

BRIEF COMMUNICATION

***Aedes aegypti* anti-salivary gland antibody concentration and dengue virus exposure history in healthy individuals living in an endemic area in Colombia**

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Introduction: Mosquito salivary proteins are able to induce an antibody response that reflects the level of human-vector contact. IgG antibodies against dengue virus (DENV-IgG) are indicators of previous exposure. The risk of DENV transmission is not only associated to mosquito or dengue factors, but also to socioeconomic factors that may play an important role in the disease epidemiology.

Objective: To determine the effect of the presence of *Aedes aegypti* mosquitos in different stages in households and the history of dengue exposure on vector-human contact determined by the level of anti-salivary protein antibodies in people living in a Colombian endemic area.

Materials and methods: A pilot study of 58 households and 55 human subjects was conducted in Norte de Santander, Colombia. A questionnaire for socioeconomic factors was administered and houses were examined for the presence of *Ae. aegypti* specimens in the aquatic stages. The level of DENV-IgG antibodies (DENV-IgG), in addition to IgG and IgM anti-*Ae. aegypti* salivary gland extract (SGE) antibodies (SGE-IgG, SGE-IgM) were evaluated by ELISA using blood collected in filter paper.

Results: We found a significant higher level of SGE-IgG antibodies in subjects living in houses with *Ae. aegypti* in aquatic stages. We also found a higher concentration of SGE-IgG antibodies in people exposed to DENV, a positive correlation between IgM-SGE and IgG-DENV and a negative correlation with IgG-SGE.

Conclusion: Anti-salivary proteins antibodies are consistent with the presence of *Ae. aegypti* aquatic stages inside houses and DENV-IgG antibodies concentrations.

Key words: *Aedes aegypti*, dengue, dengue virus, disease vectors.

doi: <http://dx.doi.org/10.7705/biomedica.v35i4.2530>

Concentración de anticuerpos contra proteínas de las glándulas salivales de *Aedes aegypti* e historia de la exposición al virus del dengue en residentes de una zona endémica colombiana

Introducción. Las proteínas salivales de los mosquitos son capaces de inducir la producción de anticuerpos, lo que a su vez refleja el grado de contacto hombre-vector. Además, los anticuerpos IgG contra el virus del dengue son indicadores de una exposición previa a este virus. El riesgo de transmisión del virus del dengue está asociado no solo con factores relacionados con la biología del

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mosquito, o factores virales, sino también, con factores socioeconómicos, como la disponibilidad de agua en el hogar, que pueden desempeñar un papel importante durante la temporada epidémica.

Objetivo. Determinar el efecto de la presencia de mosquitos *Aedes aegypti* en las casas y la exposición previa al virus del dengue, sobre los niveles de anticuerpos contra mosquitos en el contacto humano-vector en habitantes de un área endémica de Colombia.

Materiales y métodos. Se hizo un estudio piloto de 58 casas y 55 participantes en Norte de Santander, Colombia. Se empleó un cuestionario para recopilar la información sobre los factores socioeconómicos y se examinaron las casas para detectar la presencia de sitios de cría de *Ae. aegypti*. Se recolectó una muestra de sangre humana total en papel de filtro y se estableció el nivel de anticuerpos IgG contra el virus del dengue, además del de los anticuerpos IgG e IgM anti-*Ae. aegypti* de extracto de glándula salival mediante ELISA.

Resultados. Los resultados revelaron un mayor nivel de anticuerpos IgG de extracto de glándula salival en sujetos que vivían en casas con presencia de mosquitos *Ae. aegypti* en la fase acuática. Asimismo, se encontró una mayor concentración de anticuerpos IgG de extracto de glándula salival en personas previamente expuestas al virus del dengue. Los resultados evidenciaron una correlación positiva significativa entre los niveles de IgM de extracto de glándula salival y los de IgG anti-virus del dengue de extracto de glándula salival, y una correlación negativa con los de IgG de extracto de glándula salival, aunque esta última no fue significativa.

Conclusión. La concentración de anticuerpos fue mayor en quienes vivían en casas con estadios acuáticos de *Ae. aegypti*, como también en las personas con anticuerpos IgG anti-virus del dengue.

Palabras clave: *Aedes aegypti*, dengue, virus del dengue, vectores de enfermedades.

doi: <http://dx.doi.org/10.7705/biomedica.v35i4.2530>

Dengue virus (DENV) is a systemic viral infection maintained in a human-mosquito transmission cycle by the urban vector *Aedes aegypti* (1). There are four serotypes (DENV 1-4) with antigenic relatedness, but co- or subsequent infection with a different serotype is thought to enhance disease pathology (2). Serotype-specific antibodies confer lifetime immunity against the infecting serotype, but heterologous protection has limited duration (3,4). In the absence of a vaccine or other preventative therapy, DENV prevention and control efforts focus on vector control, specifically habitat abatement. Containers of standing water are the preferred breeding sites for *Ae. aegypti* in urban and suburban environments (5). Despite global public health campaigns about DENV risk and water storage, unpredictable access to running water perpetuates the practice of water storage, particularly in smaller towns and rural areas (6).

Previous studies have shown that the type and concentration of antibodies against vector salivary gland extract (SGE) can serve as markers for disease transmission and risk (7-10) and to evaluate the efficacy of vector control efforts (11,12). Additionally, estimating the prevalence of

antibodies against all DENV serotypes that have circulated in a specific area gives insight of the population exposure to the virus (13).

In Colombia, the four serotypes of DENV may co-circulate at any given point of time (14). Norte de Santander, a region of national and international commerce, is also an area with a high DENV index within the country, where more than 4,000 cases of DENV were reported, 700 of which were severe cases (15). Our study focused on three locations within Norte de Santander: Cúcuta, Los Patios, and Pamplona. The objective was to measure the prevalence of IgG antibodies specific for DENV 1-4 and *Ae. aegypti* salivary proteins among residents from the study sites and correlate these antibody concentrations with water storage practices and larval presence in the households.

The study hypothesis is that socioeconomic factors such as household water availability and storage are related to the level of exposure to *Ae. aegypti* bites measured by the concentration of IgG and IgM (SGE-IgG, SGE-IgM) antibodies against salivary proteins, and that these levels are associated with the risk of dengue exposure and infection. The study showed that there was an association between mosquito bite exposure and history of exposure to DENV also related to water availability for household use. This is the first report of association between history of dengue exposure measured as DENV-IgG antibody levels and mosquito bite exposure.

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Recibido: 22/09/14; aceptado: 06/07/15

Materials and methods

Study area

The study was conducted in the cities of Cúcuta, Los Patios, and Pamplona in Norte de Santander, Colombia. The city of Pamplona is the third largest city in this province. It is located in the northeast of the country at 2,342 meters above sea level (m.a.s.l.); due to the high altitude this area is not endemic for *Ae. aegypti* and dengue cases are sporadic, mainly imported from surrounding DENV-endemic populations like Cucuta (320 m.a.s.l) and Los Patios (410 m.a.s.l.), since at least 49% of the population travel to these areas for commerce or work (Londoño-Rentería B, Londoño-Rentería F, Cárdenas C, Cárdenas L. Main mosquito breeding sites for *Aedes aegypti* in the Pan-American Highway: Cúcuta-Pamplona Area (Norte de Santander, Colombia) in 2010. *Am J Trop Med Hyg.* 2011;86(Suppl. 6):376). Contact among these three cities is along a 75 km route on the Pan-American Highway. Los Patios is a suburb of Cúcuta with almost a tenth of its population. This city is a necessary pass through to Pamplona and the center of the country.

Human participants

A group of 58 households located around the hospitals in Cúcuta (n=13), Los Patios (n=25), and Pamplona (n=20) were included in a study of housing characteristics influencing the presence of mosquitoes. These houses were randomly selected according to the distance from the hospitals and after obtaining consent in each of them. In Cúcuta, the houses were selected from an ongoing investigation in *Primavera* neighborhood, an area with high incidence of dengue cases in 2009. Houses whose dwellers declined to have them inspected, as well as abandoned houses, were excluded from the study.

Trained interviewers administered questionnaires to one adult per household to obtain information on water storage practices and household construction. After written informed consent was obtained, capillary blood was collected from participants by finger prick directly on filter paper (Protein Saver Cards, Whatman® 903), and stored at -20°C until analysis. In total, blood samples from 55 individuals were collected (Cúcuta: n=20, Los Patios: n=18 and Pamplona: n=17). In order to determine exposure status to DENV, we also included five filter paper samples from subjects living in New Orleans in 2009 as DENV-negative controls.

Field mosquito sample collection

Mosquito larvae were collected intra- and peridomiciliary at the time of the visit, and the containers positive for mosquito larvae were recorded. Visits were performed during the beginning of the rainy season (September – October, 2010). *Aedes aegypti* larvae were identified using an illustrated taxonomic key (16).

Salivary gland extract preparation

Aedes aegypti (Rockefeller strain) mosquito dissection and salivary gland extract (SGE) preparation was performed as published elsewhere (7,8). Protein concentration was determined using the Thermo Scientific NanoDrop™ (Thermo Fisher Scientific, Wilmington, DE). The salivary gland extract (SGE) was stored in PBS at -80 °C until use.

Anti-SGE antibody detection

Detection of anti-SGE antibodies was optimized and tested following the methods published elsewhere (7,8). Samples were tested in duplicate. Three controls were included in each plate: 1) control blank: two wells without SGE to control for nonspecific induction of color for any of the reagents used in the test; 2) negative control: Two wells with SGE but without human serum to control for any nonspecific color induction of the coating antigen, and 3) positive control: two wells with pooled human serum from confirmed *Ae. aegypti*-exposed individuals from Colombia. Level of antibodies is represented as optical density (OD) measured at 450 nm.

Anti-DENV antibody levels by ELISA

Working conditions were previously standardized to test IgG antibody levels against each DENV serotype (DENV1 strain WestPac-74 [Nauru Island 1974], DENV2 strain 1232 [Indonesia, 1978], DENV3 strain CH5548904500 [Thailand, 1973] and DENV4 strain LN 634441 [Malaysia, 1988]) cultured under conditions described elsewhere (17). ELISA conditions were performed as published elsewhere (7) with minor modifications.

In summary, plates were coated with 1 µg/ml of Flavivirus group capture antibody 4G2 overnight at 4°C, blocked for 1 h with 5% dry milk in PBS at 37°C, and incubated with one of the DENV serotypes (DENV 1-4) or control media from uninfected cell culture (media control plate) 2 hours at 37°C. A 100 µl/well of each diluted human sample was incubated 2 hours at 37°C on a shaker. Plates

were washed 3 times with wash solution (1× PBS and 0.1% Tween) and incubated with 100 µl/well of goat anti-human IgG diluted 1:1,000 horseradish peroxidase (HRP)-conjugated antibodies (Caltag Laboratories, Burlingame, CA) at 37°C for 1 hour.

Colorimetric development was obtained using 100 µl/well tetra-methyl-benzidine (TMB, one-solution microwell, Gene-Script, Piscataway, NJ) incubated for 10 minutes at room temperature. The reaction was stopped with 100 µl/well of stop solution (1 M phosphoric acid), and absorbance was measured at 450 nm. Each sample was tested in duplicate.

Three controls were included in each plate: 1) control blank: Two wells without 4G2 or DENV to control for nonspecific induction of color for any of the reagents used in the assay; 2) negative control: Two wells with 4G2 and no DENV to control for any nonspecific color induction of the coating antigen, and 3) positive control from a human serum with a known concentration of IgG antibodies.

Data analysis

Antibody concentration for each sample was expressed as adjusted OD calculated for each sample by subtracting the mean OD value of the controls from the mean OD value of the duplicates for each sample. DENV IgG-ELISA cut-off value was calculated to determine virus exposure status (Receiver Operating Characteristic [ROC] curve, area=0.9268, 95% confidence interval [CI]=0.8682 to 0.9854, $p=0.0014$).

To measure risk by odd ratios (OR), IgG antibody concentration was categorized to determine level of bite exposure. The average OD value was 1.012. All OD values lower than the average OD (<1.012) were classified as low; OD values higher or equal to the average OD (≥ 1.020) were classified as high. After verifying that the values did not meet the normal distribution ($p=0.02$; skewness and kurtosis normality test), the difference in antibody concentration between two groups (*Ae. aegypti* presence or absence in the households) was tested using the nonparametric Mann-Whitney U test.

Spearman's rank correlation test was used to measure the strength and significance of an association between two numeric variables. All differences were considered significant with a probability of committing a type 1 error set at $p<0.05$. All statistical tests were computed using Prism version 6 (GraphPad Software Inc. 2007) and STATA™, version, 13 (Stata Corporation, College Station, Texas).

Ethics statement

Protocols and research methods were reviewed and approved by *Universidad de Pamplona* and the Ethics Review Board of *Hospital Erasmo Meoz*. The investigation was explained to each individual, and a written informed consent was obtained from each participant or their legal guardian before collecting samples. Blood samples were collected in compliance with the regulations on ethics of research in human participants for Colombia and the United States.

Results

Water availability and *Aedes aegypti* presence in the households

Fifty-eight households were included in the study with an average of 5.4 people (range 1-11) and 3.1 rooms per house. All households had connections installed for running water, but water was not received on daily basis in Los Patios and Cúcuta ($n=38$). Additionally, in 77.5% (45/58) of the houses water was stored for household activities such as cooking and/or cleaning. In these houses, water was mainly kept in floor tanks, roof tanks, and/or plastic or metal water cans (table 1). Thirty-one percent of houses had more than one type of water storage. In Pamplona, all households received daily water service and a majority of the households did not store water (15/20). However, two households were found using roof tanks for water storage and three more houses using plastic water cans. Furthermore, 60.3% (35/58) of the study households had containers with standing water around the house (front or back patios).

Houses were inspected for the presence of *Ae. aegypti* larvae in water holding containers. No *Ae. aegypti* were found in any of the Pamplona households (0/20). All Cúcuta households were positive for *Ae. aegypti* (13/13), as well as 84% (21/25) of the households in Los Patios. A total of 80 possible mosquito breeding sites among the 58 households were inspected in and around the property. Twenty nine percent (23/80) of all containers were found positive for *Ae. aegypti* larvae. Out of 35 inspected tanks inside houses, 16 (45.7%) were positive for mosquito water stages. Additionally, 23.8% (5/21) unused containers around the households and 40% of tires were found positive for these stages.

The concentration of IgG-SGE antibodies was significantly higher in participants living in houses positive for mosquito larvae (Mann-Whitney U

Table 1. Socioeconomic factors evaluated in households (n=58) from Cúcuta, Los Patios and Pamplona

Variable	Number of houses	%
Running water		
Daily	18	31.0
Twice a week	15	25.9
Once a week	6	10.3
Every 2 weeks	19	32.7
House material		
Brick	15	25.9
Cement	16	27.5
Combined	27	46.5
Floor material		
Sand or earth	1	1.7
Cement	30	51.7
Sheet tiles	27	46.5
Bathroom type		
Flush toilet	56	96.5
Traditional latrine	1	1.7
No facility or field	1	1.7
Sewer		
Public sewer	41	70.6
Septic hole	14	24.1
River/well/open space	3	5.2
Garbage service		
Twice a week	58	100
Water storage (household use)		
Higher tanks	17	29.3
Lower tanks	6	10.3
Both types of tanks	14	24.1
Water cans	8	13.8
No water storage	13	22.4

test $p=0.0041$). However, the SGE-IgM antibody concentration was similar between the groups (Mann-Whitney U test, $p=0.1757$) (figure 1). Odd ratios showed that a person living in a household with breeding sites positive for mosquito larvae was 3.6 times more likely to have a higher concentration of IgG antibodies (95% CI: 1.172-11.630, $p=0.0229$).

Household characteristics, water availability and anti-SGE antibody concentration

The current study included 55 human subjects; all were tested for antibodies against *Ae. aegypti* SGE. The study population's mean age was 32.7 years old (range: 2 to 75 years old), and 13% (7/55) of subjects were children under the age of 10. Participants living in Pamplona presented the lowest SGE-IgG antibody concentration (Mann-Whitney U test $p>0.05$) followed by Los Patios. There were no significant differences in the concentration of SGE-IgG between Cúcuta and Los Patios (Mann-Whitney U test $p<0.05$), and no significant difference among locations in SGE-IgM antibody concentration.

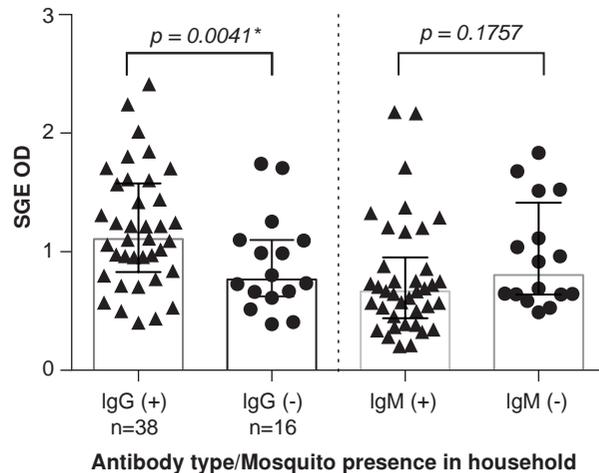


Figure 1. Concentration of anti-*Aedes* SGE IgG and IgM antibodies in subjects living in the houses with (+) and without aquatic stages of *Ae. aegypti* mosquitoes. P value denotes Mann-Whitney U test. OD: Optical density

However, this study showed that SGE-IgG antibody concentration correlates with SGE-IgM antibodies (Spearman correlation 0.7225, $p=0.0346$). Also, age was negatively correlated with both SGE-IgG and SGE-IgM antibody concentrations (Spearman correlation -0.2247 $p=0.0285$ [IgG], -0.2481 $p=0.0153$ [IgM]). There was no significant correlation between the number of people in the household (Spearman correlation -0.254 $p=0.8598$ [SGE-IgG], -0.011 $p=0.9391$ [SGE-IgM]) or the number of rooms (Spearman correlation -0.0399 $p=0.7810$ [SGE-IgG], -0.0816 $p=0.5693$ [SGE-IgM]). The concentration of antibodies was similar when comparing the house or floor material (Kruskal-Wallis test, $p>0.05$).

A comparison between SGE-IgG antibody concentration of subjects living in houses with different types of water storage showed that the concentration of antibodies was higher in people using floor tanks, although we did not find a statistically significant difference among groups. However, we found a significantly higher SGE-IgG antibody concentration in subjects living in houses with unused plastic or glass containers (i.e., beer bottles) in their patios (Kruskal – Wallis test, $p>0.05$). The analysis also showed that IgG-SGE antibody concentration in households storing water for more than eight days was significantly higher than those in households with no water storage (Mann-Whitney U test, $p=0.0062$) (figure 2).

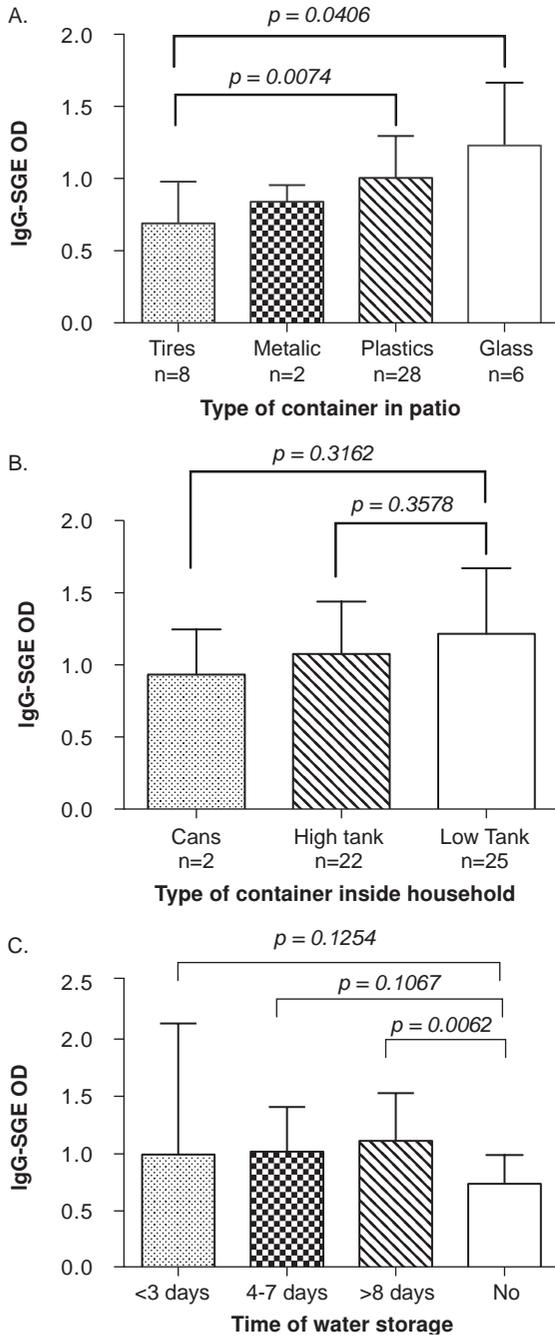


Figure 2. Anti-SGE antibody concentration by: (A) Outside container type, (B) inside container type, and (C) length of water storage. P value denotes Mann-Whitney U test. OD: Optical density

SGE antibody concentration and exposure to DENV

Fifty-five subjects were screened for antibodies against each DENV serotype. Results indicated that 84% (46/55) of the study subjects had been exposed to at least one DENV serotype. Fifty-seven

percent of children <10 years old (4/7) had been exposed to at least one DENV serotype. Serum reactivity to each serotype was evaluated on each subject. Among study participants, the majority of antibodies were against DENV1 (45/55), followed by DENV2 (44/55), DENV4 (42/55) and DENV3 (33/55). People with antibodies for at least one DENV serotype had a significantly higher SGE-IgG antibody concentration than people with negative exposure (Mann-Whitney U test, $p < 0.05$) (figure 3). A significant positive correlation was observed between SGE-IgM and DENV-IgG (Spearman Rank Correlation test, $p < 0.05$), but not between SGE-IgG antibodies and IgG-DENV (figure 4).

Discussion

In Colombia, DENV is a major public health concern due to the steady increase in severe cases, the presence of all four serotypes, and the distribution of the primary vector, *Ae. aegypti*, across more than 90% of the country (18). Barrera, *et al.*, found that the most important factor determining DENV endemicity was the practice of water storage, which provided a source of mosquito breeding sites (19), especially when the water was not frequently replaced (20). In addition, improvements to water distribution infrastructure have been shown to decrease the incidence of vector-transmitted diseases by decreasing the amount of exposure to infected bites (21). Production of *Ae. aegypti* from urban containers can be instrumental in triggering DENV outbreaks during the dry season (19). In fact, this study found a significant number of houses with tanks holding water for human consumption

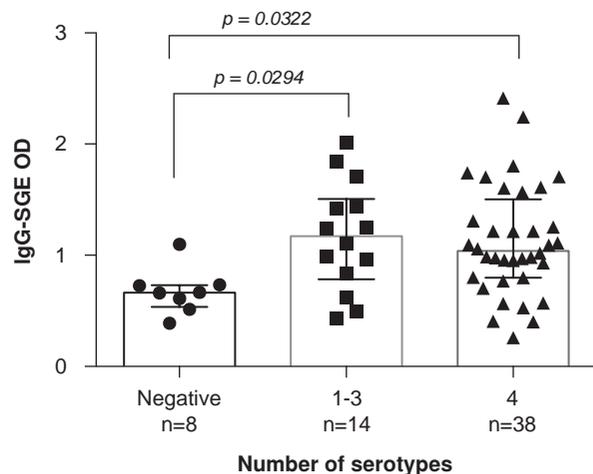


Figure 3. Concentration of antibodies against *Ae. aegypti* SGE in people exposed or not exposed to each DENV serotype. Bars represent the median. P value denotes Mann-Whitney U test. OD: Optical density

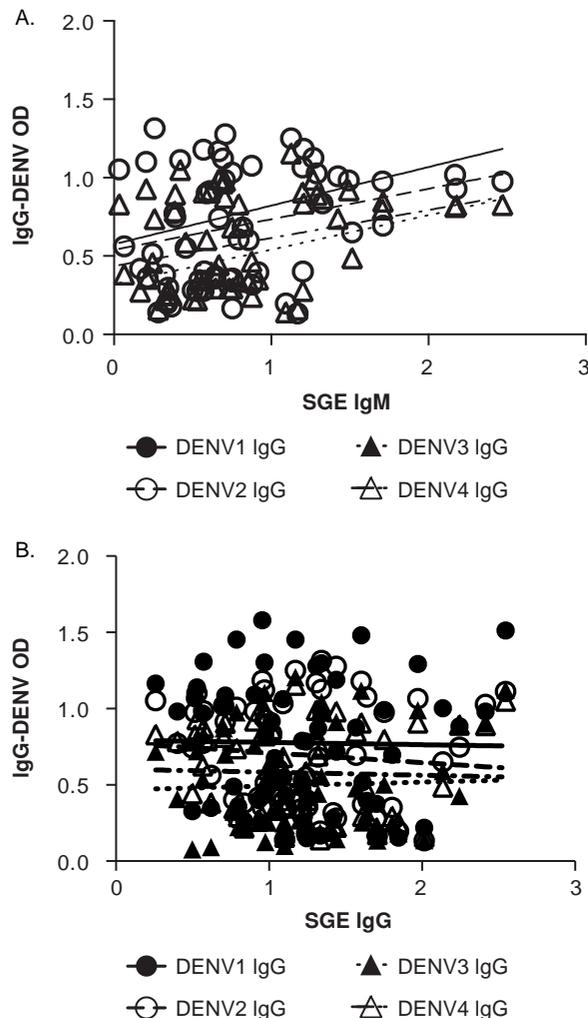


Figure 4. Correlation between the concentration of DENV IgG antibodies and the OD value of: (A) anti-SGE IgM antibody levels (DENV1 [$r=0.3181$, $p=0.0070$], DENV2 [$r=0.2424$, $p=0.0322$], DENV3 [$r=0.3855$, $p=0.0013$] and DENV4 [$r=0.2465$, $p=0.0299$]), and (B) anti-SGE IgG antibody levels (DENV1 [$r=0.0945$, $p=0.2381$], DENV2 [$r=-0.1412$, $p=0.1430$], DENV3 [$r=-0.0563$, $p=0.3360$] and DENV4 [$r=-0.0998$, $p=0.2259$]). P value denotes Spearman rank correlation test. OD: Optical density

that were infested with mosquito larvae, and that the concentration of SGE antibodies was significantly higher in participants with previous exposure to DENV.

Water security, or the reliable access to clean, running water, is associated with water storage behavior (22). Lack of daily water supply inside the houses forces residents to build or improvise water storage options, such as cement/brick water tanks or plastic containers. Container type has been shown to influence the development of aquatic stages and adult mosquito presence in the home

(23). *Aedes aegypti* breeding sites are closely associated with human habitation, as the species prefers to breed in human-made containers (24). In this study we found a rather significant number of houses (>40%) with mosquito aquatic stages inside them. Maintaining stored water for longer time periods provides larvae with sufficient time for development to the adult stage. As adults, female mosquitoes are more likely to feed and rest indoors, reducing the requirement to travel when searching for a blood meal (25).

The study showed gradients of antibody concentration, which translates as exposure to mosquito bites, that depended on the type of water storage kept inside the house. A significant difference in the concentration of antibodies in subjects living in houses with different types of containers in the peridomestic space was also found, as well as a significantly higher concentration of SGE-IgG antibodies in people residing in houses with glass and plastic containers around them, which shows that poor breeding site-reducing practices influence mosquito-human contact and lastly, the risk for pathogen transmission (26,27).

In our study, the level of SGE-IgG antibodies was significantly higher among participants living in households with containers positive for mosquito larvae. Similar results were registered in a recent study in Bolivia, where researchers found a higher antibody concentration against *Ae. aegypti* SGE in people living in areas with a greater abundance of this mosquito (28). They also found an important association between mosquito presence and the level of anti-salivary proteins antibodies. Another important findings of this study was that people exposed to DENV had a significantly higher SGE-IgG antibody concentration. These results support the assumption that the risk of virus transmission increases in DENV-endemic areas where people may be exposed to several DENV serotypes in a single infection (29,30). A recent retrospective study showed that the IgG response to the *Ae. aegypti* salivary peptide Nterm-34 kDa was an indicator for risk of dengue virus transmission in urban endemic areas (31). Our results are consistent with those findings; this is the first study showing an association between total salivary proteins and DENV antibody levels with the presence or absence of mosquitoes in houses and water management.

The study evidenced a negative correlation between anti-SGE antibody levels and age, which is consistent with previous studies (8,32) In

general, SGE-IgM antibody concentration is usually associated with a recent exposure to mosquito bites due to its short life span (33) while IgG antibodies have been found to have remain in blood for months (7). SGE-IgM antibodies were significantly positively correlated with the level of DENV-IgG antibodies while this association with SGE-IgG antibodies was negative, although not significant. In hyperendemic areas, immunity against DENV virus is developed early in life (2,21). Previous reports of seroprevalence in Colombia have shown that it is possible to find areas where up to 40% of children under five year-old have DENV-IgG antibodies (34). The level of DENV-IgG is known to rise rapidly in the presence of a secondary infection (2). In contrast, SGE-IgG antibodies seem to decrease in the presence of chronic exposure to salivary antigens mainly due to the induction of tolerance (32,35). Consequently, the observed negative correlation between SGE-IgG and DENV-IgG, although not significant, is consistent with an increase in the risk of dengue exposure in the presence of chronic exposure to salivary proteins. Additionally, the increase of DENV-IgG along SGE-IgM antibodies suggests a recent contact with potentially infected mosquito bites. The significance of these findings in dengue epidemiology and transmission are currently under investigation.

This study has several limitations. First, the lack of storage and transport logistics limited blood collection to filter paper. Consequently, it was not possible to test for DENV infection/viremia, which are important to determine both the timing and order of exposure (36). Second, previous studies have shown that entomological parameters, such as the human biting rate, can vary within small geographical areas (37) and that factors that affect individual attractiveness to mosquitoes should be also taken into account (38). We are currently recruiting a larger study population in the same three areas of Norte de Santander to address these issues.

The use of anti-vector saliva antibodies has been used as a marker for exposure after vector control methods have been performed (11,39). As seasonal variation in human-vector contact is a dynamic process that informs DENV transmission potential, developing methods to detect changes in and risk factors for increased contact are critically important. Given the demonstration of the relationship between anti-SGE antibodies and DENV exposure, and the findings that anti-vector saliva antibodies are

associated with water insecurity, it is recommended to test for such antibodies in surveillance programs to regularly monitor human-vector contact both during and between outbreaks.

Research endeavors are ongoing to elucidate the role of *Ae. aegypti* salivary proteins in DENV transmission and disease severity. Here we found that the immune response against salivary proteins of *Ae. aegypti* was associated with the presence of mosquito larva in or around houses. Our results also suggest that socioeconomic factors are associated with the concentration of antibodies to *Ae. aegypti* salivary proteins, and, most notably, a higher concentration of anti-DENV antibodies. This finding underscores the importance of addressing water insecurity and housing quality to control vector-borne diseases. The use of anti-vector saliva antibodies may aid in the identification of populations at greatest risk for exposure to the DENV vector, *Ae. aegypti*.

Acknowledgements

The authors want to thank the communities of Pamplona, Cúcuta and Los Patios for their collaboration. We are grateful to the microbiology laboratory of the local hospital of Los Patios and microbiologist Marlén Carrillo Hernández for their assistance in the collection of samples, and to Tonya Colpitts for her support in the preparation of this manuscript. We also thank Samuel Jameson at Tulane University who kindly donated the control samples.

Conflicts of interest

Authors declare no conflicts of interest.

Financial support

This work was supported by Universidad de Pamplona-Colombia (50-year Anniversary Grant), Colciencias-LASPAU International Doctoral Program, and by the National Institutes of Health (USA).

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