High Rates of Inapparent Dengue Cases and Dengue Spatial Clustering in a Prospective Cohort Study in Northern Venezuela

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INTRODUCTION

Dengue is an emerging arthropod-borne viral disease, caused by the dengue virus (family Flaviviridae, genus Flavivirus) and transmitted to humans through the bite of female Aedes aegypti mosquitoes. Dengue virus (DENV) comprises 4 serotypes (DENV-1 to DENV-4) which are the causative agents of dengue fever and dengue severe disease (previously dengue hemorrhagic fever). This disease poses a major public health burden with an estimated 390 million annual infections (Bhatt et al 2013) affecting 128 countries within tropical and subtropical regions (Simmons et al, 2012; WHO, 2018). However, dengue is not constrained to tropical regions anymore, having recent autochthonous transmission in new areas not previously considered under risk such as Europe (ECDC, 2017). In Venezuela, dengue is endo-epidemic and a major cause of morbidity, with the co-circulation of the 4 serotypes. Dengue incidence has shown an increasing trend since 2001 with an average of 39.5 cases x 100,000 inhabitants in the early 1990’s to a 10-fold higher mean incidence of 368 cases x 100,000 inhabitants in the last 6 years (2010-2016) (Vincenti-Gonzalez et al 2018). These data are still an underestimation of the real burden of disease given routine underreporting and the presence of an important proportion of inapparent dengue cases (those with subclinical or asymptomatic disease). It has been estimated that around 50-75% of all dengue infections correspond to inapparent cases (Bhatt et al 2013; Endy et al 2002; Espino et al 2010). Since control measures are triggered by the report of symptomatic cases, the occurrence of innaparent cases and the “silent” transmission they originate will go undetected by the regular surveillance systems (Endy et al, 2011; Yap et al., 2013) having important implications for disease management and control.

Dengue shows a heterogeneous pattern of occurrence. This heterogeneity, similar to that of other infectious diseases, is influenced by multiple factors that interact concomitantly such as: spatial and social structure of the human population, population size, differences in climate patterns (rainfall, humidity and temperature), population movement and social interaction, socio-economic variables and the dynamics of the vector (Hagenaars et al, 2004; Favier et al, 2005; Stoddard et al, 2013; Reiner et al, 2014). These factors modulate the spread of dengue at a local and global level differently (Teurlai et al, 2012; Stoddard et al, 2013). It has been reported that the transmission of dengue is spatially local, occurring mainly at the neighbourhood or household level (Salje et al, 2012; Rabaa et al, 2012; Vincenti-Gonzalez et al, 2017). Such local transmission is influenced by: 1) the domestic nature of the vector, which exhibits a limited flight range (<100 m) (Harrington et al., 2005; Getis et al., 2003), 2) socio-economic risk factors such as overcrowding, housing type (Velasco-Salas et al, 2014) and 3) deficiency in public services as garbage collection and running water that in turn promote the development of mosquito breeding sites (Barrera et al, 1995). Earlier studies conducted in Venezuela, had shown that there are areas at higher risk of dengue transmission than other (hot spots) (Barrera et al, 2000, Vincenti-Gonzalez et al, 2017). As result of these local patches of disease, the areas/houses with higher transmission risk may account for the majority of the transmission (Yoon et al, 2012).

The above statement reinforces the necessity of cohort studies for a better understanding of the role that dengue inapparent infections can have in the estimation of the real burden of dengue and the degree of dengue transmission within a community (Endy et al, 2011; Bhatt et al, 2013). In addition, the relevance of the study of dengue heterogeneity at local level is based on the creation of sufficient information to establish the most likely risk areas of disease transmission to aim for the maximization of limited resources for dengue control. In this study, we aim to identify hot spots of
recent DENV transmission that are maintained in space and time and estimate the proportion of inapparent dengue infections within high dengue incidence neighborhoods in Maracay between 2010 and 2013.

**MATERIAL AND METHODS**

The area of study is located in Maracay, a dengue hyperendemic city in northern Venezuela. From 2010 to 2014, we conducted a longitudinal study that initially had a cohort of approximately 2000 individuals. Every year, a cross-sectional seroepidemiologic survey was carried out in three neighbourhoods within Maracay city: Caña de Azucar, Candelaria and Cooperativa (Figure 1a). A total of four surveys were completed: Baseline (2010), Survey 1 (2011), Survey 2 (2012) and Survey 3 (2013). The full description of the cohort study is presented elsewhere (Velasco-Salas et al., 2014).

Briefly, in 2010 a total of 2014 consenting individuals aged 5-30 years inhabiting 840 households within the above-mentioned neighborhoods were enrolled in the study (Figure 1b). The study protocol was clearly explained to all members of the household. Patient enrollment occurred after invited individuals read and signed a written informed consent. Individual and household questionnaires were applied to collect information regarding demographics, clinical symptoms of dengue and epidemiologic data, while serological and hematological data were acquired through blood sample collections and laboratory tests (Velasco-Salas et al., 2014). A unique household code was associated to the spatial coordinates obtained through a Global Positioning System device (GPS, Garmin Ltd.). Due to violence during anti-governmental protests in the country during the time of Survey 3, data and sampling collection activities were affected resulting in a lower number of participants included. The study was approved by the Ethics Review Committee of the Biomedical Research Institute, Carabobo University (Aval Bioetico #CBIIB(UC)-014), Maracay, Venezuela; the Ethics, Bioethics and Biodiversity Committee (CEBioBio) of the National Foundation for Science, Technology and Innovation (FONACIT) of the Ministry of Science, Technology and Innovation, Caracas, Venezuela; and by the Regional Health authorities of Aragua State (CORPOSALUD Aragua).

![Figure 1](https://www.qgis.org/).

The identification of hot spots was assessed using the local Getis statistic ($G_i^*(d)$) (Getis & Ord, 1992), a local measure of spatial association, which can detect significant local clustering of high
positive (hot spots) values of dengue prevalence around each point (e.g. household infection) within a specific radius of specified distance $d$ from that location (e.g. 20 and 90 meters). The search for disease clusters was analyzed at two spatial scales: at household level, and at block level. In our case, this analysis identified an excess of recent dengue infections at block level between the years of 2011, 2012 and 2013 (Survey 1, 2 and 3). Multinomial Monte Carlo simulations were applied to obtained the probability for the most likely hot spot (Getis) ($P < 0.05$). The analyses of $G_i^*(d)$ were carried out in ARCGIS (ESRI Corporation, Redlands, CA) using the Spatial Statistics Tools toolbox. The results were shown in maps using the software QGIS 1.8.0-Lisboa (GNU – General Public License). The satellite images of each neighborhood were obtained from Google Earth TM. For the sake of this short communication, maps and spatial analysis show the most relevant results, which were obtained for the areas of Candelaria and Caña de Azúcar.

RESULTS

The total number of individuals from which blood samples were obtained in each yearly Survey was as follows: Baseline (n=1994), Survey 1 (n=1873), Survey 2 (n=1187), Survey 3 (n=575). Blood samples were collected, stored in cooling box storage containers during transport, processed, aliquoted and stored at -20oC until they were used. Recent dengue infections were determined using two different methods depending on the year of the corresponding sero-Survey. Dengue seropositivity from samples from the baseline period (2010) was determined using the Hemaglutination Inhibition (HI) Assay (Recent dengue infection: HI titres ≥ 1280) as described in detail in Velasco-Salas et al., 2014. Dengue seropositivity from samples from Survey 1, 2 and 3 were tested using a capture dengue IgM enzyme-linked immunosorbent assay (ELISA) (PanBio, Windsor, Queensland, Australia) following manufacturer’s instructions. We assumed that an HI titer ≥ 1280 or the presence of IgM against dengue, indicated a recent dengue infection.

Figure 2 shows the results of recent dengue seroprevalence and the proportion of inapparent cases by Survey. Overall, the average recent dengue seroprevalence throughout the study was 10.06% (range= 2.78-14.34). In general, since 2010, recent dengue seroprevalence showed an increase, except for the year 2011. The values of recent seroprevalence for every survey year describe concordantly the epidemiological situation that was present during the years of data collection. Figure 2 shows that years 2010 and 2013 (national epidemic years) exhibited high dengue recent seroprevalence. Similarly, the year of 2012 -described as a local epidemic year for Aragua state- showed also high values of dengue seroprevalence (MPPS-Boletines Epidemiologicos)

![Figure 2](image-url) - Recent dengue seroprevalence for the years under study.
During Survey 1 and Survey 3, those individuals who tested positive by IgM against dengue but who had no recollection of having had fever and/or other symptoms (headache, chills, body aches, myalgia, retro-orbicular pain, nausea) in the previous three months, were considered inapparent dengue cases (IDC). Accordingly, for both Survey 1 and Survey 3, 66 and 68% of the total IgM positive individuals were categorized as inapparent infections. The overall inapparent-symptomatic ratio (I:S) was approximately 2:1. In Survey 1, from a total of 53 DENV infections among enrollees, 35 (66%) were inapparent, and 18 were symptomatic, whereas for Survey 3, from a total of 69 DENV infections, 47 (68%) were inapparent, and 22 were symptomatic (Figure 2). Between individuals with inapparent infections, there were no gender differences (P>0.05). Furthermore, when stratified by age groups, in Survey 1, age groups 2 (11-15yr) and 5 (26-30) showed the highest proportion of inapparent dengue infections, while in Survey 3, these infections tended to decrease with age, although, individuals with age ranging between 26-30 years (Age group 5) also showed a high proportion of inapparent dengue (Figure 3). Regarding the neighborhoods were these inapparent infections were found, there were differences between Survey 1 and Survey 3. During Survey 1, the majority of inapparent dengue cases were found in Caña de Azucar neighborhood, while in Survey 3, the majority of these infections were found in Candelaria neighborhood. Also, inapparent dengue infections were present in more than one individual living in the same house.

Seroprevalence maps and hotspot identification of recent dengue infections portrayed spatial heterogeneity in each one of the Surveys (Figure 4). Furthermore, the highest frequency of recent dengue transmission at block level (seroprevalence >21%) was found in Caña de Azucar neighborhood for the years 2010 (Baseline), and 2012 (Survey 2) (Vincenti-Gonzalez et al., 2017 and Fig 3), while Candelaria neighborhood showed high values of recent dengue during the year 2013 (Survey 3).

With regards to the spatial distribution, Survey 1showed a recent dengue seroprevalence below 5% at block level in all neighborhoods under study (Figure 4a). In comparison, dengue seroprevalence substantially changed in Survey 2 where several blocks displayed a seroprevalence above 21%. Specifically, Caña de Azúcar neighborhood depicted a higher seroprevalence average (17.55 %, range=0-43%) compared with Candelaria (9.90 %, range=0-33%). During Survey 3, Candelaria was the neighbourhood with the greatest recent dengue seroprevalence (average 16.54%, range=
0-53%), while Caña de Azúcar showed an average of 8.14% (range=0-36.66) (Figure 4c), although the results of this last Survey in Caña de Azúcar neighborhood must be taken carefully, since a considerable number of individuals were not Surveyed as previously explained.

In this study, significant hot spots at two spatial scales (household and block) were identified for recent dengue seroprevalence in all Survey years. During Survey 1, spatial statistics showed that most of the recently infected individuals were spatially located towards the northern side of Caña de Azúcar neighborhood (Figure 4a). The local Getis statistic test identified 2 hot spots at block level (blocks 30 and 79) and 3 hot spots at household level in Caña de Azúcar area. Candelaria was the neighbourhood with more hotspots at household level (total of 12 hotspots), while at block level 2 hot spots (blocks 07 and 26) were found (Figure 4a). Figure 4b depicts the spatial distribution of hotspots at block and household level belonging to Survey 2. The number of household hotspots increased significantly compared with the previous Survey, with a total of 36 hot spots between Candelaria and Caña de Azucar neighborhoods. In total, 3 blocks (13,39,85) were identified as hotspots for both Caña de Azúcar and Candelaria. Regarding hotspots at household level, Caña de Azúcar showed 2 times more hotspots (n=24) compared with Candelaria (n=12). Finally, in Survey 3 a total of 12 households and 2 block hotspots were found in Candelaria, while in Caña de Azucar 3 household and 1 block hotspots were identified (Figure 4c). The maximum distance at which the hot spots were located varies depending on the spatial scale. The hot spots at the household level were detected at a distance of 20 meters, whilst detection of hot spots at block level was set at 90 meters.

About the composition of the hotspots encountered at both spatial scales, in Survey 1 and Survey 3, the hotspots that were identified were mostly constituted by houses that had at least one case of inapparent dengue. This was expected, since inapparent cases occurred twice as often compared with symptomatic cases (I:S ratio: 2:1). However, in both Surveys, more than the half of the houses identified as hot spots were constituted only (100%) by cases of inapparent dengue (Table 1, inapparent/ positives (%)). At the household scale, 8 hot spots out of 12 (Survey 1) and 9 hot spots out of 14 (Survey 3) were solely constituted by inapparent dengue cases (Table 1, Figure 4).
Figure 4.- Recent dengue seroprevalence and distribution of inapparent dengue cases in Candelaria and Caña de Azucar neighboorhoods. a) Survey 1- year 2011 b) Survey 2- year 2012 c) Survey 3- year 2013. Blocks depicts recent dengue seroprevalence (%). Blue encircled blocks show the results of the local Getis statistic (Gi*(d)) analyses at a distance of 90 meters, with significant (P <0.05) clusters of recent dengue infection at block level (hot spots). Red dots show the results of Gi*(d) analyses at a distance of 20 meters with significant clustering of recent dengue infection at household level (Gi*(d) >1.96, P <0.05). Black dots indicate Surveyed households. Green hexagons indicate households that contained at least one positive case of recent dengue (IgM), while orange circles shows the households constituted solely by inapparent dengue cases. Original figure made by Maria Vincenti-Gonzalez with QGIS software (version 2.18, https://http://www.qgis.org/).
Table 1.- Detailed description of the dengue inapparent-symptomatic individuals within a hot spot household. Part a: hot spot households identified in Survey 1. Part b: hot spot households identified in Survey 3.

<table>
<thead>
<tr>
<th>Part a.- Survey 1</th>
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<td>Hot spot households</td>
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<td>2</td>
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| Hot spot households | Positive IgM (n) | ID | Positive IgM (n) | ID |
|-------------------|-------------------|-------------------|
|                    | • | IgM prevalence (%) | • | IgM prevalence (%) |
| 237                | 1 | 1 | 100 | yes | 1 | 100 |
| 246                | 1 | 1 | 100 | yes | 1 | 100 |
| 267                | 1 | 2 | 50 | yes | 1 | 100 |
| 269                | 1 | 2 | 50 | no | 0 | 0 |
| 281                | 2 | 2 | 100 | yes | 2 | 100 |
| 309                | 2 | 2 | 100 | yes | 1 | 50 |
| 314                | 1 | 1 | 100 | no | 0 | 0 |
| 318                | 1 | 1 | 100 | yes | 1 | 100 |
| 446                | 2 | 2 | 100 | yes | 2 | 100 |
| 466                | 2 | 2 | 100 | no | 0 | 0 |
| 672                | 1 | 3 | 33.33333333 | yes | 1 | 100 |
| 673                | 1 | 1 | 100 | yes | 1 | 100 |
| 711                | 1 | 1 | 100 | yes | 1 | 100 |
| 714                | 1 | 1 | 100 | no | 0 | 0 |

**DISCUSSION**

Our main results showed space and time heterogeneity of dengue at the local level (households and blocks) within the neighborhoods under study. In this study we show that: a) Overall recent dengue seroprevalence throughout the study period was >10% b) Among seropositive individuals, 66% in both survey 1 & 3 were defined as inapparent dengue cases c) During the study period, we detected significant spatial clusters of dengue infections in all neighborhoods at a distance of 20 and 90 meters d) half of the households identified as hot spots were constituted entirely (100%) by clusters of inapparent dengue cases.

Important values of recent dengue seroprevalence were found during the years under study. These results were expected if we consider that in 2010 one of the greatest dengue epidemics occurred, affecting a large number of individuals (>120,000 individuals). This large epidemic could have conferred partial immune protection (herd immunity) to a great part of the country population, which may explain the low values for dengue seroprevalence obtained for 2011 (post-epidemic year). Regarding the years of Survey 2 and 3, the year 2012 exhibited a local dengue epidemic in Aragua state (6,878 cases, 14% of national total cases), while the year 2013 was also a major national dengue epidemic (>63,000 cases) (MPPS-Boletines epidemiologicos).

With respect to inapparent dengue infections, we found a high rate of inapparent dengue cases...
High Rates of Inapparent Dengue Cases

(66%, I:S ratio 2:1), a value that is concordant with what has been reported previously (Yap et al., 2013, Wang et al., 2015; Bhatt et al 2013). The majority of the research related to dengue inapparent infections has been done in children, while studies focused on adults are less frequent (Yap et al., 2013; Grange et al., 2014). These studies have shown that the majority of asymptomatic infections occur in children, while adults tend to present more symptoms (Endy et al, 2011; Yap et al., 2013; Mohsin et al., 2016). In contrast, our results show that the proportion of inapparent dengue by age group had a U-like shape, were most of these infections were found in individuals belonging to younger and older age groups. In our case, children between 5-15 years and adults (26-30 years) showed higher proportions of inapparent dengue. The high proportion of inapparent infections in our adult population may be explained by the high transmission intensity of our area of study where most individuals have been exposed to the different dengue serotypes at an early stage (children and young adults) conferring older people partial cross-protective immunity (Yap et al., 2013). This is has been shown in previous studies in Aragua State where by the age of 18 years individuals are likely to have suffered more than 2 dengue infections in their lifetime (Comach et al., 2009; Espino et al., 2010). Moreover, the results about hot spot households being constituted solely by inapparent cases raises important questions about the transmission dynamics of dengue in urban settlements where transmission rate is high. The latter raises some questions about how effective is the current approach of “first symptomatic case report then mosquito control measures” that is commonly applied by the public health programs, since inapparent cases are undetected by the same public health programs (house fogging, etc.) (Barrera et al, 2002). The social interactions have proven to favor the transmission of dengue at the local level, where the infections can occur when one individual visits places where contact with infected mosquitoes is likely (Stoddard et al, 2013). Likewise, individuals that are infectious but who do not feel disease, can continue with their normal social interactions within friends and family, promoting disease transmission and propagation at the local level (Reiner et al, 2014). This has important implication for disease control: since inapparent individuals do not seek medical care, the case is not registered and therefore does not trigger house fogging and other mosquito control measures. Previous studies have shown that hot spot houses exhibit a series of risk factors that enhance dengue transmission (Vincenti-Gonzalez et al, 2017) so when a hot spot household is solely constituted by inapparent dengue cases, this house goes undetected by the public health services and therefore, keeps providing sufficient conditions for Aedes aegypti to breed at the household level, letting disease transmission to continue.

Seroprevalence maps and hotspot identification of recent dengue infections portrayed a great spatial heterogeneity in all years and significant hot spots at household and block level were identified at a distance of 20 and 90 meters suggesting that relevant features modulating dengue transmission occurs at such small and focal scale (Getis et al, 2003; Braga et al, 2010; Yew et al, 2009). Hot spots at both spatial scales were found in each one of the Surveys in Caña de Azucar neighborhood, and specially for Survey 2, a remarkable accumulation of hot spots at household level is depicted. This suggests that there is a sustained maintenance of the risk factors that facilitate the persistent transmission of dengue in this area. Previous studies have shown certain risk factors to be related with the heterogeneity and clustering of dengue cases such as water storage, poverty, litter accumulation, mosquito density (Teixeira et al, 2011; Stewart-Ibarra et al, 2014, Velasco-Salas et al, 2014; Vincenti-Gonzalez et al, 2017). The distance between cases/houses is an important factor for dengue transmission. As human social interactions tend to decrease with distance at the house/neighborhood level, so dengue transmission does, having that dengue transmission
is optimal in areas within <100 meters from a positive case (Yoon et al, 2012; Sharma et al, 2014). Nonetheless, we do not disregard the influence of social structured human movements in shaping heterogeneous transmission patterns of dengue, and this needs to be further investigated (Reiner et al, 2014). Furthermore, the transmission of dengue may increase in areas considered as low risk for dengue if the socio-economic and environmental conditions surrounding these areas worsen. An example of this is Candelaria neighborhood. When comparing the results between the baseline study (Velasco-Salas et al, 2014; Vincenti-Gonzalez et al., 2017), and the rest of the Surveys (Surveys 1,2, 3), a significant increase in hot spots at the house and block level is noteworthy. The increase in hot spots may be related to a series of factors that have facilitated the persistence of dengue infections over time in this neighborhood, such as: unreliable piped water, delays in garbage collection, neglect in vector management and control. Furthermore, since the beginning of this longitudinal study in 2010, the deterioration of public health services and public services has been reported, as well as an increase in poverty rates country-wide (ENCOVI-2017, PRODAVINCI-2018). This is due to the intense political destabilization and the collapse of the economy in Venezuela, which has allowed the hazardous and sustained increase of multiple vector-borne diseases, including dengue, in all the national territory (Tami, 2014; Hotez et al, 2017, Hotez, 2017, Grillet et al, 2018).

Dengue is a world-wide public health concern. Therefore, to understand the disease dynamics at the local level can help to design new tailored strategies of vector control and disease management, using the limited resources available but in an effective way. The application of a more focalize vector/disease control at the small geographic scale, may have important impacts at bigger geographical scales (municipality, state, country). Furthermore, the exploration of inapparent infections within a dengue endemic area can help to understand the real burden of this disease, the transmission dynamics of dengue during epidemics-non-epidemics periods and finally, for a more realistic calculation of the minimum amount of DENV protected individuals that are needed to fully protect the population in future vaccination programs.
REFERENCES


