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Prevalence of dengue antibodies in healthy children and adults in different Colombian endemic areas

Myriam L. Velandia-Romero[a], Carolina Coronel-Ruiz[a], Lorena Castro-Bonilla[a], Sigrid Camacho-Ortega[a], María Angélica Calderón-Peláez[a], Angélica Castellanos[b], Víctor Alberto Olano[c], Alexandra Porras[d], Myriam Arevalo-Herrera[b], Wilmer Villamil-Gómez[e], Jaime E. Castellanos[a,*]

[a] Universidad El Bosque, Vicerrectoría de Investigaciones, Grupo de Virología, Bogotá, Colombia
[b] Centro de Investigación Científica Caucaseco, Cali, Colombia
[c] Instituto de Salud y Ambiente, Universidad El Bosque, Bogotá, Colombia
[d] Universidad El Bosque, Facultad de Medicina, Grupo de Medicina Comunitaria, Bogotá, Colombia
[e] Hospital Universitario de Sincelejo, Sucre, Programa Doctorado de Medicina Tropical Universidad del Atlántico, Barranquilla, Colombia

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ABSTRACT

Objectives: Colombia is a dengue hyperendemic country; however, the prevalence of antibodies against dengue in the general population including the inhabitants of rural areas is unknown. This study aimed to determine the prevalence of dengue IgM and IgG antibodies in healthy children and adults in urban and rural areas of seven different endemic regions in Colombia between 2013 and 2015.
Design or method: Blood samples from healthy volunteers (1,318) were processed by serology (by indirect IgG and capture IgM and IgG ELISA) and molecular tests to detect viral RNA and circulating serotypes.
Results: The seroprevalence of IgG for dengue were 85% in children and over 90% for adults. In addition to the high IgM positive rate (14.9%) and secondary recent infection marker rate (capture IgG, 16%), 8.4% of the healthy volunteers were positive for dengue virus (DENV) RNA.
Conclusion: This study confirmed the broad and permanent circulation of DENV in Colombia and the high rates of infection and reinfection suffered by its inhabitants. This information can be used by the health authorities to strengthen vector control and vaccine policies and review the algorithms of diagnosis and disease management in children and adults.

Introduction

Dengue is a vector-borne disease with a significant impact on Latin America. According to the annual report of the Pan American Health Organization, Colombia is the main contributor of dengue in the Andean region, and the second in Latin America, with an incidence of 321.4 cases per 100,000 inhabitants in 2016 (WHO, 2017). Colombia is hyperendemic (Guzmán and Kouri, 2003; Pérez et al., 2016), and approximately 65% of Colombian municipalities have ecological conditions that favor the sustained circulation of *Aedes aegypti* and the transmission of dengue virus (DENV) (Díaz-Quijano et al., 2018).

Although the mosquito is reported more frequently in urban areas, it has also been identified in rural areas without essential services (Olano et al., 2015), including municipalities located at 2,200 meters above sea level (MASL) (Ruiz et al., 2016), from which transmission occurs and DENV and other arboviruses, such as chikungunya and zika viruses (CHIKV, ZIKV), are spread to new areas (Waggoner et al., 2016).

The age groups affected by the dengue infection have varied geographically in recent years. Dengue hemorrhagic fever in Southeast Asia reportedly occurred mainly in children, while in the Americas, the most affected were adults (Halstead, 2007). However, cases of dengue, severe dengue (SD), and dengue deaths have occurred more frequently in children under 15 years since 2008 in Brazil (Teixeira et al., 2009), and in Colombia since 2010. During the 2010 outbreak, the average ages were 21 years for dengue and 15 years for SD patients, while in the 2012 epidemic, the average age of the patients was 13 and 11 years, respectively

* Corresponding author at: Universidad El Bosque, Vicerrectoría de Investigaciones, Grupo de Virología, Carrera 9 No. 131 A 02, Bogotá 110121, Colombia.
E-mail address: castellanosjaime@unbosque.edu.co (J.E. Castellanos).

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(INS 2010, 2012). From then until today, 50% of cases with SD in Colombia occurred in children under 15 years of age, which confirms the drastic change in the profile of the epidemic (INS, 2012, 2013, 2014, 2015).

Although Colombia is considered a dengue hyperendemic country, the prevalence of dengue antibodies among the general population, including inhabitants of rural areas, has not been reported. Antibody prevalence is therefore an important question that should be addressed in order to guide health policies and the care of the population during dengue epidemics as well as decisions on vaccine implementation in the pediatric population.

The present study assessed the prevalence of dengue immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies in healthy children and adults in urban and rural areas across different endemic regions in Colombia to reveal a complicated panorama of DENV infections and reinfections in Colombia and help health authorities reinforce vector control policies that aim to reduce the distribution of the virus.

Methodology

Study sites

The study was conducted between April 2013 and October 2015. Seven municipalities of five provinces located in the center, west, south, and northwest of Colombia were included (Figure 1). The municipalities of Anapoima (710 MASL) and Apulo (420 MASL) have an average temperature of 26–35 °C, belong to the central zone, with approximate populations of 13,106 and 7,812 inhabitants, and are located at 87 and 101 km, respectively, of Bogotá, the

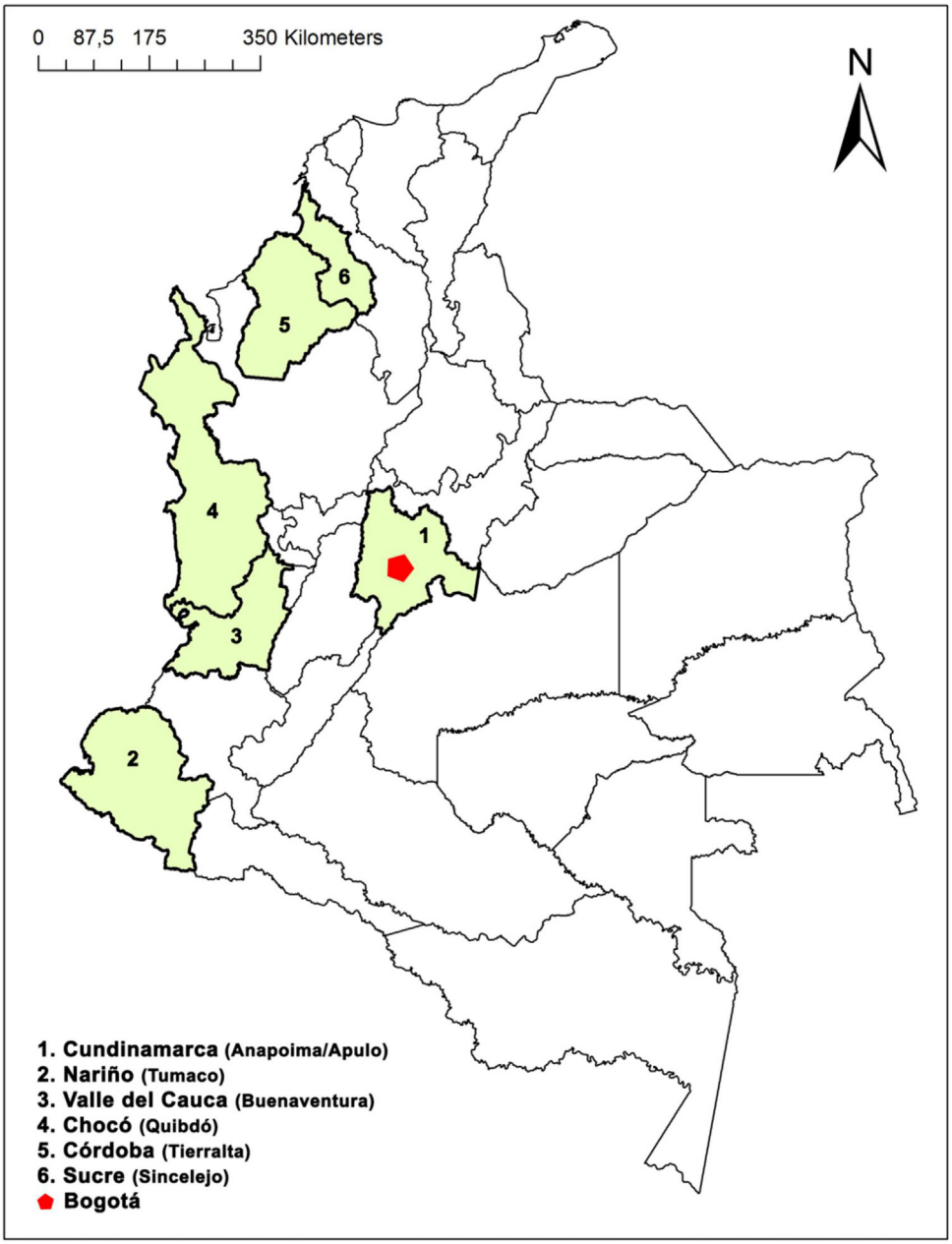

Figure 1. Geographical location of Colombia, of the departments and municipalities evaluated in this study.
Colombian administrative map. The country is located at the northwest corner of South America, the capital of the country is highlighted in red, Bogotá. The municipalities and provinces evaluated were: Anapoima and Apulo of Cundinamarca (1), Tumaco (2) of the province of Nariño, Buenaventura (3) of the Valle del Cauca and Quibdó (4) of the province of Chocó. Tierra Alta (5) and Sincelejo (6) of the provinces of Córdoba and Sucre, respectively.

capital of Colombia (Cundinamarca Planning Secretary, 2017). The other municipalities were Tumaco (Nariño) in the southwest, which has an altitude and temperature of 1 MASL and 26.2 °C (Tumaco town hall, 2018), respectively, and an approximate population of 203,971 inhabitants. Buenaventura (Valle del Cauca) is located 528 km from Bogotá, with an average altitude of 7 MASL, average temperature of 28 °C (Governorship of Valle del Cauca, 2018), and an approximate population of 271,401 inhabitants. Quibdó (Chocó), is located 718 km from Bogotá and has an altitude of 50 MASL, an average temperature of 28 °C, and a population of 97,714 inhabitants (Quibdó town hall, 2018). Tierralta (Córdoba) has an average altitude of 51 MASL, average temperature of 27 °C, and a population of approximately 104,817 inhabitants, and, finally, Sincelejo (Sucre) is located 987 km from Bogotá, with an approximate population of 282.833 inhabitants, an altitude of 213 MASL, and an average temperature of 27.1 °C (Sincelejo town hall, 2018) (Figure 1).

Study population

All study procedures (described below) were approved by the Institutional Ethics Committee of Universidad El Bosque (Minutes 007-2014), the Malaria Vaccine and Drug Development Center (CECIV, Cali, Number: 009) (Forero et al., 2014), and the Hospital Universitario of Sincelejo (Minute 003 of 2014). The sample size was calculated using the OpenEpi V.3.01 (Dean et al., 2013) software, with a 99% confidence interval, a design effect of 2.1, a population disease frequency of 70%, and a power of 80%. Consequently, the total number of suggested participants was 1,170, and 1,318 volunteers were included.

The adult volunteers who participated in our study were between 18 and 95 years (n = 834), from Anapoima, Apulo, Buenaventura, Quibdó, Tumaco, and Tierralta municipalities. These healthy (non-febrile) volunteers recruited from the community were invited to participate and their selection was not randomized. In Sincelejo municipality, 103 samples from a previously enrolled 250 volunteers were randomly selected. The samples were taken between 2013 and 2014 from the volunteers of three vulnerable neighborhoods from urban area with flood risk, deficient aqueducts, and sewerage services (Sincelejo town hall, 2016). All the participants signed the written informed consent form and answered a sociodemographic survey before their blood samples were taken.

Children and adolescents under 18 years (n = 484) were recruited as follows. In March 2014 and 2015, a rural school and three urban schools located in Apulo or Anapoima municipalities were visited and the study was presented to teachers, parents, and children. Only children who agreed to participate and voluntarily signed the written informed consent form or assent were allowed to provide blood samples. In each sampling area, the sera were separated by centrifugation, aliquoted, and frozen. Then, the samples were transported to the Laboratory of Virology in Bogotá, where they were stored until analysis.

Laboratory tests

The Indirect IgG ELISA (Panbio Alere test 01PE30), which reports in dengue secondary infections a sensitivity of 100% (95% CI, 96.1%–100%), whilst the sensitivity in primary infections from endemic areas is 87.0% (95% CI, 66.4%–97.2%). In seronegative samples, the specificity of this test is 100% (95% CI, 96.6%–100.0%), and serologic concordance of this diagnostic test is 75.1% (95% CI, 70.8%–79.4%) to define previous contact with the virus (Castellanos et al., 2016; Castro et al., 2018). For the detection of IgM antibodies, a MAC-ELISA system was used (UMELISA Dengue IgM Plus UM 2016, Tecnosuma, Cuba), following the manufacturer's instructions. This kit reports a sensitivity of 99.6% (95% CI, 98%–100%) and a specificity of 97.4% (95% CI, 96.1%–98.3%). In detecting high-affinity IgG antibodies, which are increased during secondary infections, the capture IgG ELISA (Panbio Alere test 01PE10), which has a reported sensitivity of 79.5% (95% CI, 73.9%–85.1%) and a specificity of 87.1% (95% CI, 80.6%–93.7%), was used. Samples that were positive for the IgM or capture IgG ELISA were further processed, to extract and amplify viral RNA using the RT-PCR technique, following the protocol described previously (Chien et al., 2006; Calvo et al., 2016; Castro et al., 2018).

According to the positivity of the serological and molecular tests, the following three scenarios were considered: *recent infections*, which included IgM positive samples or IgG capture ELISA, suggesting contact with the virus 90 days before sampling. Within these, they were defined as primary or secondary infections produced by DENV. The second scenario consists of samples with *history of dengue infection*, and the third group called *negatives* those individuals with negative samples for all tests (Figure 2).

Data analysis

The information was recorded in an Excel database and included sociodemographic variables and laboratory tests results. Descriptive methods were used to present the general characteristics of all participants, and a statistical analysis was performed using Stata 13.0 software. A univariate analysis was performed with the relative and absolute frequencies for the nominal and ordinal variables. Shapiro-Wilk test was used to verify the normality of the ratio variables, and the median and interquartile range was estimated when asymmetry was found. Chi-squared test was used to establish the differences between nominal or ordinal categorical variables (laboratory tests) and variables independent of sociodemographic characteristics. The seroprevalence was

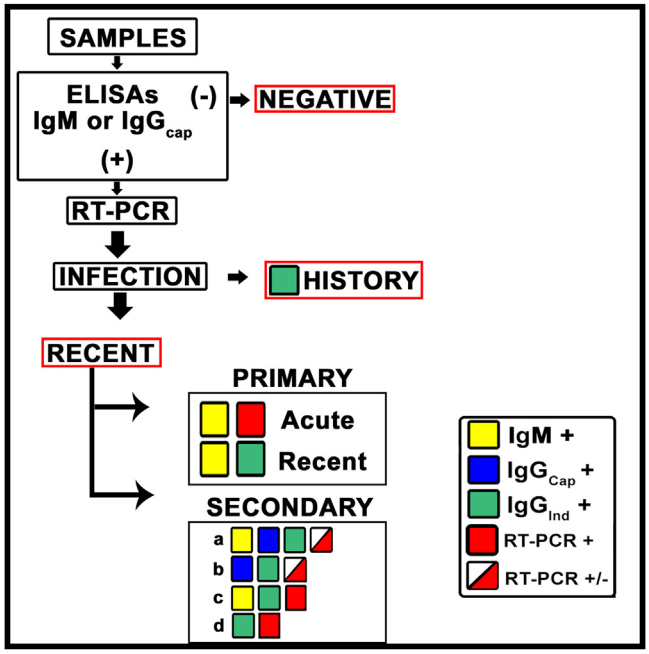

Figure 2. Scheme of the processing and classification of DENV infections according to the positivity of the samples.
The samples of the 1318 volunteers were processed by IgM ELISA and Capture IgG ELISA. If the sample had negative results in all measures it was defined as negative. If a sample was positive only for indirect IgG, it was considered as a history of infection. However, if a sample was positive for any of the ELISAS applied (IgM or IgG Capture), viral RNA was extracted and amplified by RT-PCR. If DENV RNA was detected, it was defined as recent infection, and classified as primary or secondary infection, according to the positivity of any of the tests mentioned in the conventions. ELISAS IgM, IgG capture (IgG $_{Cap}$), IgG indirect (IgG $_{Ind}$), and RT-PCR.

calculated by the proportion of positive volunteers for each laboratory test and divided into the total number of volunteers included in the sample.

Results

Sociodemographic description

Of the participants, 78.6% (n = 1,036) lived in rural areas of the different municipalities, with 54.8% (n = 710) recruited in 2013, 35.1% (n = 454) in 2014, and 10.1% (n = 131) in 2015. The mean age was 25 years (minimum 4 years–maximum of 95 years), and 59.4% (n = 783) were women. Of the evaluated children, 76.5% (n = 270) were from the municipality of Anapoima and 23.5% (n = 83) were from the municipality of Apulo. Adults were recruited from Tumaco (23.2% n = 224), Buenaventura (22.4% n = 216), Tierralta 14.4% (n = 139), Apulo (13.4% (n = 129), Anapoima (13.9% (n = 134), Sincelejo (10.7% n = 103), and Quibdó (2.1% n = 19). At the time of sampling, the participants reported neither fever nor signs or symptoms suggestive of dengue nor had a fever in the last 15 days (Table 1).

The 1,318 healthy volunteers (children and adults) were recruited as described in the methodology and blood samples processed for dengue antibodies ELISA. The highest percentages of seroprevalence are highlighted in bold font for each of the immunoglobulins IgM, IgG capture and indirect IgG according to the municipality, home location and age groups.

Seroprevalence was calculated by the proportion of positive volunteers for each laboratory test and divided into the total number of volunteers.

Seroprevalence and virus infection frequency

A total of 1,318 samples were analyzed, of which 89.8% (n = 1.183) had previous contact with the virus (positives for indirect IgG ELISA). Children between 4 and 11 years presented a dengue seroprevalence of 85%, 88.3% which was for adolescents and young adults, and 91.4% and 94.4% for adults and elderly, respectively. Significant differences were not observed in seroprevalence values regarding municipality, sex, or origin (rural or urban) of the participants using Chi square test (Table 1). On the other hand, the IgM seroprevalence in the studied sample was 14.9% (n = 197) and did not display differences by age or origin using the same test. The most significant number of positive volunteers for this test came from the municipality of Sincelejo (n = 22, 21.4%), followed by the inhabitants of Apulo (16%, n = 34) (Table 1). The prevalence of recent/current dengue secondary infections that was measured by the capture IgG ELISA was 16% (n = 211). We found the highest prevalence for IgG (measured by capture ELISA) in children 4–11 years old in the urban areas (31%) (Table 1), and this was higher 2014, considered an epidemic year (Figure 4).

Table 1
Seroprevalence of IgM and IgG antibodies specific for DENV in children and adults from different regions of Colombia.

Municipalities by department (n = 1318)	n (%)	IgM			IgG Capture			IgG Indirect		
		Frequency	Prevalence (%)	CI 99%	Frequency	Prevalence (%)	CI 99%	Frequency	Prevalence (%)	CI 99%
Cundinamarca										
Anapoima	404 (30.7%)	61	15.1	10.9–20.1	90	**22.3**	17.3–28.0	340	84.1	79.1–88.4
Apulo	212 (16.1%)	34	**16.0**	11.2–23.3	61	**28.8**	21.3–37.2	198	93.4	88.0–96.9
Valle del Cauca										
Buenaventura	216 (16.4%)	26	12.0	7.1– 18.6	11	5.1	2.1–10.0	185	85.6	78.7–91.0
Chocó										
Quibdó	19 (1.4%)	3	15.8	2.3–44.7	2	10.5	0.8–38.1	14	73.7	43.7–93.0
Sucre										
Sincelejo	103 (7.8%)	22	**21.4**	12.3–33.0	11	10.7	4.5–20.7	103	100.0	95.6–100
Córdoba										
Tierralta	139 (10.5%)	19	13.7	7.4–22.4	9	6.5	2.4–13.4	121	87.1	78.4–93.1
Nariño										
Tumaco	225 (17.0%)	32	14.3	9.0–21.1	27	12.1	7.2–18.5	220	98.2	94.7–99.6
Sex (n = 1318)										
Female	783	121	15.5	12.3–19.0	124	15.8	12.7–19.4	709	90.5	87.6–93.0
Male	536	74	13.9	10.3–18.1	87	16.3	12.5–20.7	471	88.4	84.4–91.6
Home location (n = 1281)										
Rural	1036	154	14.9	12.2–17.9	120	11.6	9.2–14.3	931	89.9	87.3–92.1
Urban	245	37	15.1	9.9–21.7	76	**31.0**	23.8–39.0	218	89.0	83.1–93.4
Age groups (years) (n = 1318)										
4–11 (children)	353	61	17.3	12.5–22.9	94	**26.6**	20.9–33.0	300	**85.0**	79.6–89.4
12–25 (teenagers and young adults)	307	32	10.4	6.5–15.5	49	16.0	11.1–21.9	271	**88.3**	82.9–92.4
26–45 (adults)	336	47	14	9.6–19.4	30	8.9	5.5–13.5	307	**91.4**	86.8–94.7
46–95 (adults and older adults)	322	57	17.7	12.7–23.6	38	11.8	7.7–17.0	304	**94.4**	90.4–97.1

Using an algorithm that was established in our laboratory (Castro et al., 2018), all positive samples for IgM or capture IgG were processed to detect viral RNAs. A total of 445 samples met these criteria, and 111 (n = 24.9%) showed amplified viral RNA, of which 50 (45%) came from individuals under 18 years and 13.7% (n = 61) over 18 years. This finding demonstrates that these individuals had an asymptomatic infection at the time of sampling. In the municipalities of Anapoima and Buenaventura, the four DENV serotypes were detected. In Apulo, Sincelejo, and Tumaco, DENV-1 and DENV-2 were identified, while DENV-1 and DENV-4 were detected in Quibdó, and Tierralta municipality presented only DENV-4. Thus, the DENV-2 serotype was detected more frequently (n = 48, 43.2%), followed by DENV-1 with 37.8% (n = 42). The least frequently observed serotypes were DENV-3, (7.2%, n = 8) and DENV-4 (4.5%, n = 5). Eight individuals (4 children and 4 adults) had coinfections, seven of whom showed the combination of DENV1–DENV2 (6.3%) and one had DENV2–DENV3 (1%) (Figure 3). Three individuals who were coinfected with DENV1–DENV2 were identified in Anapoima. In Apulo, one individual was found to be coinfected with DENV1–DENV2 and another with DENV2–DENV3. With respect to annual circulations, DENV-1 was the most frequent virus serotype in 2013, while DENV-2 was the most frequent during 2014. RT-PCR identified only four positive samples of those taken in 2015, two of which were positive for DENV-1. From those participants with recent contact with the virus (IgM or capture IgG positive), 127 (9.6%) were considered primary infections and 252 (19.1%) were secondary infections. Interestingly, only 120 (9.2%) of the participants had not had contact with the virus (Table 2).

Infections (recent or past previous contact with the virus three months before taking the sample) were those positive for the IgM ELISA or Capture IgG ELISA (independent of the PCR result). Primary infections were individuals which included samples

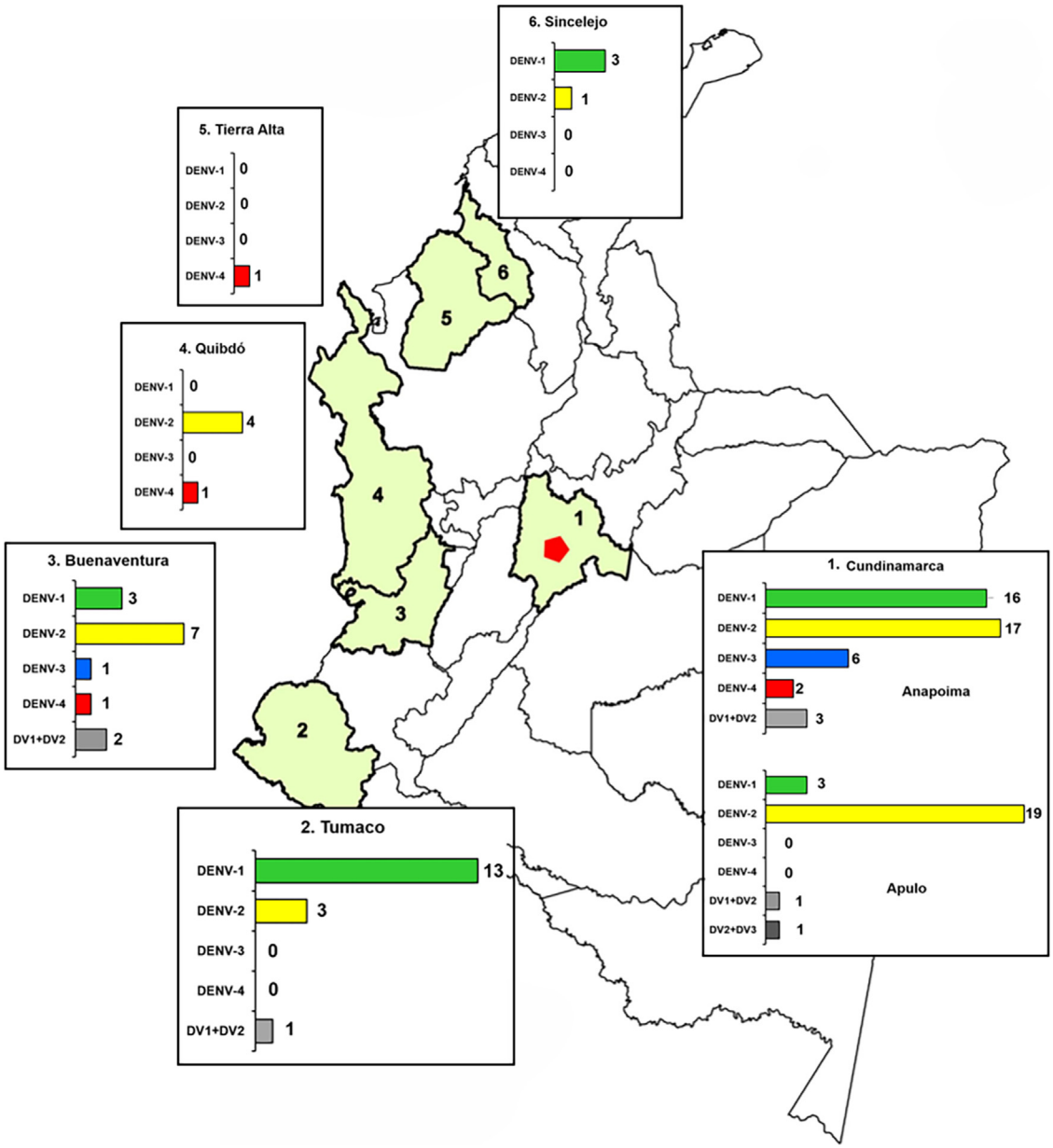

Figure 3. DENV serotypes frequencies identified in healthy donors in the different evaluated municipalities of Colombia, such as Anapoima and Apulo from Cundinamarca province (1), Tumaco from Nariño province (2), Buenaventura municipality from Valle del Cauca province (3), Quibdó municipality from Chocó province (4). The Colombian northern areas were represented by Tierra Alta municipality from Córdoba province (5) and Sincelejo municipality from Sucre department (6). Note the circulation of all four serotypes in the country.

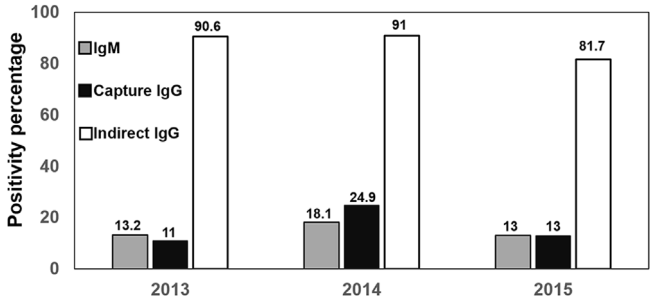

Figure 4. Annual prevalence of IgM and IgG antibodies specific for DENV in healthy volunteers (children and adults) from different regions of Colombia.
High seroprevalence are shown for DENV specific immunoglobulins by year, which shows the high and permanent circulation of the virus and the constant infections and reinfections of the inhabitants of the different regions of Colombia evaluated. Note the highest numbers during the year 2014.

Table 2
Frequencies of healthy volunteers classified according to the results of the laboratory tests.

Classification	Frequency	(%)	CI 99%
Primary infection	127	9.6	7.7–11.9
Secondary infection	252	19.1	16.4–22.0
History of dengue infection	819	62.1	58.7–65.5
Negative	120	9.2	7.2–11.3

positive by IgM and/or RT-PCR, while secondary infections encompassed all indirect IgG ELISA-positive samples and also positive for IgM or capture IgG ELISAs. (Independent of the PCR result). The history of infection condition corresponded to individuals who were positive only for indirect IgG ELISA-Individuals without a history were negative for all tests applied. The classification was defined according to the results of serological and molecular test applied. Analysis was performed using Stata 13.0.

Strikingly, of all the participants, the cohort that included children from 4 to 11 years had the highest frequency of secondary infections (36.2%), and the inhabitants of the municipalities of Anapoima and Apulo had the highest positivity for the capture IgG test (22.3% and 28.8%, respectively) (Table 1). Finally, the highest percentage of primary and secondary infections occurred during 2014, which was declared as an epidemic, and consequently, a high number of seropositive people were identified in 2015 (Figure 4), as it happened in 2013, after the 2010 epidemic (Castro Rodríguez et al., 2015; Villar et al., 2015a).

Discussion

Prevalence of dengue virus antibodies: higher than expected

We used different serological and virological tests to investigate the dynamics of DENV infections between 2013 and 2015 in nonfebrile individuals between 4 and 95 years. Our results show that Colombian inhabitants in different geographical regions and sociodemographic conditions have a very high prevalence of antibodies against DENV. Confirming the hyperendemicity is the constant circulation of the virus in the territory. This high seroprevalence could lead to an increase in the number of cases of SD or deaths in the coming years and affect the use and distribution of the vaccine in endemic areas.

We are thus reporting in an urban and rural population, near 90% seroprevalence for DENV specific total IgG antibodies and a 15% IgM prevalence, which are similar to those previously reported in Quindío, Casanare, and Meta Colombian provinces (Dayan et al., 2015). In an analysis of around 1,100 serum samples collected from 2010 to 2011 during an active dengue disease surveillance identified in Colombia, there was an overall IgG prevalence of 92.5%, which was significantly higher than that found in Puerto Rico (48.9%), Mexico (50.9%), and Brazil (55.5%). These results provide strong evidence of steady virus circulation in Colombia (Dayan et al., 2015).

Other Colombian seroprevalence data was obtained from a vaccine study, where researchers enrolled around 10,000 9- to 16-year-old Colombian children and reported a 92.2% prevalence of dengue antibodies, measured by PRNT (Villar et al., 2015b). Furthermore, randomized all-ages serum samples that were taken during a 2014 study, which was performed throughout four Colombian Quindío province municipalities, showed an IgG prevalence from 81.5% to 97.8% and an IgM prevalence of up to 29% in individuals of all ages (Jiménez et al., 2017). These numbers are close to those found in our study. More recently, the overall dengue seroprevalence in the largest North coast Colombian City, Barranquilla, was found to be 88.2%, which was higher in women (93.1% vs. 87.6%), possibly due to this group extended period of household permanence, compared to male volunteers and showed a sevenfold risk of seropositivity in individuals older than 30 years (Tuesca-Molina et al., 2018). Also, the 11.8% IgM prevalence reported there was similar to the prevalence reported in the present study.

In addition to our finding of high percentages of individuals with evidence of recent infection (IgM positive), it was surprisingly found that the values in adults and older adults were similar to those found in the children group, indicating that there is an optimal ecological environment to favor the DENV lifecycle and a large and persistent pattern of virus circulation in those selected municipalities. It is possible that the high numbers of positivity for recent infections in older adults are due to a higher frequency of lifetime exposure to the virus, including variants of serotypes circulating years ago, as reported in Taiwan, where those older than 72 have the highest rates of positivity (Chien et al., 2019). A similar finding was shown in the study by Yap et al. (2013), in which asymptomatic infection rates (IgM/IgG positive) are around 78% for all groups but reach up to 100% in the older adult group. Although IgM for dengue is considered detectable only for 90 days, it was recently demonstrated that almost half of the patients were still positive one year later by ELISA and even that 35% were positive by immunochromatographic rapid tests (Chien et al., 2018), which indicates that this marker can modify prevalence values in hyperendemic areas such those evaluated in this study.

The seroprevalence was high in both Colombian children and adults, indicating that DENV infections affect all age groups equally, as reported in other parts of the world (Martins-Melo et al., 2018; Sacramento et al., 2018), and in Medellín, Colombia, where 34% of the subjects under 15 years old and 82.8% over 51 years old were positive for indirect IgG (Carabali et al., 2017). This can be explained by the periods of prolonged residences and, therefore, a greater probability of exposure to the vector. However, although we did not find statistically significant differences by age, our data suggest that people in Colombia from an early age are exposed to the virus and suffer secondary infections (36.2%). Therefore, the preexistence of antibodies against one of the four DENV serotypes favors the development of DS, due to the phenomenon of antibody-dependent improvement, which would explain prolonged hospitalization times and fatal cases in children (Liu et al., 2018).

Similar data have been reported in a Nicaraguan cohort, where 64% of children under 5 years and 55% of children aged 6 to 11 years were found to present SD or dengue with warning signs, in contrast with the adult group (36%) (Hammon et al., 2005). This finding confirms the high vulnerability of children, who also showed more frequent secondary infections in our study. Because one of the

available dengue vaccines (Dengvaxia) is more effective in seropositive than seronegative children (83.7% vs. 43.2%) (Hammon et al., 2005; Jiménez et al., 2017; Villar et al., 2015b), the antibody prevalence data presented here can help guide decisions about the age groups or geographic zones that will benefit from vaccination. It will be important to take into account that the DENV-2 serotype is the more frequently detected virus, for which Dengvaxia is less effective (Halstead, 2016).

High rates of asymptomatic infections and dengue in rural areas of Colombia

We highlight a situation that, although previously known, has not been considered by the health authorities in tropical countries. According to our results, 78.6% of the population in the municipalities evaluated here lived in rural areas, and we found that the frequency of both DENV antibody types and RNA presence was equal to that found in urban areas, demonstrating the broad spreading of *Aedes aegypti* and DENV. Until a few years ago, dengue cases were reported mainly in people from the urban areas, and even today, vector control activities only take place in these areas. Moreover, none of the recent Colombian seroprevalence reports described data about rural areas. Together, previous data and our results underscore a possible change in the pattern of dengue cases could be occurring (Hadinegoro et al., 2015; Muhammad et al., 2011), as the vector in the Colombian rural area was first reported in 1981 (Padilla et al., 2012) and has migrated over to other areas (Pérez et al., 2016; Olano et al., 2015).

Strikingly, a high percentage (31.2%) was observed for IgM- or capture IgG-positive individuals who reported no fever in the last 15 days before the sampling. These individuals could be considered to have asymptomatic infections, 41% of whom were children or adolescents. Around a quarter of this group were DENV RNA positive (111 samples) at sampling time. Both volunteers with asymptomatic infections could contribute effectively to horizontal transmission, which would explain the permanent and silent circulation of DENV throughout the year, which is not associated with outbreaks or epidemics.

We recognize some limitations of the present study, including possible cross-reactivity of immunoglobulin tests between arboviruses yellow fever virus and ZIKV. Although our study collected samples before the introduction of ZIKV and there are no yellow fever cases reported in the study areas, it would be advisable to use other strategies such as neutralization tests to make comparisons. The bias of the information was also minimized, taking into account that the sensitivity and specificity values of the serological tests were higher than 80% in areas of high endemicity. Likewise, we can highlight the importance of performing molecular tests as an additional element to the epidemiological surveillance of arboviruses.

Conclusion

We report high prevalence and asymptomatic infections by DENV, which demonstrates the high and steady circulation of DENV in both rural and urban areas of Colombia.

Although the Colombian health authorities have led campaigns such as the National Integrated Management Strategy for the prevention and control of dengue to reduce morbidity and mortality caused by this disease, the final impact of these actions did not reach the goal. Therefore, it is necessary to reinforce educational activities through the integration of vector and disease control systems (Díaz-Quijano et al., 2018), and the community must be actively and permanently involved in order to guarantee the success of the implemented strategies and to control the transmission of this disease and other arboviral infections.

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Conflict of interest

The authors have no financial or other interests with regard to the submitted manuscript that might be construed as a conflict of interest.

Author contributions

Myriam L. Velandia-Romero: Principal investigator and project manager, data analysis and manuscript writing.

Carolina Coronel-Ruiz: Participated in samples collection and processing, and database management. Data analysis.

Lorena Castro-Bonilla: Data analysis and interpretation. Manuscript writing and revision.

Sigrid Camacho-Ortega: Serology test processing and data analysis. Manuscript revision.

María-Angélica Calderón-Peláez: Molecular biology tests processing, data analysis and manuscript revision.

Angélica Castellanos: Buenaventura and Tumaco samples collection and processing. Data analysis and manuscript revision.

Víctor Alberto Olano: Participated in project management and Cundinamarca samples collection, data analysis and manuscript revision.

Alexandra Porras: Data analysis and interpretation.

Myriam Arevalo-Herrera: Co-investigator, collection of samples from Tierralta and Buenaventura. Data analysis and manuscript revision.

Wilmer Villamil-Gómez: Sincelejo samples collection. Data analysis and manuscript revision. Jaime E. Castellanos: Co-investigator, project management. Data analysis, manuscript writing and editing.

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References

Calvo E, Sánchez F, Duran S, Castellanos J. Easy and inexpensive molecular detection of dengue, chikungunya and zika viruses in febrile patients. Acta Trop 2016;163:32–7, doi:http://dx.doi.org/10.1016/j.actatropica.2016.07.021.

Carabali M, Kyungah J, Vélez D, Trujillo A, Egurrola J, Sung K, et al. Dengue virus serological prevalence and seroconversión rates in children and adults in Medellin, Colombia: implications for vaccine introduction. Int J Infect Dis 2017;58:27–36, doi:http://dx.doi.org/10.1016/j.ijid.2017.02.016.

Castellanos J, Coronel C, Parra S, Castilla M, Calvo E, Arévalo M, et al. Description of high rates of inapparent and simultaneous multiple dengue virus infection in a Colombian jungle. Trop Biomed 2016;33:375–82.

Castro L, Coronel-Ruiz C, Castellanos J, Porras A, Parra-Álvarez S, Velandia-Romero ML. Factors associated with dengue virus infection and reinfection in asymptomatic

children in two Colombian municipalities. Am J Trop Med Hyg 2018;99(6):1422–9, doi:http://dx.doi.org/10.4269/ajtmh.17-0617.
Castro Rodríguez R, Galera-Gelvez K, López Yescas J, Rueda-Gallardo J. Costs of dengue to the health system and individuals in Colombia from 2010 to 2012. Am J Trop Med Hyg 2015;92(4):709–14, doi:http://dx.doi.org/10.4269/ajtmh.14-0386.
Chien L, Liao T, Shu P, Huang J, Gubler D, Chang G. Development of real-time reverse transcriptase PCR assays to detect and serotype dengue viruses. J Clin Microbiol 2006;44:1295–304, doi:http://dx.doi.org/10.1128/JCM.44.4.1295-1304.2006.
Chien YW, Liu ZH, Tseng FC, Ho TC, Guo HR, Ko NY, Ko WC, et al. Prolonged persistence of IgM against dengue virus detected by commonly used commercial assays. BMC Infect Dis 2018;18:156, doi:http://dx.doi.org/10.1186/s12879-018-3058-0.
Chien YW, Huang HM, Ho TC, Tseng FC, Ko NY, Ko WC, et al. Seroepidemiology of dengue virus infection among adults during the ending phase of a severe dengue epidemic in southern Taiwan, 2015. BMC Infect Dis 2019;19:338, doi:http://dx.doi.org/10.1186/s12879-019-3946-y.
Dayan G, Arredondo J, Carrasquilla G, Deseda C, Dietze R, Luz K, et al. Prospective cohort study with active surveillance for fever in four dengue endemic countries in Latin America. Am J Trop Med Hyg 2015;93:18–23, doi:http://dx.doi.org/10.4269/ajtmh.13-0663.
Dean AG, Sullivan KM, Soe MM. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version. 2013 Avalaible at: www.OpenEpi.com. [Updated 6 April 2013]. [Accessed 26 May 2018].
Development plan of Sincelejo 2016–2019. Available at: http://www.alcaldiadesincelejo.gov.co/Ciudadanos/ProyectosNormatividad/Plan%20de%20Desarrollo%20Municipal%202016%20-%202019.pdf. [Accessed 13 April 2018]. [Spanish].
Díaz-Quijano FA, Martínez-Vega RA, Rodríguez-Morales AJ, Rojas-Calero RA, Luna-González ML, Díaz-Quijano RG. Association between the level of education and knowledge, attitudes and practices regarding dengue in the Caribbean region of Colombia. BMC Public Health 2018;18(1):143, doi:http://dx.doi.org/10.1186/s12889-018-5055-z.
Forero D, Chaparro P, Vallejo A, Benavides Y, Gutiérrez J, Arévalo-Herrera M, et al. Knowledge, attitudes and practices of malaria in Colombia. Malar J 2014;13:165, doi:http://dx.doi.org/10.1186/1475-2875-13-165.
Governorship of Valle del Cauca. Available at: http://www.valledelcauca.gov.co/publicaciones.php?id=32. [Accessed 13 April 2018]. [Spanish].
Guzmán M, Kouri G. Dengue and dengue hemorrhagic fever in the Americas: lessons and challenges. J Clin Virol 2003;27:1–13.
Hadinegoro SR, Arredondo García J, Capeding M, Deseda C, Chotpitayasunondh T, Dietze R, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. N Engl J Med 2015;373:1195–206, doi:http://dx.doi.org/10.1056/NEJMoa1506223.
Halstead S. Licensed dengue vaccine: public health conundrum and scientific challenge. Am J Trop Med Hyg 2016;95:741–5, doi:http://dx.doi.org/10.4269/ajtmh.16-0222.
Halstead S. Dengue in the Americas and Southeast Asia: Do they differ?. Rev Panam Salud Pública 2007;20:407–15.
Hammon S, Balmaseda A, Pérez L, Téllez Y, Saborio S, Mercado J, et al. Difference in dengue severity in infants, children, and adults in a 3-year hospital-based study in Nicaragua. Am J Trop Med Hyg 2005;73:1063–70.
Jiménez M, Arias J, Carrasquilla G. Seroprevalence of dengue infection in the municipalities of Armenia, Calarcá, La Tebaida and Montenegro in Quindío, 2014. Biomédica 2017;37:34–41, doi:http://dx.doi.org/10.7705/biomedica.v37i1.3208 [Spanish].
Liu J, Deng Y, Jing Q, Chen X, Du Z, Liang T, et al. Dengue infection spectrum in Guangzhou: a cross-sectional seroepidemiology study among community residents between 2013 and 2015. Int J Environ Res Public Health 2018;15(6), doi:http://dx.doi.org/10.3390/ijerph15061227 pii: E1227.
Martins-Melo F, Carneiro M, Ramos Jr A, Heukelbach J, Ribeiro ALP, Werneck G. The burden of Neglected Tropical Diseases in Brazil, 1990-2016: a subnational analysis from the Global Burden of Disease Study 2016. PLoS Negl Trop Dis 2018;12(6)e0006559, doi:http://dx.doi.org/10.1371/journal.pntd.0006559.
Muhammad Azami N, Salleh S, Neoh H, Syed S, Jamal R. Dengue epidemic in Malaysia: not a predominantly urban disease anymore. BMC Res Notes 2011;4:216, doi:http://dx.doi.org/10.1186/1756-0500-4-216.
National Institute of Health. Dengue final report, Colombia. 2010 Available at: https://www.ins.gov.co/buscadoreventos/Informesdeevento/Dengue%202010.pdf. [Accessed 29 May 2018]. [Spanish].
National Institute of Health. Dengue final report, Colombia. 2012 Available at: https://www.ins.gov.co/buscadoreventos/Informesdeevento/Dengue%202012.pdf. [Accessed 29 May 2018]. [Spanish].
National Institute of Health. Dengue final report, Colombia. 2013 Available at: http://www.ins.gov.co/lineasdeaccion/SubdireccionVigilancia/Informe%20de%20Evento%20Epidemiolgico/DENGUE%202013.pdf. [Accessed 4 May 2018]. [Spanish].
National Institute of Health. Dengue final report, Colombia. 2014 Available at: http://www.ins.gov.co/lineasdeaccion/SubdireccionVigilancia/Informe%20de%20Evento%20Epidemiolgico/Dengue%202014.pdf. [Accessed 29 May 29 2018]. [Spanish].
National Institute of Health. Dengue final report, Colombia. 2015 Available at: http://www.ins.gov.co/buscadoreventos/Informesdeevento/Dengue%202014.pdf. [Accessed 29 May 2018]. [Spanish].
Olano VA, Matiz M, Lenhart A, Vargas S, Jaramillo J, Sarmiento D, et al. Schools as potential risk sites for vector-borne disease transmission: mosquito vectors in rural schools in two municipalities in Colombia. J Am Mosq Control Assoc 2015;31(3):212–22, doi:http://dx.doi.org/10.2987/moco-31-03-212-222.1.
Padilla J, Rojas D, Sáenz-Gómez R. Dengue in Colombia: Epidemiology of Hyperendemic Re-emergence] Guías de impresión. (Bogotá, Colombia) ISBN: 9789584606617. [Spanish]. 2012.
Pérez R, Castellanos J, Olano V, Matiz M, Jaramillo J, Vargas S, et al. Detection of all four dengue serotypes in *Aedes aegypti* female mosquitoes collected in a rural area in Colombia. Mem Inst Oswaldo Cruz 2016;111:233–40, doi:http://dx.doi.org/10.1590/0074-02760150363.
Quibdó town hall. Available at: https://www.quibdochoco.gov.co/Institucional/Paginas/Informacion-del-Municipio.aspx. [Accessed: 13 April 2018]. [Spanish].
Ruiz F, González A, Vélez A, Gómez G. Presence of Aedes (Stegomyia) aegypti (Linnaeus, 1762) and its natural infection with dengue virus at unrecorded heights in Colombia. Biomédica 2016;36:303–8, doi:http://dx.doi.org/10.7705/biomedica. v36i2.3301.
Sacramento R, de Carvalho Araújo F, Lima D, Alencar C, Martins V, Araújo LV, et al. Dengue fever and *Aedes aegypti* in Indigenous Brazilians: seroprevalence, risk factors, knowledge and practices. Trop Med Int Health 2018;23(6):596–604, doi:http://dx.doi.org/10.1111/tmi.13061.
Secretariat of Planning of Cundinamarca Office of Information Systems Analysis and Statistics. Basic Statistics Province of Tequendama. Available at: http://www.cundinamarca.gov.co/wps/wcm/connect/b0515f7a-9fb9-481a-955e-edb962c9c50d/Tequendama.pdf?MOD=AJPERES. [Accessed 14 April 2017]. [Spanish].
Sincelejo town hall. Available at: http://www.alcaldiadesincelejo.gov.co/MiMunicipio/Paginas/InformacionelMunicipio.aspx. [Accessed: 13 April 2018]. [Spanish].
Teixeira T, Costa MdaC, Barreto F, Barreto ML. Dengue: twenty-five years since reemergence in Brazil. Cad Saude Publica 2009;25 supp1:S1–S18, doi:http://dx.doi.org/10.1590/S0102-311X2009001300002.
Tuesca-Molina R, Navarro-Lechuga E, Goenaga-Jiménez EC, Martínez-Garcés, Acosta-Reyes J. Sero-prevalence in a hyperendemic area of dengue: Barranquilla-Colombia. Aquichan 2018;18(1)95–109, doi:http://dx.doi.org/10.5294/aqui.2018.18.1.9 [Spanish].
Tumaco town hall. Available at: http://www.tumaconarino.gov.co/municipio/nuestro-municipio. [Accessed 13 April 2018]. [Spanish].
Villar L, Rojas D, Besada-Lombana S, Sarti E. Epidemiological trends of dengue disease in Colombia (2000-2011): a systematic review. PLoS Negl Trop Dis 2015a;9(3)e0003499, doi:http://dx.doi.org/10.1371/journal.pntd.0003499.
Villar L, Dayan G, Arredondo-García J, Rivera D, Cunha R, Deseda C, et al. Efficacy of a tetravalent dengue vaccine in children in Latin America. N Engl J Med 2015b;372:113–23, doi:http://dx.doi.org/10.1056/NEJMoa1411037.
Waggoner JJ, Gresh L, Vargas MJ, Ballesteros G, Tellez Y, Soda KJ, et al. Viremia and clinical presentation in Nicaraguan patients infected with zika virus, chikungunya virus, and dengue virus. Clin Infect Dis 2016;63(December (12)):1584–90, doi:http://dx.doi.org/10.1093/cid/ciw589.
World Health Organization and Pan American Health Organization. Dengue cases in the Americas. . p. 1–3 Available from: http://www.who.int/mediacentre/factsheets/2017-cha-dengue-cases-mar-27-ew-11.pdf?ua=1. [Accessed 17 May 17 2018].
Yap G, Li C, Mutalib A, Lai YL, Ng LC. High rates of inapparent dengue in older adults in Singapore. Am J Trop Med Hyg 2013;88:1065–9, doi:http://dx.doi.org/10.4269/ajtmh.12-0150.