Trans R Soc Trop Med Hyg 2018; **112**: 223–229
doi:10.1093/trstmh/try046 Advance Access publication 16 June 2018

ORIGINAL ARTICLE

Seroprevalence of dengue in school children in Mexico ages 6–17 years, 2016

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Received 16 January 2018; revised 29 March 2018; editorial decision 27 April 2018; accepted 3 May 2018

Background: Dengue is the most important arboviral disease in the world. Seroprevalence has been proposed as a marker of endemicity, however, studies are scarce.

Methods: We conducted a cross-sectional, stratified cluster, random sample study to measure the seroprevalence of antibodies to dengue virus (DENV) in Mexico. The target population was school children ages 6–17 y from 22 endemic states in Mexico, clustered in four regions: Pacific, South-Central, Southeast and Low.

Results: A total of 2134 subjects provided blood samples for immunoglobulin G antibody detection in serum by enzyme-linked immunosorbent assay. Overall, the seroprevalence of antibodies against DENV was 33.5% (95% confidence interval [CI] 27.5 to 40.1). The Southeast had the highest regional seroprevalence, reaching 70.9% (95% CI 60.3 to 79.7). Seroprevalence was higher in older children in the Southeast region: 62.1% (95% CI 46.9 to 75.2) in children 6–8 y and 82.6% (95% CI 73.8 to 88.9) in 13–17 years old (y). However, this was not consistent in all regions. Seroprevalence was associated with dengue incidence.

Conclusions: DENV seroprevalence in Mexico was found to be heterogeneous at the country, regional and state levels. Seroprevalence was linked to long-term exposure and did not adequately reflect recent patterns of transmission, suggesting that utilization of a single epidemiological indicator to define endemic regions should be avoided.

Keywords: dengue, ELISA, epidemiological indicator, Mexico, seroprevalence

Introduction

Dengue is the most common vector-borne viral disease in Mexico.[1] It is cyclical, with peak epidemic periods exceeding 60 000 cases, and although fatality rates are relatively low (<1%), the frequency of severe dengue (dengue haemorrhagic fever/dengue shock syndrome) has increased from 1 case of severe dengue per 30–40 cases of dengue fever (DF) to 1 case of severe dengue per 4–6 cases of DF from 1995 to 2005.[2,3]

In terms of geographical distribution, dengue cases have been reported in almost all of Mexico. However, sustained transmission has been observed in states on both coasts and some central states, particularly Morelos and the tropical region known as Huasteca (San Luis Potosí and Hidalgo).[4]

Seroprevalence is dependent on exposure of the population to an infectious agent, and ecological and epidemiological forces act on this general principle to modify the force of infection.[5] For example, Brunkard et al.[6] compared the seroprevalence of anti-dengue virus (DENV) antibodies in Matamoros in Mexico and Brownsville in the United States, which are neighbouring cities, similar in climate and vector presence. Interestingly however, Matamoros presents higher rates of

sustained dengue transmission, while Brownsville presents sporadic cases with few autochthonous cases.[7] The distribution of the frequency of anti-DENV antibodies differs between the two cities. In general, the prevalence is lower in Brownsville (40%) compared with Matamoros (78%), and in Brownsville the groups with the lowest frequency of anti-DENV antibodies are those younger than 24 y and older than 55 y of age, while in Matamoros the frequency remains high from the age of 15 y onwards.[6] It can be said that the seroprevalence distribution in Matamoros is typical of a dengue-endemic community. This study showed that risk factors associated with prevalence were those associated with the social welfare of both cities. For example, a greater population density is associated with a higher seroprevalence, while having domiciliary air conditioning is associated with a lower seroprevalence.

In Jaltipan, Veracruz, Mexico in 2002–2004, the overall seroprevalence of DENV was 79.6%.[8] In Morelos, in the cities of Axochiapan and Tepalcingo, 1196 persons were studied and a total seroprevalence of 76.6% was found. Additionally, it was reported that between ages 5 and 9 y, prevalence was 35.7%; for ages 10–14 y it was 52.2%; for ages 15–19 y it was 58.9%; for ages 20–30 y it was >70% and in those >30 y, prevalence was >93%.[9] Regarding specific serotype immunity, it has been reported that between the ages of 5 and 9 y, 82.5% tested positive for serotype DENV-1, 45% for DENV-2 and 65% for DENV-3; for the population ≥10 y of age it was 60% for the four serotypes; and in the group ages 10–25 y, DENV-4 was seen in 43%.[9]

In general, seroprevalence studies published in Mexico are consistent with global seroprevalence for dengue, at >70% and up to 90% in those ≥30 y of age. However, the principle limitation of previous studies is that they only represent areas of hyperendemic dengue disease. Consequently the objective of this study was to determine the seroprevalence in a group of 6- to 17-year-old students attending public schools, for two reasons: (1) it is important to know the baseline immunological status of these children for implementation of a public vaccination policy against dengue, since the World Health Organization recommends administration in children older than 9 y and (2) in endemic areas the seroprevalence in adults is almost 100%.[10]

Methods

A cross-sectional study was conducted from February to July 2016 with Mexican school children ages 6–17 y in randomly selected public elementary or middle schools from 22 states in the country. Initially three geographic regions were established, based on the distribution of newly reported dengue cases. However, once the seroprevalence was determined, it was decided to rearrange the regions to prevent the states with low exposure from biasing some regional results. As such, the results presented here show four regions: Pacific, South-Central, Southeast, and Low (a region composed of low seroprevalence states).

The sampling design was probabilistic, stratified and clustered, with the sampling frame (elementary and middle schools) ordered by state, district and location. The number of schools selected was 91 (48 elementary and 42 middle schools).

In the conceptual design of the survey, schools were considered as a unit of analysis and the person responsible for the child was assigned the role of informer. To calculate the sample size, the proportion to be estimated was 5%, with an acceptable relative error of 0.3, a 95% confidence interval (CI) and a design effect of 1.5. Finally, a sample size of 15% was added to compensate for non-response losses. The average sample size was 576 elementary school students and 252 middle school students out of a total of 828 students per region. This represents a total of 2484 students for the entire study.

For the selection of schools and participating school children, districts of ≥10 000 inhabitants in each of the regions were identified using the INEGI (Instituto Nacional de Estadística y Geografía [National Institute of Statistics and Geography]) databases.[11] Afterwards, with information provided by the National School Information System of the SEP (Secretaría de Educación Pública [Secretariat of Public Education]), elementary and middle schools of each of the districts per region were identified.[12] Later, elementary and middle schools were identified in each district and grouped by region. Out of the clusters, a sample of 16 elementary schools and 14 middle schools was taken for each region. Within each school, groups were randomly selected from each class and 6 students were randomly selected within each group, resulting in 36 elementary school students and 18 middle school students.

The inclusion criteria were as follows: school children 6–17 y of age enrolled in selected elementary or middle schools who agreed to provide an initial blood sample, whose parents or guardians signed the informed consent form and who themselves signed the informed consent form for children older than 6 y.

The exclusion criteria were as follows: school children diagnosed with immunological, oncological or chronic degenerative diseases and those whose parents or guardians did not give consent to participate in the study.

The primary information came from two sources: the interview questionnaire and the peripheral venous blood sample. The questionnaire included a section on sociodemographic data; household conditions; the presence of dengue cases in the household, neighbourhood and school; family history of diseases and a section on the child's disease and vaccination history. The selected student's parent or guardian answered the questionnaire.

Certified clinical laboratory personnel collected 7.5 mL peripheral venous blood samples from the participants to identify the presence of anti-DENV antibodies. The samples were preserved and dispatched according to the guidelines and regulations established by the Mexican Institute of Epidemiological Diagnosis and Reference (InDRE).[13] Later, the samples were centrifuged at local laboratories to separate the serum. Two aliquots of 1.5 mL each were frozen (≤−20°C) and sent to the School of Medicine of the National Autonomous University of Mexico for long-term storage. In order to process for anti-DENV immunoglobulin G (IgG), the samples were transported by courier services, in accordance with safety regulations, to the serology laboratory at the Infectious Disease Research Center (CISEI) of the National Institute of Public Health.

The presence of DENV-specific antibodies was determined using an indirect IgG enzyme-linked immunosorbent assay kit

(Panbio E-DEN 01G kit, Alere, Waltham, MA, USA), following the manufacturer's instructions. This kit does not discriminate between the four DENV serotypes. The sensitivity and specificity of the test are 100% and 98%, respectively. In relation to cross-reactivity with other flaviviruses, it is important to note that in Mexico there is no sustained circulation of yellow fever.[14]

Global descriptive analyses were performed, by region and age group, using measures of central tendency for continuous variables and absolute and relative frequencies for qualitative variables. Seroprevalence and CIs were determined by gender, age group and region. A simple linear regression model was also created to evaluate the link between seroprevalence and incidence rate. All estimates presented here take into consideration the sample design used.

The expansion factors were constructed as a product of the expansion factors at each sample stage: of schools, groups and students. The expansion factor of each stage was equal to the inverse of the probability of selection of the unit in question.

Results

Initially 2722 students were selected, of which 2617 agreed to participate and 81.5% (n=2134) supplied a blood sample and a completed questionnaire. Among those not included (n=483), 69 could not provide a blood sample for various reasons, including failure to properly take the sample, absence of the parent or guardian responsible for the subject or other logistical failings. The remaining 414 subjects provided a blood sample, but without the nominal information. This group consisted of siblings of enrolled subjects whom parents had asked to be included or samples not processed due to errors in code registration (Figure 1). These subjects were concentrated in the Pacific and South-Central region. Considering the expansion factor, the sample represents 4 385 349 public elementary and middle school students from the 22 states included in the study. Sample distribution by state and region can be found in Table 1.

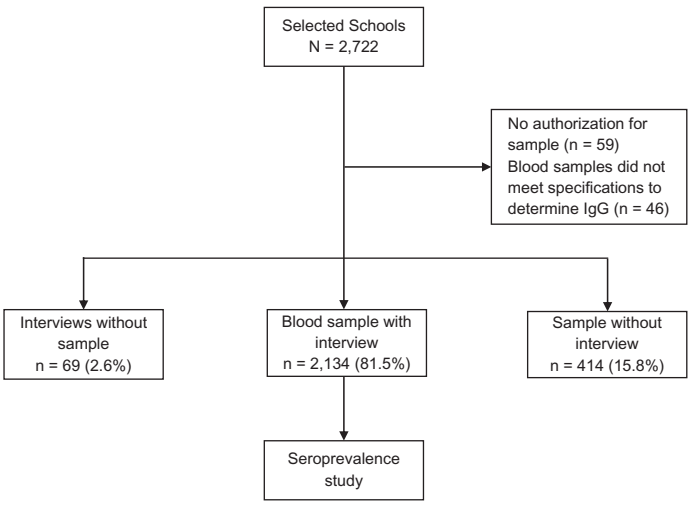

Figure 1. Scheme for selection and discontinuation of study subjects.

The mean age of the study population was 10.7 y (standard deviation [SD] 2.74); 25.8% of subjects were 6–8 y of age, 42.8% were 9–12 y of age and 31.4% were 13–17 y of age. The age distribution by region was heterogeneous (χ^2=110.5; p<0.001).

Female subjects made up 51.7% (n=1102/2134) of participants. Sex distribution among the age groups showed no statistically significant differences (χ^2=0.19, p=0.909).

Global and regional seroprevalence

Overall seroprevalence was 33.5% (95% CI 27.5 to 40.1). Of the four selected regions, the highest recorded seroprevalence was in the Southeast, at 70.9% (95% CI 60.3 to 79.7) positivity, followed by the South-Central (44.5% [95% CI 33.0 to 56.5]) and Pacific (38.8% [95% CI 25.3 to 54.4]) regions, while the region with the lowest seroprevalence had 13.3% positivity (95% CI 9.0 to 19.2). The seroprevalence results in the 9–17 y age group were similar: in the Southeast, 74.3% (95% CI 64.2 to 82.4); South-Central, 53% (95% CI 38.4 to 67.2); Pacific, 39.4% (95% CI 26.8 to 53.6) and in the lowest seroprevalence region, 13.1% (95% CI 8.8 to 19.0) (Figure 1A).

Upon analysing the seroprevalence of each age group (Figure 1B), an increasing tendency was observed with increasing age: <9 y of age, 26.5% seropositive; 9–12 y of age, 33.6%; 13–17 y of age, 42%.

There was a significant positive trend in overall seroprevalence across the three age groups in the South-Central and Southeast regions, where the average seropositivity is higher than the national average (z=5.94, p<0.005). In comparison with the younger age group, the odds ratio for the 9- to 12-year-old group was 1.42 (95% CI 0.97 to 2.06) and for the 13- to 17-year-old group was 2.07 (95% CI 1.09 to 3.91). The distribution in the Pacific region is more difficult to interpret due to the sampling problems referred to earlier, and the seroprevalence among age groups in the Low seroprevalence region is most likely the result of early transmission of dengue in that area.

Seroprevalence and incidence

In the proposed model for evaluating the association between seroprevalence and officially reported incidence, seroprevalence was considered by district (n=65 districts) and the median incidence per 100 000 inhabitants recorded between 2010 and 2015 by the Ministry of Health. The model adjusted the lower median incidence values to 80 cases per 100 000 inhabitants (n=60 districts), as no linear relationship was observed in the upper values.

In accordance with the estimated model, seroprevalence increased by an average of 0.75 percentage point (95% CI 0.457 to 1.050) for each increment of 1 unit in the median incidence per 100 000 inhabitants. The p-value associated with the test was <0.0001, suggesting the median incidence between 2010 and 2015 provides statistically significant information associated with seroprevalence. During this period the total variability of seroprevalence, explained by the median incidence among the districts included in the study, was 30.9% (Figure 2).

Table 1. Distribution of the study population by state and region

Pacific	n	South-Central	n	Southeast	n	Low seroprevalence	n
Baja California	68	Guerrero	177	Campeche	59	Coahuila	108
Baja California Sur	21	Morelos	43	Chiapas	190	Guanajuato	125
Nayarit	36	Oaxaca	53	Quintana Roo	55	Jalisco	172
Sinaloa	70	Puebla	29	Tabasco	106	Michoacán	59
Sonora	76	Veracruz	203	Yucatán	140	Nuevo Leon	147
						San Luis Potosi	50
						Tamaulipas	147
Total	271		505		550		808

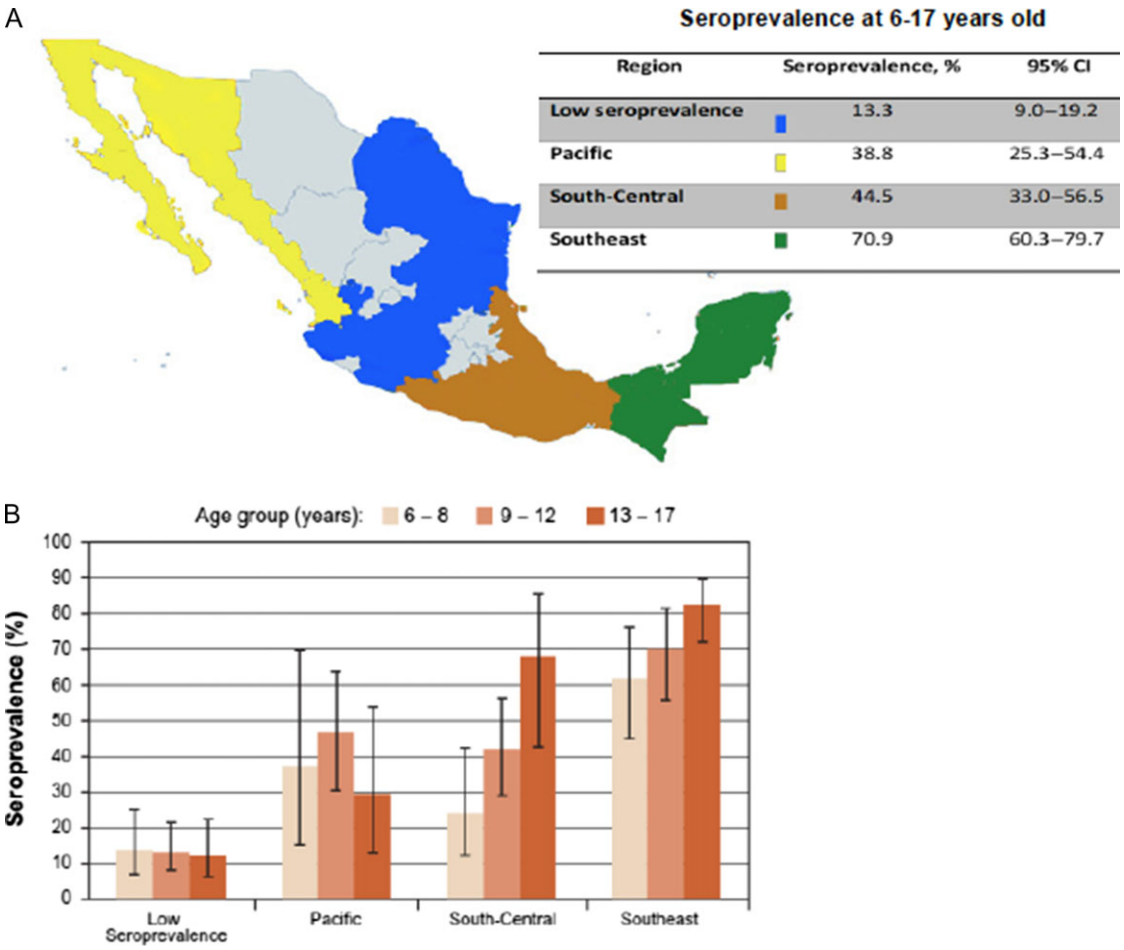

Figure 2. Dengue seroprevalence by region and age group. Map of Mexico shows (A) the areas analysed in this study. (B) The insert shows the average prevalence by region and the bars show the average positivity by age group.

Discussion

To our knowledge, this is the first probabilistic study to measure the seroprevalence of anti-DENV antibodies at the national level in a continental country in America. The overall seroprevalence of the subjects between 6 and 17 y of age was relatively low (33.5% [95% CI 27.5 to 40.0]), which should not be surprising since the majority of the country's population lives in districts at 1000 m above sea level, where the force of infection is weak or non-existent (Figure 3).

The data do show a marked heterogeneity between and within regions. The central region has a reported seroprevalence

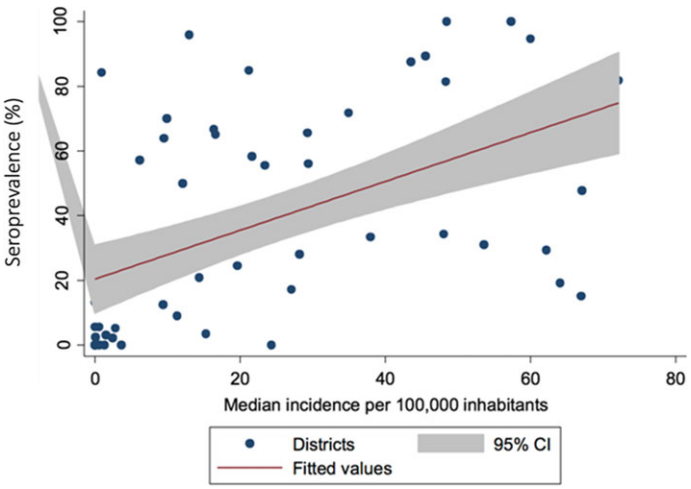

Figure 3. Seropositivity to dengue and association with incidence. Linear association between seroprevalence by district (n=60 districts) and the median incidence per 100 000 inhabitants recorded between 2010 and 2015. Incidence data from the Direccion General de Epidemiologia, Secretaria de Salud, Mexico.

of 44.5% (95% CI 33.0 to 56.5), however, published findings of two studies in districts within the central region (Jaltipan in Veracruz and Tepalcingo/Axochiapan in Morelos) reported mean seroprevalences (in that age group) of 68% and 42%, respectively; one district falls within the expected interval while the other exceeds it.[8,9]

We found a significant association between the incidence during the last 5 y and the seroprevalence found in the districts studied. However, in the model, the variability of seroprevalence explained by the median incidence only accounts for 31% of the total variability. In terms of seroprevalence, there are other factors aside from the recorded incidence that determine the frequency of anti-DENV antibodies. For example, in accordance with the Water Commission report from 2013–2016,[15] all schools in the South region are in states where annual precipitation reaches well above the national average (641.6 mm). In contrast, the schools in the low seroprevalence region are found in states where annual precipitation was below the national average, with the exception of Tamaulipas, which reached 712.1 mm. Similar observations have been reported in Africa.[16,17]

Another factor of potential importance is the circulation of serotypes. In general, Mexico has shown regular behaviour in the replacement of types and lineages,[3] and according to the virological surveillance system, for approximately 9 y the predominant serotype has been DENV-1.[18] Notwithstanding the states in the Southeast region, where seroprevalence is highest, a hyperendemic state[19] has been maintained since 1995, while the states in the low seroprevalence region (with the exception of Jalisco) have maintained a situation of monotypical transmission.[18] This interpretation has been used in other studies relating seroprevalence to the circulation of serotypes.[20,21]

Seroprevalence by age shows a similar pattern of behaviour. Where transmission is more intense (in the South-Central and Southeast regions), seroprevalence is directly proportional to age group. Where transmission is less intense (in the Pacific region and the low seroprevalence region), this does not appear to be the case (Figure 1). Little is known about incidence and other factors associated with transmission that may affect seroprevalence, but we do know that transition from a hypoendemic to a hyperendemic state modifies the seroprevalence profiles of the age groups.[22]

Some limitations were identified but they were considered insufficient to bias the study. Limitations identified included the fact that excluded subjects were 18.5% of the sample obtained. Comparing this with the estimated lack of response rate at 15%, the difference did not represent a greater threat to the validity of the results, considering the estimated sample prevalence was less than that ultimately recorded. Our initial supposition in the selection of schools and districts of ≥10 000 inhabitants at <1800 m above sea level in the defined region was found to be insufficient, but this was principally due to the fact that this was a previously unpublished population-based probabilistic and randomized study of seroprevalence of anti-dengue antibodies. Districts with high transmission and an almost hyperendemic profile (schools in Jalisco, Nuevo Leóon and Tamaulipas) within the low seroprevalence region were not sufficiently represented, while schools in districts that may be considered hypoendemic were distributed throughout all regions. Consequently, further research is needed that considers samples by state and without limitations on the population size and distance above sea level.

It is not surprising that a country as diverse as Mexico in terms of ecological microenvironments, virus circulation history and mobility of inhabitants[23] produced heterogeneous results preventing generalization of the study results. However, the problem of heterogeneity of dengue transmission is most likely to repeat itself regardless of aggregation, whether at the state or district level, and even at the level of blocks in each neighborhood.[24–26] We believe, therefore, that only in countries with a defined geography, ecology and circulation of serotypes are national seroprevalence surveys useful for public health.

The initial concept that anti-DENV immunity has an impact on protection, as well as on the pathogenesis of severe cases, is beginning to be revised. Understanding these concepts is considered critical for the development and evaluation of vaccines currently registered in various countries, including Mexico. However, recent evidence suggests that anti-DENV immunity is far more complicated than we initially thought.[27,28] Public health decisions made based on data of this type should be evaluated taking into consideration factors such as the ecology and history of serotype circulation in the area where the studies are being conducted and other relevant epidemiological indicators that define priority areas where control efforts should be focused.

In conclusion, the frequency of anti-DENV IgG antibodies in Mexico reflects both the incidence of dengue and the circulation of serotypes; the Southeast region presents the highest proportion of the seropositive population in the 6–17 y age group, but it should be noted that, according to the literature, there are areas with a high frequency of anti-DENV antibodies at locations inside regions with low seroprevalence. Therefore the results presented here should be considered carefully and generalizations

on specific locations avoided. Further work should be conducted to explore variables such as climate and geography in order to have a predictive model of dengue seroprevalence.

Authors' contributions: MLC and ES conceived the study. IYAL, MRR, MLC, JRC and LLTG designed the study protocol. JRC and IYAL carried out laboratory tests. IYAL, LCP, MVCB and GOB performed the analysis and interpretation of the database. JRC and IYAL drafted the manuscript. JRC, IYAL, EPR and ES critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

Acknowledgements: The authors would like to thank to Dr Cuitlahuac Ruiz-Matus and Dr Maria Eugenia Jimenez-Corona from Direccion General de Epidemiolgia, Secretaria de Salud, Mexico for providing the country-specific information. Editorial assistance with the preparation of the manuscript was provided by Rebecca Hornby, inScience Communications, Springer Healthcare.

Funding: The study was funded entirely by Sanofi Pasteur (DNG-31). It was also authorized by the Mexican regulatory agency COFEPRIS (Federal Commission for Protection against Health Risks; COF092097/COF092098) and the ethics institutional review board from the National and Autonomous University of Mexico (FMED/CI/SPLR/129/2015). Sanofi Pasteur had the opportunity to review and comment on this manuscript.

Competing interests: ES and EP are employees of Sanofi Pasteur. JR-C is a member of the Scientific Advisory Board on Dengue Vaccine from Sanofi Pasteur. All have received honoraria for their participation and funding for scientific research. The remaining authors declare no conflicts of interest. The article has not been published by any other journal or electronic media. A preliminary version was presented as a poster at American Society for Tropical and Medical Hygiene 66th Annual Meeting, Baltimore, MD, USA, November 2017.

Ethical approval: The protocol was approved and registered by the Research and Ethics Commissions of the School of Medicine of the Universidad Nacional Autonoma de Mexico (FMED/CI/SPLR/129/2015) as well as the Federal Commission for the Protection of Health Risks (COFEPRIS) of the Ministry of Health (COF092097/COF092098).

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