of the corresponding attribute [36]. The results are similar to Gi*, as a Z score is calculated, but LISA also calculates a p-value for significance. A positive value for I means a feature has neighboring features with similar values and is considered part of a cluster. A negative I value means that a feature has neighbors with dissimilar values and is considered an outlier. A p-value of 0.05 is used to determine the statistical significance [37].

$$I_i = \frac{x_i - \bar{X}}{S_i^2} \sum_{j=1, j \neq i}^{n} w_{i,j} (x_j - \bar{X})$$

Table 1. LISA Classifications

Classification	*Meaning*
High-High	High values surrounded by high values-considered hot spots
Low-Low	Low values surrounded by low values-considered cold spots
Low-High	Low values surrounded by high values-potential spatial outliers
High-Low	High values surrounded by low values-potential spatial outliers

4.0 RESULTS

4.1 DESCRIPTIVE

Between 2002 and 2012, Cambodia reported 192,128 cases of dengue fever. The annual incidence of dengue ranged from 70.77 to 311.99 per 100,000, with the highest in 2007 and lowest in 2005; refer to Table 2 and Figure 1 below.

Table 2. Total Incidence by Year

Year	Incidence per 100,000
2002	102.132
2003	98.3708
2004	78.157
2005	70.774
2006	130.345
2007	311.994
2008	69.634
2009	80.381
2010	88.983
2011	111.099
2012	287.615

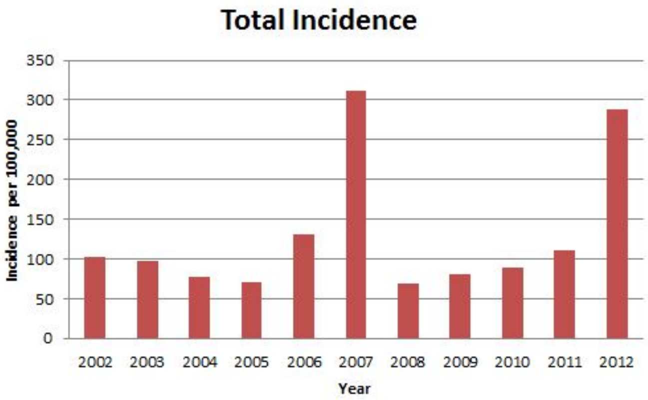


Figure 1. Total Incidence by Year

When annual incidence is visualized by month and year, Figure 2, the highest incidence rates occur between May and August for all years. Two epidemic years can be seen, in 2007 and 2012, where the rates were nearly three times of that in non-epidemic years. June and July were the peak months of incidence for all years except 2006, which peaked in August.

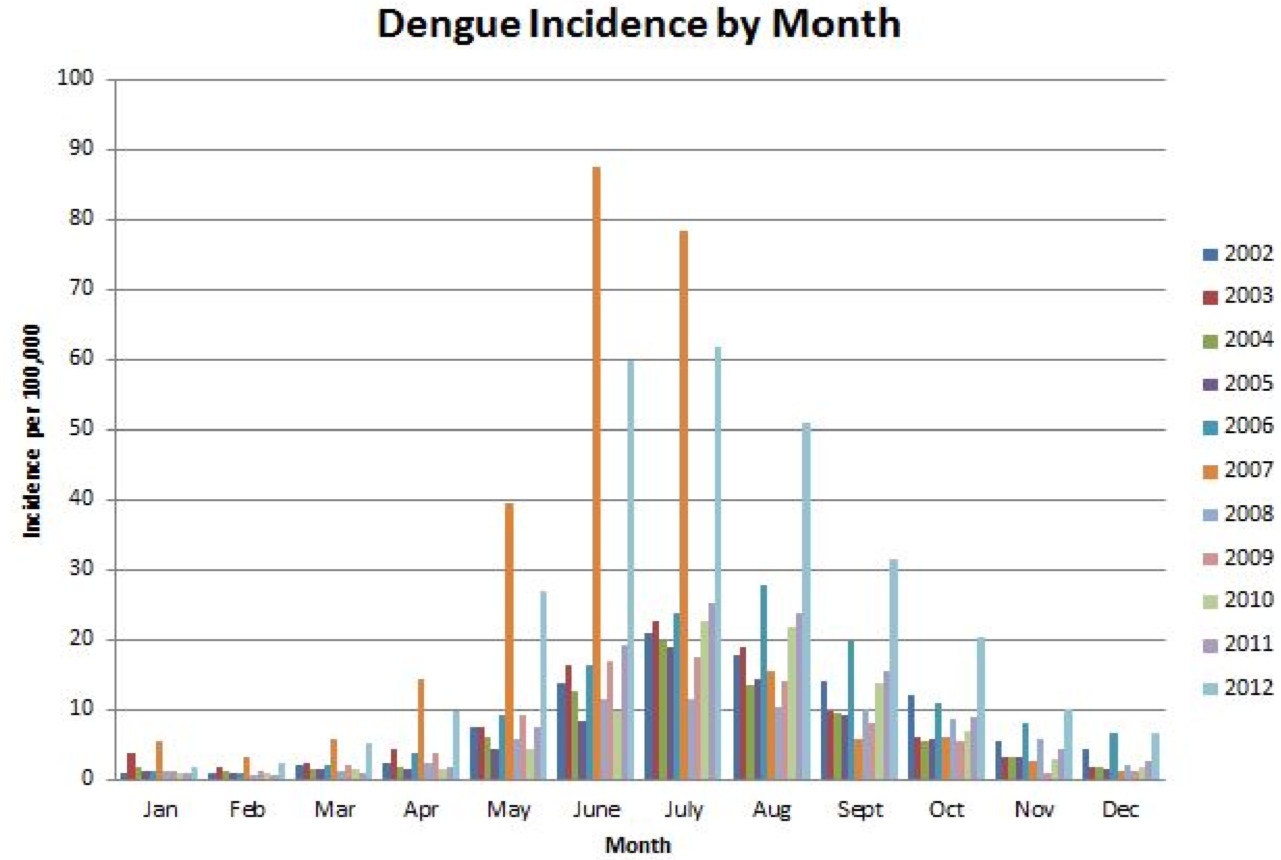
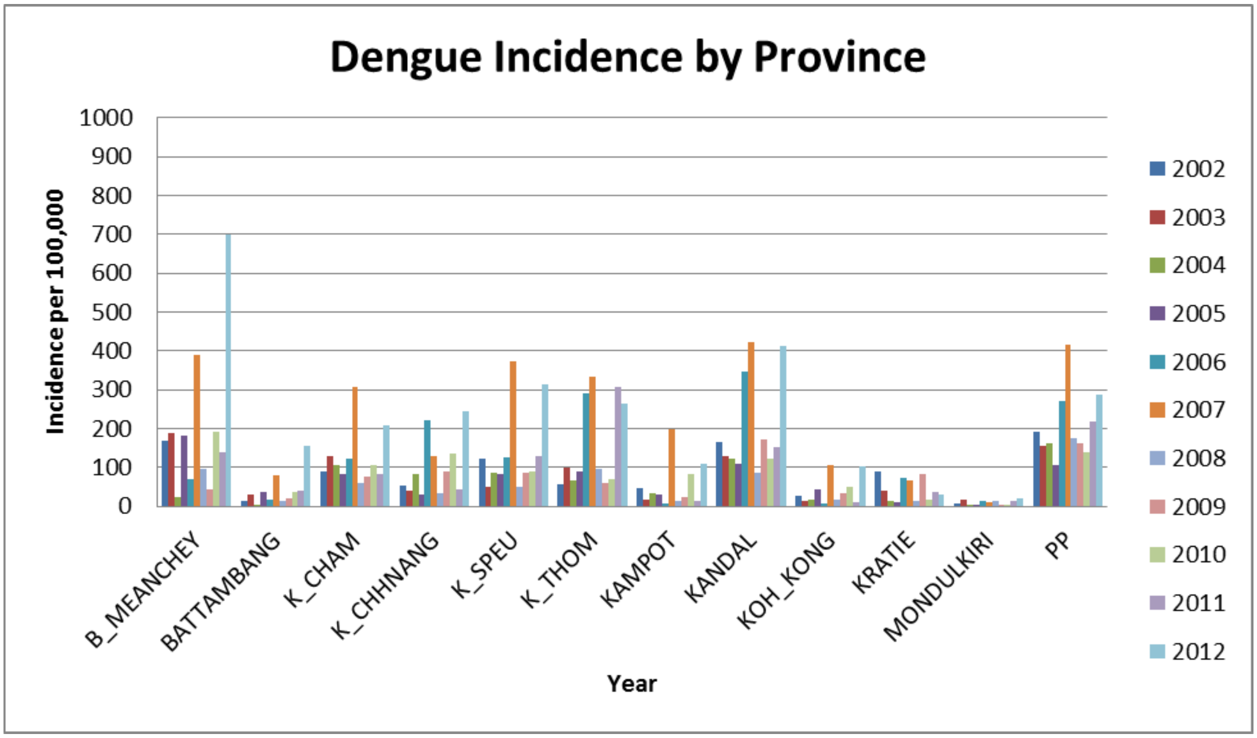


Figure 2. Incidence by Month and Year

Figure 3 illustrates the yearly incidence per 100,000 of dengue fever in each province. Again, the highest incidences are seen in the 2007 and 2012 epidemic years. In 2007, Kandal, Phnom Penh, Siem Reap and Takeo recorded the highest incidence. In 2012, Banteay Meanchey, Kandal, Siem Reap, and Oddar Meanchey were the highest.



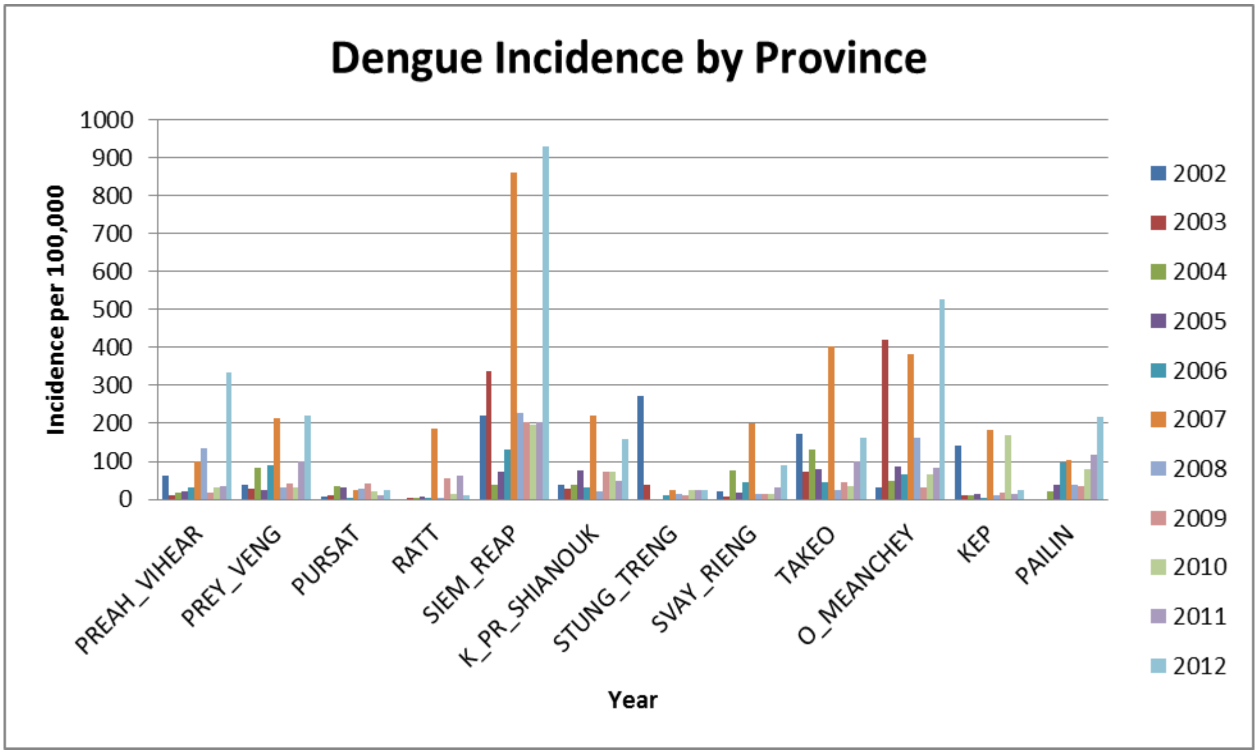


Figure 3. Incidence by Province and Year

4.2 GEOSPATIAL ANALYSIS

All maps referred to in this section are located in Maps appendix.

Figures 5-7 are Local Gi* cluster and significance maps for each year, 2002 to 2012. Phnom Penh reveals high-high clustering for all eleven years, with significant p-values ranging from 0.05 to 0.001. Districts within Siem Reap Province also show high clustering in all years except 2004, 2005 and 2006. The northeast and southwest regions for all eleven years show low-low clustering, but that the clusters are significant at the 0.05 and 0.01 p-values meaning there is clustering of low values around those locations.

The LISA maps are shown in Figures 8 and 9 for each year. These map results are classified as those shown in Table 1. These show similar results to the Local Gi* maps, in that Phnom Penh and Siem Reap both have high-high clustering, considered to be hot spots of dengue fever, and the northeast and southwest regions have low-low clustering, representing cold spots of dengue. The 2004 map revealed Sandan District in Kampong Thom Province, had high-low clustering. This represents a potential spatial outlier. In 2005, high-low clustering is seen in two districts and low-high in two districts in the northwest region of the country.

5.0 DISCUSSION

The incidence by month results, Figure 2, in this study, support other analyses in that the majority of dengue cases in Cambodia occur between May and August, which corresponds to the country's monsoon season when there are more breeding sites for the vector [31, 38, 39]. Two epidemic years were identified from this study, in 2007 and 2012. Again, this supports other studies that have shown dengue fever is cyclic in nature, causing epidemics every four to five years in most endemic countries [11, 14, 40]. The 2007 epidemic has been examined by other studies, however the 2012 data in this study is recent enough that the author was unable to find studies that analyzed data from this year.

The local Gi* maps, Figures 5-7, display significant hot and cold spots for dengue at the district level. Over the eleven years, Phnom Penh was consistently a significant hot spot for dengue cases. The districts surrounding the capital city were also significant each year. Significance for hot spots means that the neighbors surrounding the district that was analyzed all contained high numbers of dengue fever cases and this could not occur by chance. These hot spot results would promote the use of prevention programs targeted to Phnom Penh and the surrounding districts.

The northeast and southwest regions of Cambodia were regularly cold spots for dengue cases. The northeast region of Cambodia is considered to be very poor, rural and, notably, dependable roadways to that area were only just completed in 2008 [27]. Because of the remoteness of this area, surveillance activities could be decreased or absent for many of the districts. Likewise, the southwest region of Cambodia is considered rural and has unreliable roads and transportation from the more populated areas of the country. Inhabitants of these regions are less likely to seek medical care in a health center or hospital, where dengue can be identified, simply because there is not a health facility nearby. However, the isolation could be the reason for the low numbers of dengue fever as well. With one of the lowest populations in the country and little traffic in and out of these provinces, people are not bringing in the virus or the vector is not being transported from high risk areas, such as Phnom Penh.

Siem Reap Province was similar to the Phnom Penh area, with consistent hot spots each year. The districts within Siem Reap, however, varied in significance from year to year. For example, from 2004 to 2006 no districts had significant hot or cold spots. Siem Reap contains two of the sentinel surveillance sites, which provides more accurate and timely data to the NDCP. This is likely an accurate picture of dengue in Siem Reap Province. Since the majority of the years examined in this study, showed significant hot spots for districts in Siem Reap, prevention and control programs should be targeted here as well.

Phnom Penh and Siem Reap were both consistently hot spots for dengue fever at the district level. This was expected as population density is higher and five of the sentinel surveillance sites are located in these two areas, as seen in Figure 4. With sentinel sites, surveillance is likely more sensitive and the analysis maps could simply be representative of the active surveillance at those sentinel locations.

Unexpectedly, Battambang Province and Banteay Meanchey Province both had significant hot spots nine out of the eleven years analyzed. This could due to traffic between Siem Reap and Battambang, both of which are heavily populated and transport centers for the rest of the country. Battambang Province to Banteay Meanchey Province is also the predominant route of those traveling to and from Thailand. This raises the question of whether the dengue viruses are being transported from Thailand into Cambodia or vice versa. Unfortunately, this goes beyond the scope of this study, but leaves an area that needs to be explored further. Of note, the United States Center for Disease Control and Prevention has been funding the improvement of Cambodia's TB, HIV, and influenza surveillance systems, as well as supporting the national communicable disease surveillance, of which contains dengue fever [41]. The CDC has piloted many of their activities in Battambang Province, which has allowed them to be able to digitize their surveillance reports, including dengue fever, in many districts [27]. This has allowed the NDCP to collect more accurate dengue fever data from the province.

The LISA maps, Figures 8 and 9, show similar results to the Local Gi* maps, but reveals potential spatial outliers as well. The outliers vary from year to year. These districts either have low values surrounded by high values or high values surrounded by low values. These are districts that would need further analysis done to determine if surveillance differed that year, if there was an increase of traffic or if some other occurrence could have caused either the high or low values compared to the neighboring districts.

5.1 LIMITATIONS

A limitation of this study was the use of secondary data for analysis. The data was provided by the NDCP, who handles the national surveillance of dengue fever in Cambodia. Studies have demonstrated that the national surveillance system potentially underreports due to only documenting hospitalized cases of dengue fever [3, 14, 42]. This underreporting could have an effect on the analysis of clustering and LISA hot spots, causing a misrepresentation of what areas of the country are most affected by dengue fever.

Additionally, as stated before, sentinel surveillance sites are most likely representing the majority of clusters of dengue fever. This data may not be representative of the entire country because of the two types of surveillance used to collect dengue cases in Cambodia. This study asked where the most cases of dengue fever in Cambodia are and did not take into account population density. Accounting for population and performing this analysis with incidence rates, instead of counts, could give a clearer picture of dengue fever in Cambodia.

5.2 FUTURE DIRECTIONS

Future directions for this study would include performing the analysis with incidence rates, instead of counts of dengue cases. Analyzing additional variables, such as rainfall totals or vector populations, could also be performed to give a more specific representation of dengue fever in Cambodia.

In order to reduce underreporting issues, which could affect all analyses on dengue in the country, sentinel surveillance activities should be expanded. Community-based surveillance has been found to be effective and requires little resources [43, 44].

Additional studies should be done that includes Thailand, Laos and Vietnam in order to compare incidence along borders and to determine transmission patterns between the countries.

6.0 CONCLUSION

Dengue fever has reemerged as a serious public health issue worldwide. Approximately two fifths of world’s population is at risk of contracting the virus and it is considered endemic in more than 100 countries. The majority of the dengue burden lies in Asia Pacific countries [6]. Cambodia has a large dengue burden, with 10,000-40,000 hospitalized cases per year for children less than 15 years of age [31]. As dengue fever continues to grow as a public health issue worldwide, the need for more effective control and prevention programs becomes more important.

This study examined eleven years, from 2002 to 2012, of national dengue fever surveillance data from Cambodia for geospatial patterns to determine where control and prevention programs should be targeted. A LISA and Local Gi* analysis of each year revealed that districts within Phnom Penh city and Siem Reap Province were consistently hot spots for dengue fever. Prevention and education programs should be targeted to these areas. Unexpectedly, Battambang and Banteay Meanchey Provinces had significant hot spots nine out of the eleven years analyzed. As human movements have been shown to possibly affect dengue fever transmission, these results could be due to traffic between Siem Reap and Battambang, both of which are heavily populated and transport centers for the rest of the country.

Further analyses and studies should be done for these data, as other variables, such as population, rainfall or vector population, could affect the geospatial patterns of dengue fever in

Cambodia. Given the hot spots of dengue found in this study corresponded with multiple sentinel surveillance sites, expanding this surveillance could potentially give a clearer picture of dengue patterns in the country.

APPENDIX: MAPS

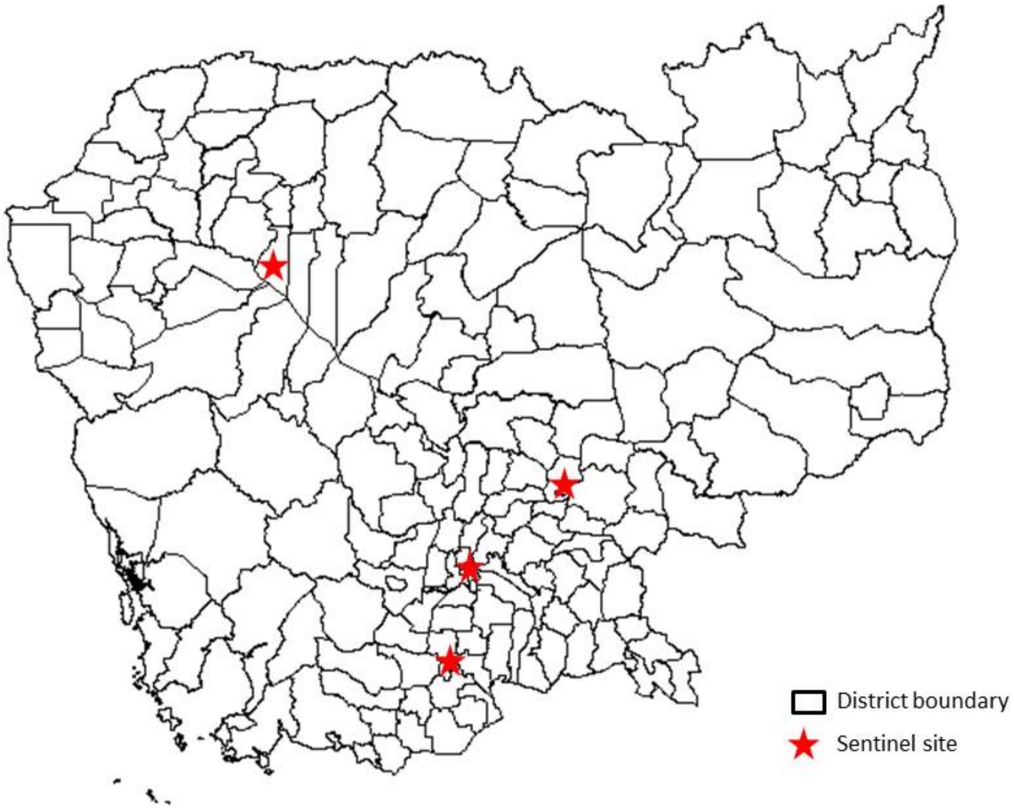


Figure 4. Sentinel Surveillance Sites

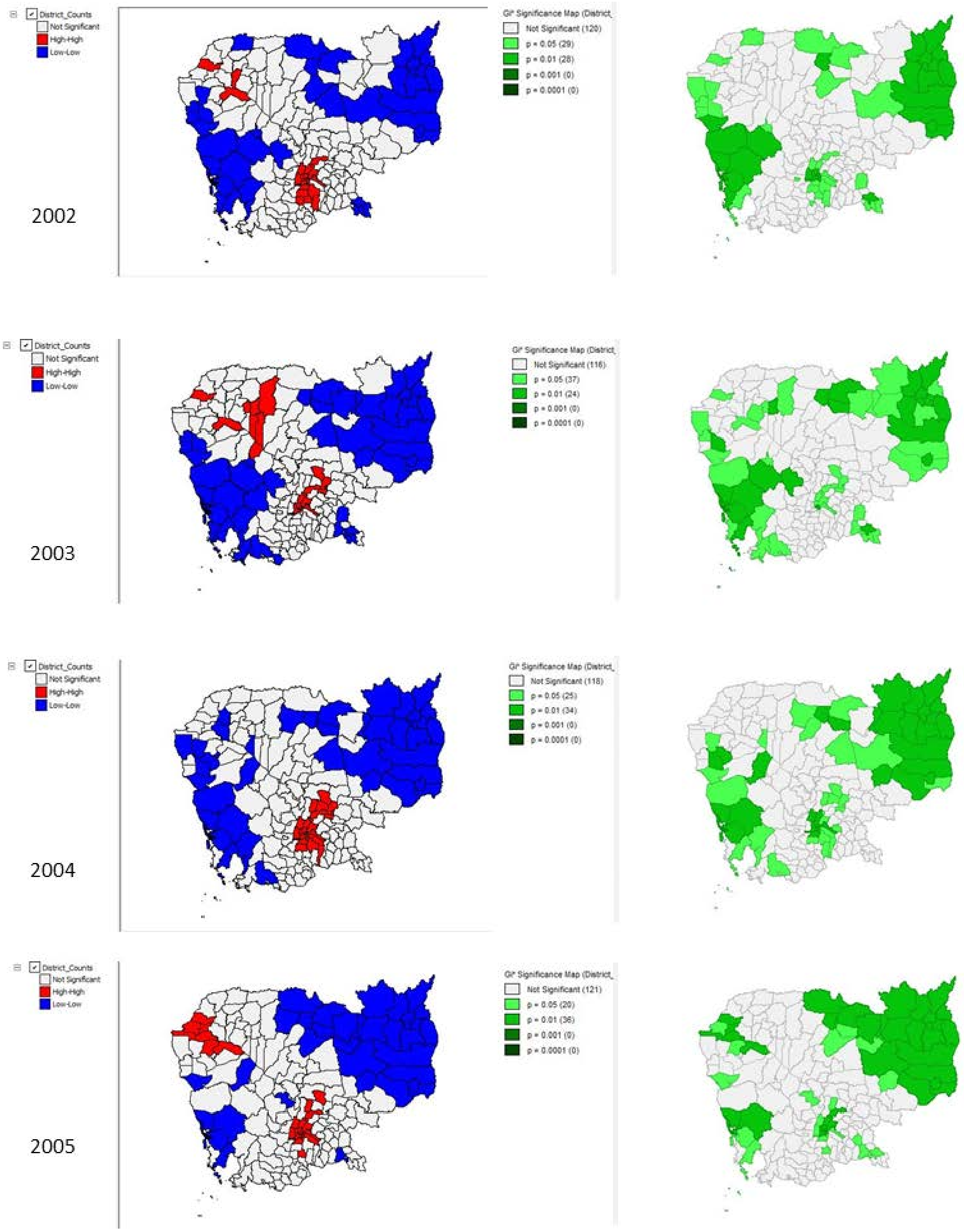


Figure 5. Local Gi* and Significance Maps 2002-2005

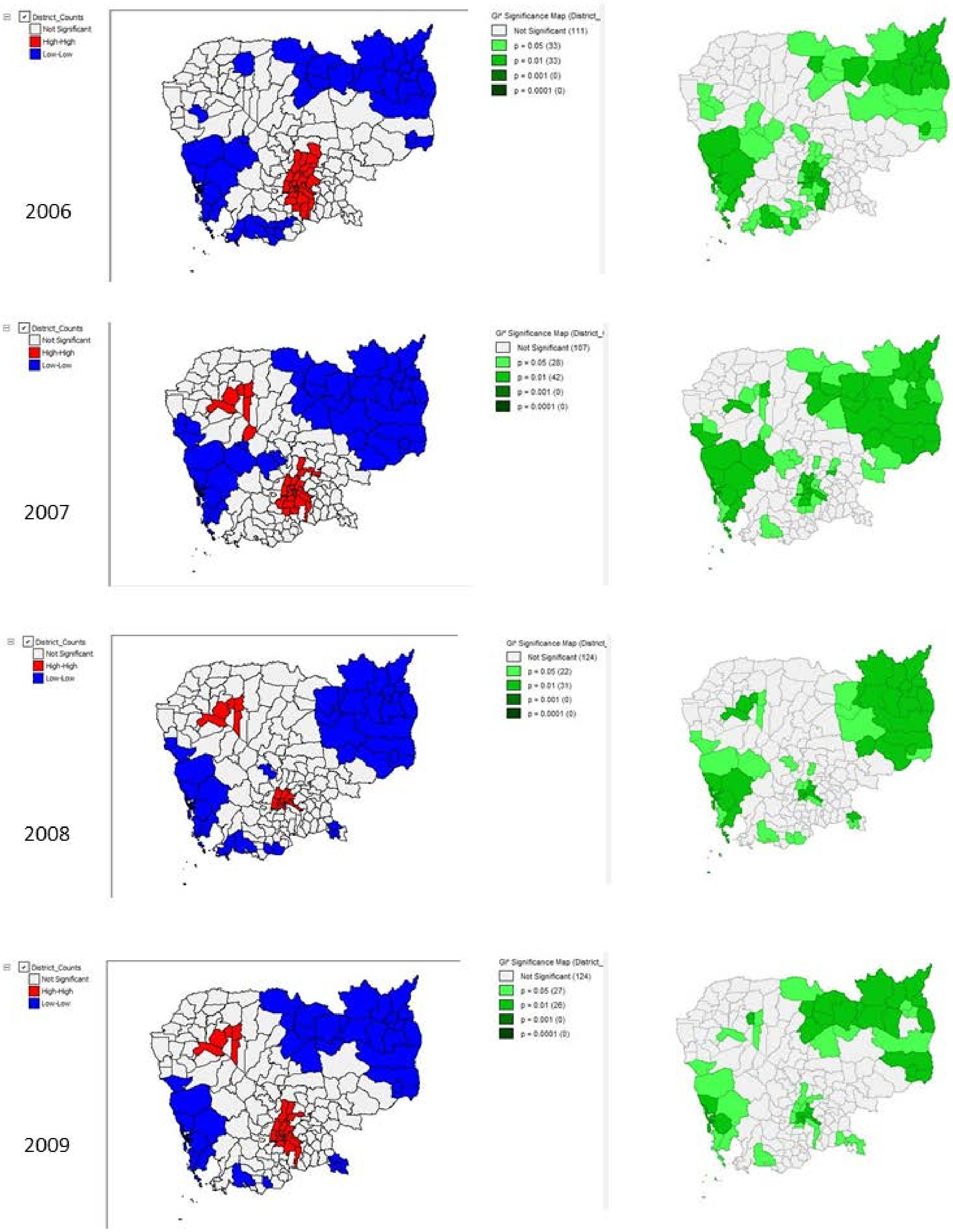


Figure 6. Local Gi* and Significance Maps 2006-2009

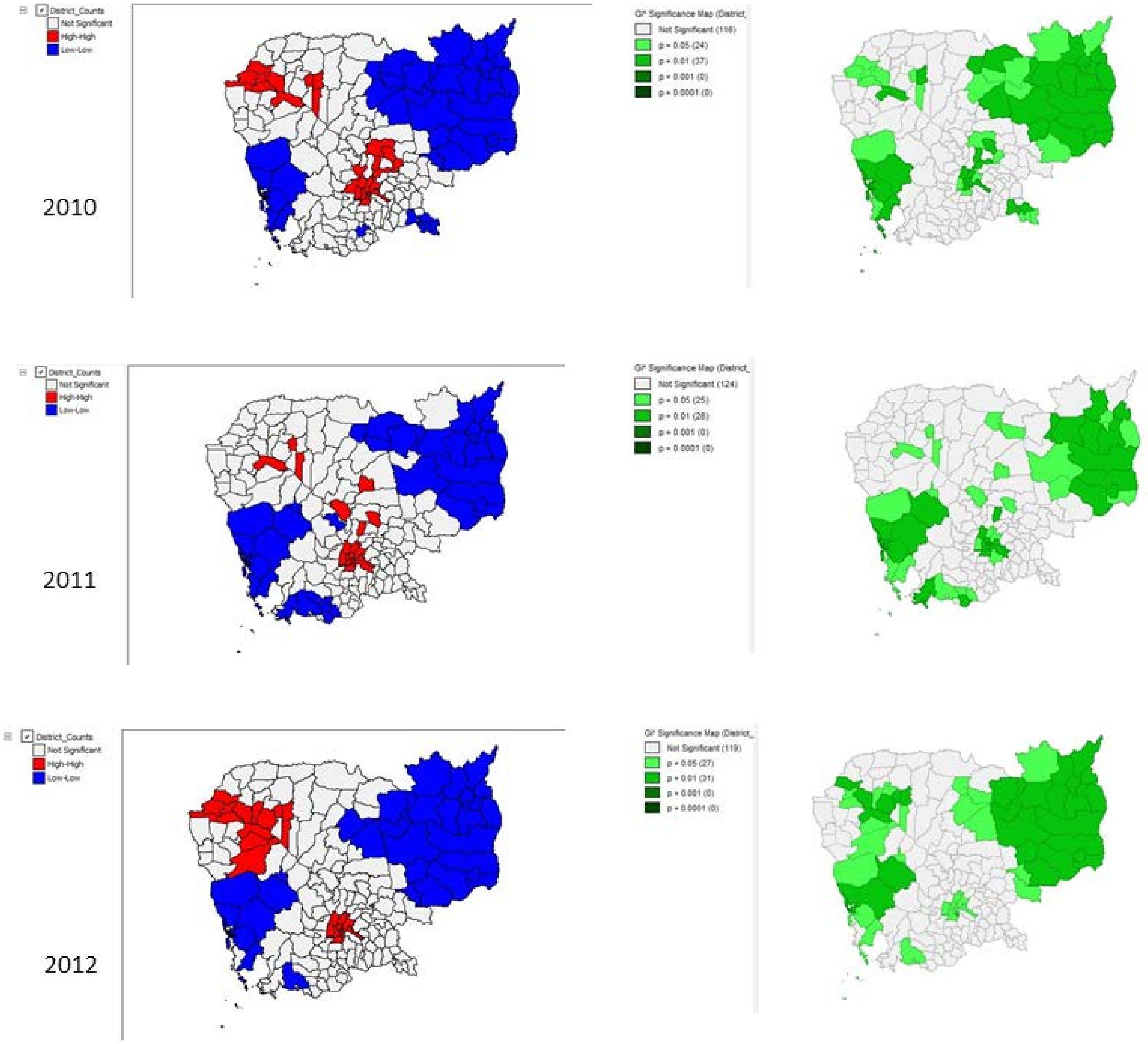


Figure 7. Local Gi* and Significance Maps 2010-2012

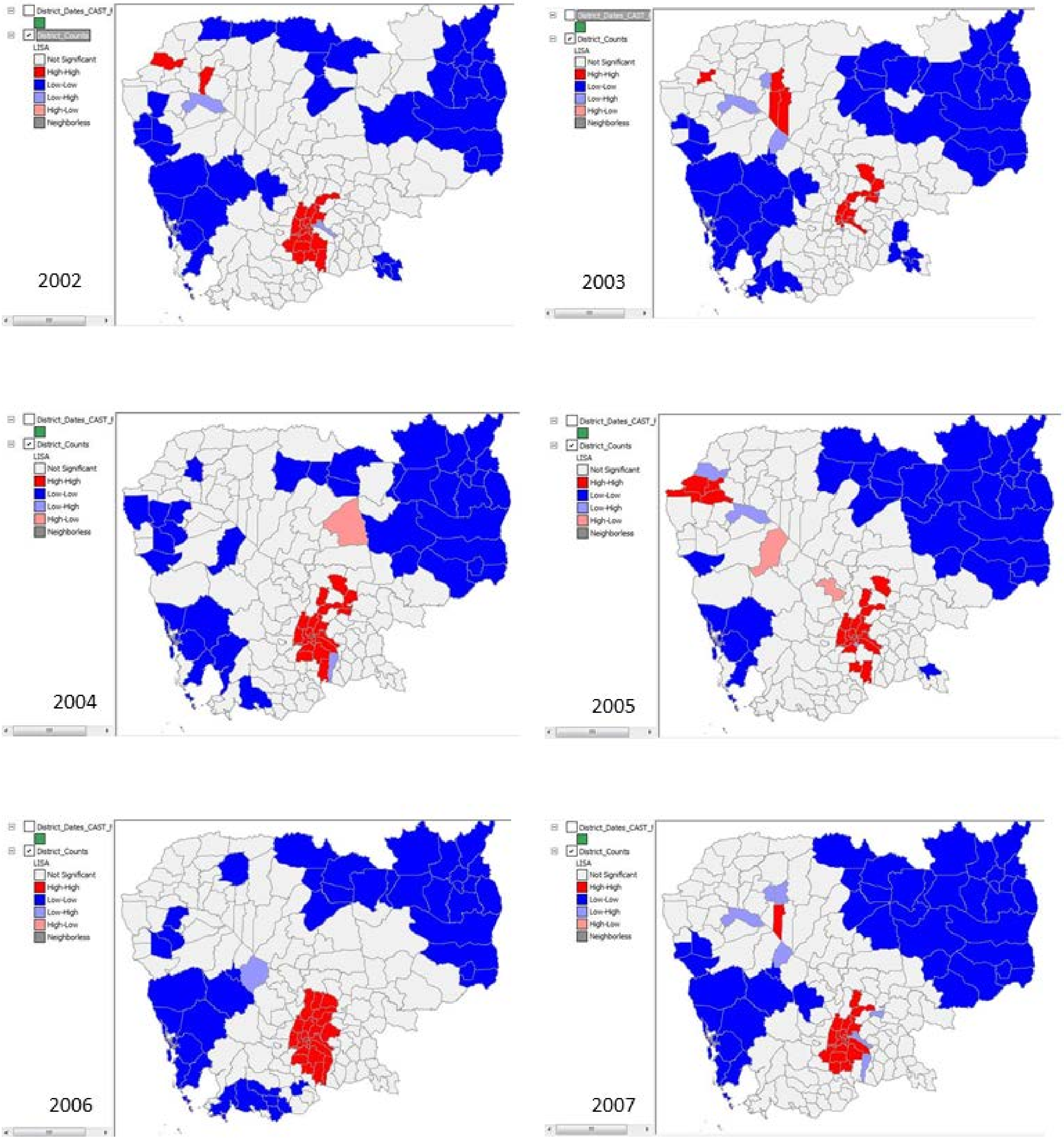


Figure 8. LISA Maps 2002-2007

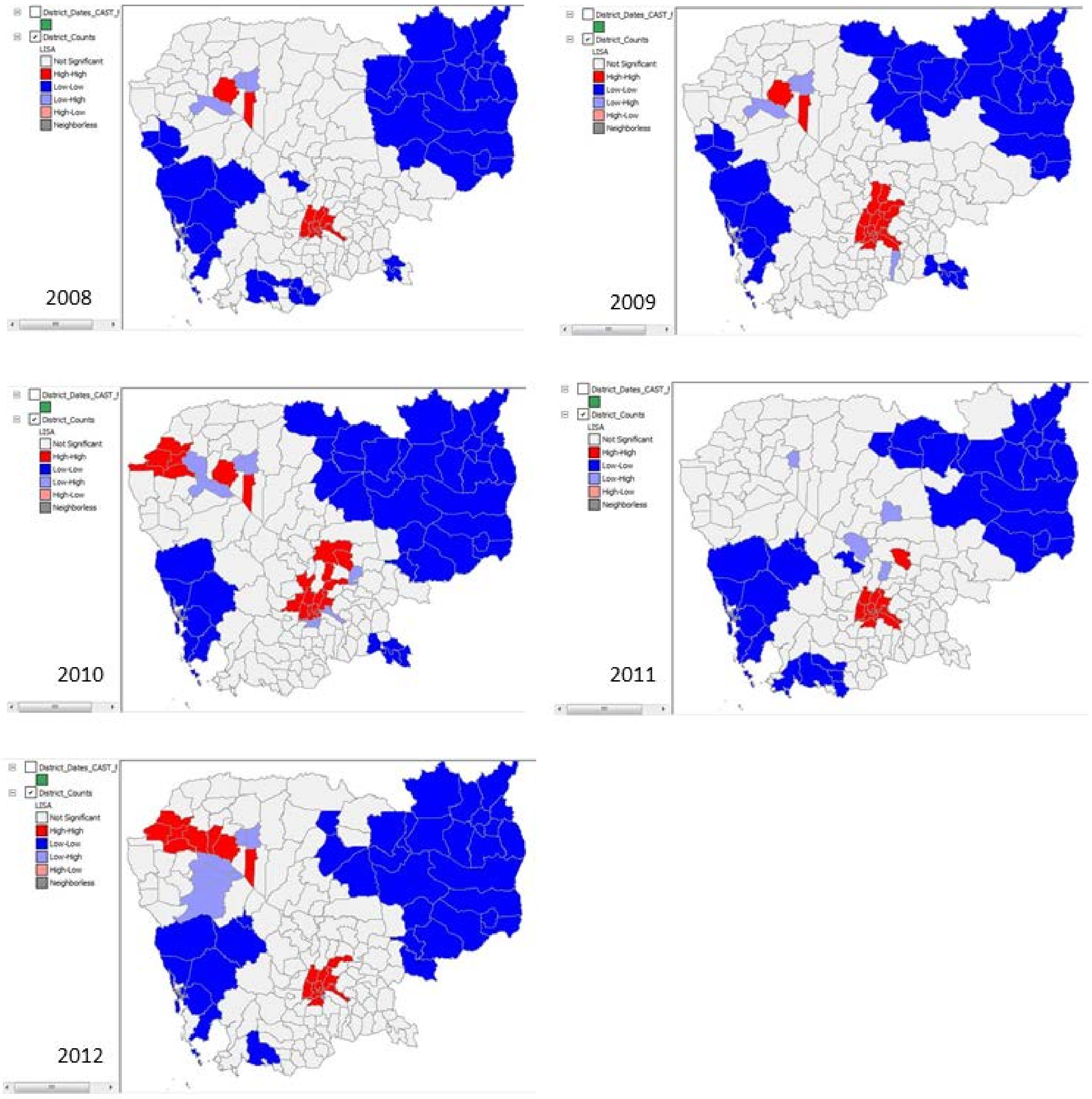


Figure 9. LISA Maps 2008-2012

BIBLIOGRAPHY

1. CDC. *World Health Day-Vector-Borne Diseases*. 2014 April 7, 2014 [cited 2014 March]; Available from: http://www.cdc.gov/features/worldhealthday2014/.
2. Guzmán, M.G. and G. Kouri, *Dengue: an update.* The Lancet Infectious Diseases, 2002. **2**(1): p. 33-42.
3. Vong, S., et al., *Under-recognition and reporting of dengue in Cambodia: a capture-recapture analysis of the National Dengue Surveillance System.* Epidemiol Infect, 2012. **140**(3): p. 491-9.
4. Gubler, D.J., *Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century.* Trends Microbiol, 2002. **10**(2): p. 100-3.
5. WHO. *Dengue and Severe Dengue*. 2013 Sept. 2013 [cited 2014 Feb. 15]; Fact Sheet No 117:[Available from: http://www.who.int/mediacentre/factsheets/fs117/en/.
6. Guzman, M.G., et al., *Dengue: a continuing global threat.* Nat Rev Microbiol, 2010. **8**(12 Suppl): p. S7-16.
7. Rigau-Pérez, J.G., et al., *Dengue and dengue haemorrhagic fever.* The Lancet, 1998. **352**(9132): p. 971-977.
8. CDC. *Dengue: Clinical Guidance*. 2013 October 28, 2013 [cited 2014 April 6]; Available from: http://www.cdc.gov/dengue/clinicallab/clinical.html.
9. Guha-Sapir, D. and B. Schimmer, *Dengue fever: new paradigms for a changing epidemiology.* Emerging Themes in Epidemiology, 2005. **2**(1): p. 1.
10. Anker, M. and Y. Arima, *Male-female differences in the number of reported incident dengue fever cases in six Asian countries.* Western Pac Surveill Response J, 2011. **2**(2): p. 17-23.
11. Shepard, D.S., E.A. Undurraga, and Y.A. Halasa, *Economic and disease burden of dengue in southeast Asia.* PLoS Negl Trop Dis, 2013. **7**(2): p. e2055.
12. *Dengue Vaccine Initiative*. [cited 2014 March]; Available from: http://www.denguevaccines.org/.
13. Sabchareon, A., et al., *Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial.* The Lancet, 2012. **380**(9853): p. 1559-1567.
14. Vong, S., et al., *Dengue incidence in urban and rural Cambodia: results from population-based active fever surveillance, 2006-2008.* PLoS Negl Trop Dis, 2010. **4**(11): p. e903.
15. Liebman, K.A., et al., *Spatial Dimensions of Dengue Virus Transmission across Interepidemic and Epidemic Periods in Iquitos, Peru (1999–2003).* PLoS Negl Trop Dis, 2012. **6**(2): p. e1472.

16. Chareonsook, O., et al., *Changing epidemiology of dengue hemorrhagic fever in Thailand.* Epidemiol Infect, 1999. **122**(1): p. 161-6.
17. Kumar, A., et al., *An outbreak of dengue fever in rural areas of northern India.* J Commun Dis, 2001. **33**(4): p. 274-81.
18. Strickman, D., et al., *Distribution of dengue and Japanese encephalitis among children in rural and suburban Thai villages.* Am J Trop Med Hyg, 2000. **63**(1-2): p. 27-35.
19. Morens, D.M., et al., *Dengue in Puerto Rico, 1977: public health response to characterize and control an epidemic of multiple serotypes.* Am J Trop Med Hyg, 1986. **35**(1): p. 197-211.
20. Githeko, A.K., et al., *Climate change and vector-borne diseases: a regional analysis.* Bull World Health Organ, 2000. **78**(9): p. 1136-47.
21. Barbazan, P., S. Yoksan, and J.P. Gonzalez, *Dengue hemorrhagic fever epidemiology in Thailand: description and forecasting of epidemics.* Microbes Infect, 2002. **4**(7): p. 699-705.
22. *World Development Indicators*, 2012, ESDS International.
23. *The World Factbook*. 2014 Jan. 28, 2014 Feb. 11, 2014]; Available from: https://www.cia.gov/library/publications/the-world-factbook/geos/cb.html.
24. National Institute of Statistics (Cambodia), et al., *Cambodia demographic and health survey*.
25. *Global Burden of Disease Study 2010: Cambodia Country Report*, 2012, Institute for Health Metrics and Evaluation: Seattle, Washington.
26. *GBD Arrow Diagram*, 2013: University of Washington.
27. Huy, R., 2013.
28. Khun, S. and L.H. Manderson, *Abate distribution and dengue control in rural Cambodia.* Acta Tropica, 2007. **101**(2): p. 139-146.
29. Khun, S. and L. Manderson, *Community and school-based health education for dengue control in rural Cambodia: a process evaluation.* PLoS neglected tropical diseases, 2007. **1**(3): p. e143.
30. *Standard Operating Procedures of the National Dengue Sentinel Surveillance System*, 2010, National Dengue Control Program: Phnom Penh, Cambodia.
31. Huy, R., et al., *National dengue surveillance in Cambodia 1980-2008: epidemiological and virological trends and the impact of vector control.* Bull World Health Organ, 2010. **88**(9): p. 650-7.
32. *Dengue haemorrhagic fever: Diagnosis, treatment, prevention and control*, 1997, World Health Organization: Geneva, Switzerland.
33. Luc Anselin, I.S., Kho Youngihn, *GeoDa: An Introduction to Spatial Data Analysis.* Geographical Analysis, 2006. **38**(1): p. 5-22.
34. *Global Administrative Areas: Boundaries without limits*, 2012.
35. *Local G Statistics*. [cited 2014 March 1]; Available from: http://www.biomedware.com/files/documentation/spacestat/Statistics/G/Local_G_Statistic.htm.
36. Anselin, L., *Local indicators of spatial association—LISA.* Geographical analysis, 1995. **27**(2): p. 93-115.
37. Esri, *ArcGIS Resource Center*, 2012.
38. Arima, Y., et al., *Epidemiologic update on the dengue situation in the Western Pacific Region, 2011.* Western Pac Surveill Response J, 2013. **4**(2): p. 47-54.

39. Gibbons, R.V. and D.W. Vaughn, *Dengue: an escalating problem.* BMJ: British Medical Journal, 2002. **324**(7353): p. 1563.
40. Banu, S., et al., *Dynamic spatiotemporal trends of dengue transmission in the Asia-Pacific region during 1955.* EXAMINING THE IMPACT OF CLIMATE CHANGE ON DENGUE TRANSMISSION IN THE ASIA-PACIFIC, 2013: p. 45.
41. CDC, *Influenza Division International Activities*, 2011, U.S. Centers for Disease Control and Prevention. p. 169-174.
42. Wichmann, O., et al., *Dengue in Thailand and Cambodia: an assessment of the degree of underrecognized disease burden based on reported cases.* PLoS Negl Trop Dis, 2011. **5**(3): p. e996.
43. Oum, S., D. Chandramohan, and S. Cairncross, *Community-based surveillance: a pilot study from rural Cambodia.* Tropical medicine & international health, 2005. **10**(7): p. 689-697.
44. Ndiaye, S.M., et al., *The value of community participation in disease surveillance: a case study from Niger.* Health Promot Int, 2003. **18**(2): p. 89-98.