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Susceptibility to endemic *Aedes*-borne viruses among pregnant women in Risaralda, Colombia

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ABSTRACT

Objectives: *Aedes*-borne viruses (ABV) affect humans on every inhabited continent and frequently cause epidemics. Recent epidemics of chikungunya and Zika viruses (ZIKV) highlight that preparedness for future epidemics requires assessment of susceptibility, particularly among high-risk groups. We sought to determine immunity against the three major circulating ABV among pregnant women in an ABV-endemic area of Colombia.

Methods: A cross-sectional seroprevalence study was performed, enrolling women presenting to Labor and Delivery. Cord blood and maternal peripheral blood samples were obtained. IgG seroprevalence to flaviviruses and chikungunya was determined by ELISA. An abbreviated neutralization test was used to estimate the frequency and magnitude of immunity to Zika and four dengue serotypes. Cluster analyses explored epidemiologic factors associated with seroprevalence.

Results: Most women exhibited high levels of neutralizing antibodies to one or more ABV; however, nearly 20% were seronegative for flaviviruses. Our research took place after the epidemic peak of the ZIKV outbreak in Colombia in 2016. However, only 20% of pregnant women had high levels of Zika-neutralizing antibodies consistent with likely protective immunity to ZIKV.

Conclusion: Hence, a high proportion of pregnant women in Risaralda remain susceptible to one or more ABV including the teratogenic ZIKV, indicating a risk for future epidemics in this region.

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Introduction

Aedes-borne viruses (ABV) affect humans on every inhabited continent, and they have frequently been responsible for epidemics leading to a substantial burden on healthcare systems in terms of morbidity, mortality, and cost (Cardona-Ospina et al., 2015; Shepard et al., 2016; Wilder-Smith et al., 2017). Dengue virus (DENV) poses a risk to nearly half of the world's population and causes recurrent but unpredictable epidemics in endemic areas. Chikungunya virus (CHIKV), an arthritogenic alphavirus, can cause chronic rheumatologic disease and long-lasting detriment to the quality of life (Rodriguez-Morales et al., 2017; Rodríguez-Morales et al., 2016). The explosive transmission of CHIKV following its introduction into the western hemisphere in 2013 again demonstrated the pandemic potential of ABV (Weaver and Lecuit, 2015). In 2015-2016, Zika virus (ZIKV), another flavivirus like DENV, followed a similar path as CHIKV, rapidly infecting a large number of people in the Americas and exhibiting previously unrecognized clinical manifestations including teratogenicity, sexual transmission, and association with Guillain-Barré syndrome (Lazear and Diamond, 2016; Musso and Gubler, 2016; Parra et al., 2016; Rodriguez-Barraquer et al., 2019). These recent events highlight the need to predict and prepare for epidemics to enable optimal prevention efforts and minimize the detriment to human health.

The risk of infectious diseases in pregnant women is an interesting and vital topic for global health (Jamieson et al., 2006; Rodriguez-Morales et al., 2016). Infections such as ZIKV, H1N1 influenza, and malaria have demonstrated unique pathology in pregnant women (Beigi, 2017). ZIKV most notoriously can profoundly affect maternofetal health, causing a range of adverse maternofetal outcomes (Brasil *et al.*, 2016; Lopes Moreira et al., 2018; Miranda-Filho Dde *et al.*, 2016; Ospina et al., 2020). ZIKV infection and/or exposure *in utero* can also impair growth and development in young children (Cardona-Ospina, 2021; Mulkey et al., 2020; Stringer et al., 2021). In addition to congenital Zika syndrome, maternal DENV infection is associated with maternal death, premature delivery, and spontaneous abortion (Paixão et al., 2016; Tougma *et al.*, 2020). Maternal and perinatal CHIKV infection has been associated with various clinical outcomes (Ferreira *et al.*, 2021). Some studies suggest minimal risk due to perinatal CHIKV infection, but small numbers of cases with severe outcomes including death advocate for systematic research on pregnancy outcomes in the context of CHIKV infection (Ferreira *et al.*, 2021; Escobar et al., 2017; Fritel et al., 2010; Foeller et al., 2021; Villamil-gómez et al., 2015). For three major ABV, the problems are exacerbated by challenges in access and equity in maternal-child health services (Rodriguez-Morales et al., 2018; Tepper et al., 2016) and a lack of countermeasures such as antivirals and vaccines approved for use in pregnancy. A thorough understanding of population immunity to ABV is critical to inform public health efforts to control transmission and prevent disease in endemic areas. Therefore, in this study, we assessed immunity to ABV in a cohort of pregnant patients in Risaralda, Colombia, by defining the serologic immune profile for DENV, ZIKV, and CHIKV.

Methods

Study design

We conducted a cross-sectional analysis of a cohort of pregnant women enrolled upon delivery in one of two study-affiliated hospitals in Risaralda, Colombia. A trained nurse or physician recruited pregnant patients following admission to the Labor and Delivery Unit. Demographic, clinical, and epidemiological information was obtained through a structured questionnaire. Each participant provided a single sample of 20ml of peripheral blood before delivery or post-partum before discharge.

Participants

Recruitment was performed using convenience sampling; thus, a specific sample size was not established. The analyses reported here were performed after achieving a sample size of n = 115 participants. This gave a power of 0.895 to detect differences in the proportion of previously DENV-infected vs. not infected participants, (degrees of freedom = 2-1), of factors with a medium effect size (w = 0.3), with a confidence of 0.95 ($\alpha = 0.05$) (Cohen, 1988). Pregnant women who were admitted to the Labor and Delivery Unit of the participating healthcare centers and met the inclusion criteria were invited to participate and enrolled: ability and willingness to complete informed consent, and aged 18-35 years. Patients with a hemorrhagic complication, preeclampsia, severe anemia requiring transfusion, HIV infection, or autoimmune disease requiring immunomodulatory or immunosuppressive therapy were excluded.

Setting

The study was conducted in two participating healthcare centers (E.S.E Hospital San Pedro y San Pablo in La Virginia and Hospital Universitario San Jorge in Pereira) located in the ABV-endemic area of Risaralda, Colombia from November 2017 to June 2019. Risaralda is one of three departments in the Coffee-Triangle region of Colombia (Figure 1), and it is divided into 14 municipalities. Among them, La Virginia, Pereira, Dosquebradas, and Santa Rosa (urban areas) compose the western metropolitan region of Risaralda, which was the place of residence of most of our study participants. La Virginia has 32,265 inhabitants, with more than 95% of the population living in urban areas (2018). It has one public primary healthcare center (E.S.E Hospital San Pedro y San Pablo) that provides care to at least 70% of the local population. Pereira is the capital city of Risaralda, and Hospital Universitario San Jorge is the leading high-complexity hospital in the public healthcare network. It serves patients not only from Risaralda but also from other departments in the region like Caldas, Quindío, and the Valle del Cauca region. Both Pereira and La Virginia have ecological, climatic, and social features that create favorable habitats for mosquito vectors (*Aedes aegypti* and *Aedes albopictus*) of DENV, ZIKV, and CHIKV (Figure 1a-g).

A total of 115 pregnant patients were enrolled between November 22, 2017, and June 5, 2019. Most (78.3%, n = 91) of them lived in the urban area of Risaralda, mainly Pereira (47.0%, n = 54), La Virginia (20.9%, n = 24), and Dosquebradas (10.4%, n = 12). The median age was 25.1 years (IQR 21.07 to 29.31 years), 7% (n = 8) of the patients did not complete elementary studies (three of them did not know how to read or write), while the remaining patients completed elementary, high-school, technical or university education (Table 1). Most of the patients were housewives who lived in houses made of bricks, cement, or adobe (91.3%, n = 105), all of them with electricity and with proper aqueduct service (95.7%, n = 110) and sewer (99.1%, n = 114). They also reported living with at least three other people (n = 100, 87%) and domestic animals (53%, n = 64). Regarding measures taken to reduce mosquito bites, only 14.8% (n = 17) used screens on windows, 27.8% (n = 32) used bednets, 13% (n = 15) used repellent, and 15.7% (n = 15) used insecticide. Insecticide application by local authorities was reported by 45.2% (n = 52) of women, with 51.9% indicating applications in the last 6 months. A total of 48 patients (41.7%) were vaccinated against yellow fever, and 29 patients (25.2%) reported traveling outside Colombia between 2013 and 2018.

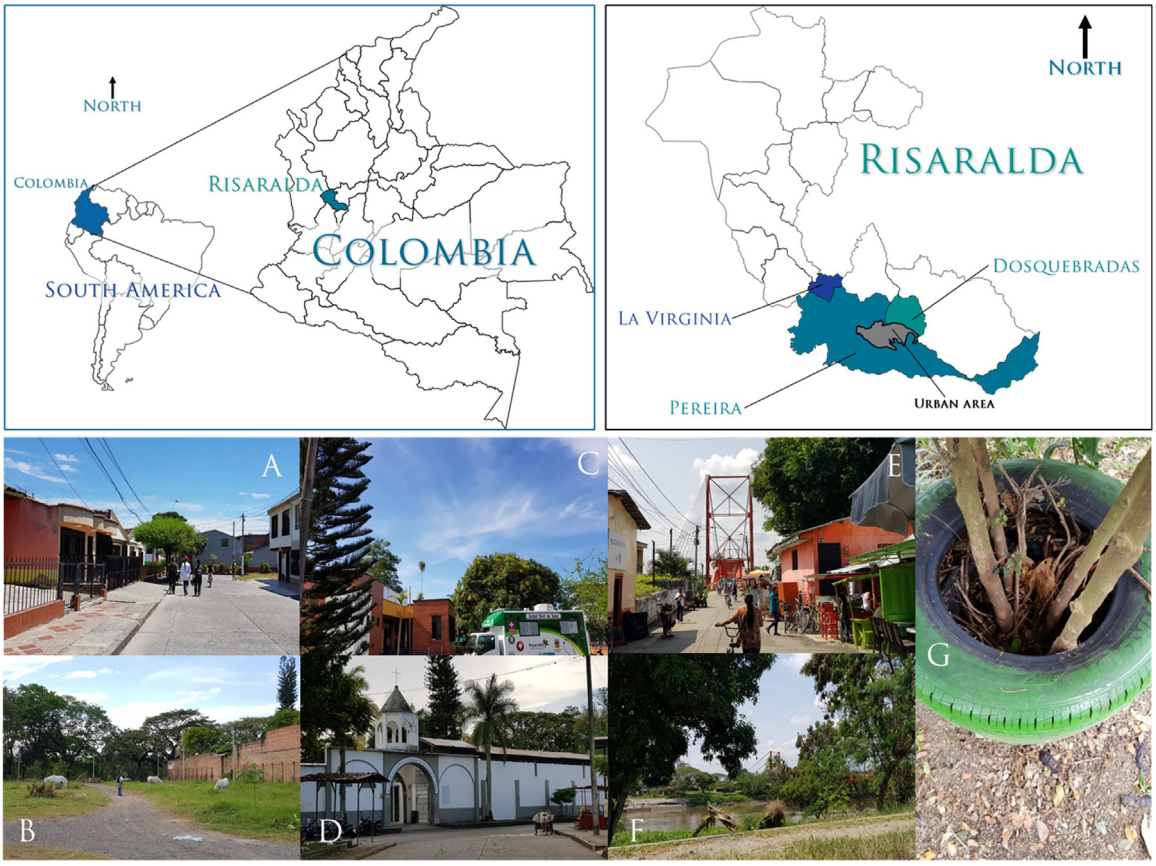


Figure 1. Location of Pereira, Dosquebradas and La Virginia municipalities in the Risaralda department, Colombia. a-g) Ecoepidemiological conditions associated with the transmission of *Aedes*-borne viruses in La Virginia, the most affected area in the department and the region.

Data sources and management

Self-reported epidemiologic and sociodemographic data were provided by each participant via a standard questionnaire. Study staff also filled in clinical variables on a standard study form. We obtained information about working conditions, housing, education, animal husbandry, and access to government-sponsored vector control measures through insecticide use. Participants were also asked about behaviors and practices to avoid mosquito exposure. Clinical and demographic data were entered into an electronic database created in REDCap®.

Antigen capture IgG ELISA

Binding IgG to DENV, ZIKV, or CHIKV was measured by antigen-capture ELISA as previously described (Collins *et al.*, 2019). Briefly, ZIKV or DENV (an equal volume mixture of supernatant from each of the four DENV serotypes cultured in C6/36 cells) antigen was captured by a murine monoclonal antibody (4G2) broadly reactive with flavivirus envelope protein (E) (Henchal *et al.*, 1982). For CHIKV ELISA, plates were coated with the murine mAb CHK-48 (Fox *et al.*, 2015). Plates were blocked with 3% nonfat dry milk and incubated with plasma at 1:100 dilution at 37°C for 1 hour. Binding was detected with an alkaline phosphatase-conjugated goat anti-human IgG secondary Ab and p-nitrophenyl phosphate substrate. Absorbance at 405 nm (optical density, OD) was measured using spectrophotometry on a plate reader.

Neutralization testing

Focus Reduction Neutralization Test (FRNT) and estimated FRNT (eFRNT) were performed as previously described (Collins, 2019, 2020). Assays were performed in a 96-well microFRNT format. Serial dilutions of plasma were mixed with approximately 50-100 focus-forming units of the virus in DMEM with 2% FBS. The virus-antibody mixtures were incubated for 1 hour at 37°C and then transferred to a monolayer of Vero cells for infection for 2 hours at 37°C. OptiMEM overlay media supplemented with 2% FBS and 5g (1%) Carboxymethylcellulose were added. Cultures were incubated for 48 hours (ZIKV and DENV4) or 72 hours (DENV1-3). Cells were fixed and permeabilized with 100 µl of 1:1 methanol:acetone for 30 min. 100 µl of permeabilization buffer was added for 10 min, followed by 100 µl of blocking buffer (3% normal goat plasma in-permeabilization buffer) and left overnight at 4°C. Then, 50 µl of 4G2 at 12.5 ng/µl was added to the plates and incubated for 1 hour at 37°C. Cells were washed with a microplate washer followed by adding 50 µl of 1:3000 horseradish peroxidase-conjugated goat anti-mouse secondary antibody for 1 hour at 37°C. Foci were visualized with 100 µl of True Blue and counted with a user-supervised automated counting program on 2x-magnified images of microwells obtained on a CTL ELISPOT reader. Negative plasma controls were included on every plate to define 100% infection. For eFRNT, samples were run in singleton over four 4-fold dilutions.

Data analysis

For serologic analyses, ELISA data were reported as OD values which are the average of technical replicates. The cut-off for positivity was calculated for each plate as the average OD of negative plasma control wells + three standard deviations + 0.1. FRNT50 values were determined by using the sigmoidal dose-response (four parameters, variable slope) equation of Prism 7 (GraphPad Software). Maximum (100%) infection was defined by the foci counts in wells with negative control plasma. FRNT50 values are

Table 1
Characteristics of participating pregnant patients and variables associated with prior flavivirus infection.

	Flavivirus naïve		Flavivirus seropositive	
	n = 19	%	n = 95	%
Education				
Unable to read and write	1	5.3	2	2.1
Incomplete Elementary		-	5	5.3
Complete Elementary	2	10.5	6	6.3
Incomplete High-school	1	5.3	19	20.0
Complete High-school	6	31.6	40	42.1
Technician	5	26.3	15	15.8
Professional	4	21.1	7	7.4
Unknown	-	-	1	1.1
Municipality of residence[a]				
Anserma	1	5.3		0.0
Apia	2	10.5	1	1.1
Armenia	-	-	1	1.1
Balboa	-	-	1	1.1
Cartago	-	-	4	4.2
Chinchina	1	5.3	-	-
Chocó	1	5.3	-	-
Dosquebradas	4	21.1	8	8.4
El Aguila	-	-	1	1.1
La Celia	-	-	2	2.1
La Virginia	2	10.5	21	22.1
Manizales	-	-	1	1.1
Marsella	-	-	2	2.1
Mistrato	1	5.3	1	1.1
Obando	-	-	1	1.1
Pereira	6	31.6	47	49.5
Pueblo Rico	-	-	2	2.1
Quimbaya	1	5.3	-	-
Quinchia	-	-	1	1.1
Santa Rosa	-	-	1	1.1
Occupation				
Housewife	9	47.4	67	70.5
Healthcare worker	2	10.5	3	3.2
Industrial worker	-	-	3	3.2
Seller	2	10.5	4	4.2
Student	1	5.3	4	4.2
Other	5	26.3	14	14.7
Dwelling features				
Walls material				
Brick and cement	15	78.9	85	89.5
Wood	2	10.5	4	4.2
Other	2	10.5	6	6.3
Aqueduct and sewer[a]				
No	3	15.8	1	1.1
Yes	16	84.2	94	98.9
Living with animals				
No	5	26.3	48	50.5
Yes	14	73.7	47	49.5
Mosquito nets in windows				
No	17	89.5	79	83.2
Yes	2	10.5	16	16.8
Mosquito bednets				
No	17	89.5	65	68.4
Yes	2	10.5	30	31.6
Use of repellent				
No	15	78.9	84	88.4
Yes	4	21.1	11	11.6
Use of insecticide				
No	17	89.5	79	83.2
Yes	2	10.5	16	16.8
Yellow fever vaccine				
No	9	47.4	37	38.9
Yes	7	36.8	41	43.2
Unknown	3	15.8	17	17.9

[a] $P < 0.005$

the calculated titer at which maximum foci counts are reduced by 50%. Dilution curves for plasma Ab and mAb binding were generated using the same equation. Reported FRNT50 values were required to have an R^2 greater than 0.75, a Hill slope greater than 0.5, and an FRNT50 falling within the range of the dilution series. The eFRNT50 value is a discrete number corresponding to the dilution factor, at which 50% maximum FFU is observed or the average of the two dilution factors between which the 50% FFU threshold is crossed. Results from eFRNT assays were entered directly into an excel file. For generating the heat map, eFRNT titers of <20 were assigned a value of 1, and titers >1280 were assigned a value of 2000. eFRNT testing on a random subset of DENV IgG ELISA-negative samples was performed, consistently yielding values <20. Some ELISA-negative samples were not tested by eFRNT and directly assigned a value of 1 for the heatmap.

Quantitative variables were summarized with mean, median, or proportions, with their respective SD, interquartile range (IQR), and confidence interval (CI), when appropriate. Data analysis was performed using R Studio based on the imported dataset from REDCap®. Variables were compared using parametric or non-parametric tests for hypothesis testing, after verifying normality using the Shapiro-Wilk's test and equal variances with Bartlett's test. Categorical variables were compared using the Chi-square test or Fisher's exact test when appropriate.

Cluster analysis and validation

All eFRNT50 titers were log-transformed (base 10), data were scaled, and we then calculated a distance matrix using the Euclidean distance as the metric for clustering. Next, we analyzed cluster tendency using principal component analysis for inspecting the data for clustering. Then the results were validated using a visual assessment of cluster tendency and the Hopkins statistic to test the spatial randomness of the data (threshold <0.5). We tested a nested clustering method (agglomerative hierarchical) and a partitioning clustering method (k-means). The best clustering strategy was selected by assessing internal measures: connectivity; silhouette coefficient; and the Dunn index; and stability measures: average proportion of non-overlap (APN), average distance (AD), average distance between means (ADM), and figure of merit (FOM), for evaluating the consistency of clustering. For external validation, we compared the clustering solutions using the corrected Rand index with variables significantly associated with serostatus. This index measures the similarity between two partitions, adjusted for the chance. Its range is -1 (no agreement) to 1 (perfect agreement).

Results

Participants and demographic data

ABV seroprevalence and immune profile

There were 114 samples available for testing by DENV and ZIKV IgG ELISA, which both serve as sensitive and non-specific assays for prior flavivirus infection. In the DENV ELISA, 96 (83.3%) were seropositive, and 99 (86.8%) were seropositive by ZIKV IgG ELISA, indicating that >80% of women in this study have had at least one previous flavivirus infection (Figure 2a). The seroprevalence for CHIKV was 29.6% (Figure 2a). Selected CHIKV IgG ELISA+ samples (n = 22) were tested by CHIKV eFRNT, and a strong concordance of results was observed for these two serologic assays (Supplementary Figure S1). Interestingly, when DENV seropositivity was analyzed in subjects grouped by CHIKV serostatus, there was a significant association between prior DENV and CHIKV infection ($\chi^2 = 6.6929$, $P < 0.001$; Figure 2b).

All available seropositive samples were then tested by eFRNT assay to give a snapshot view of individual and population immunity to DENV and ZIKV (Figure 2c, Supplementary Table S1). When neutralization testing was performed on DENV ELISA negative samples, there was a high concordance of results (data not shown). Thus, ELISA negative samples were defined as neutralization negative when not tested. A selected subset of samples

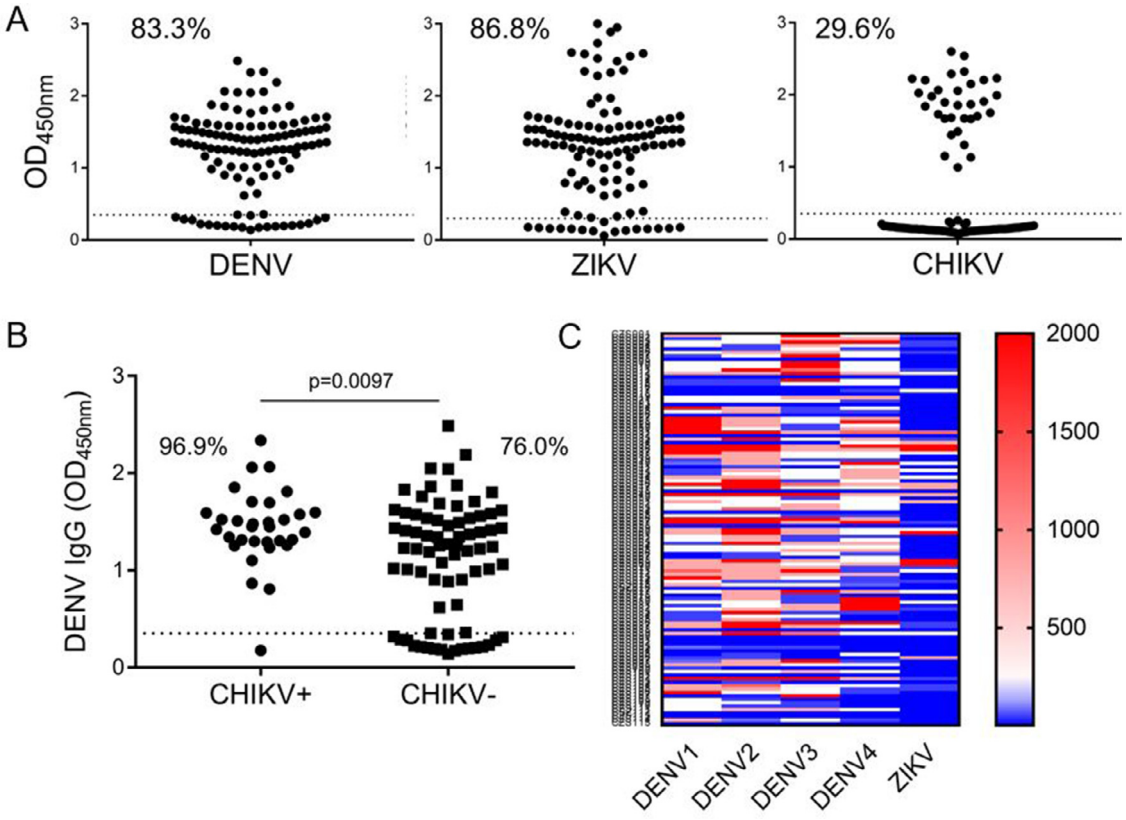


Figure 2. *Aedes*-borne viruses are highly endemic in Risaralda. a) IgG seroprevalence for the indicated virus (DENV and ZIKV, n = 114; CHIKV, n = 108) was measured by ELISA. The percent (%) positive is shown in the upper left of each panel. The cut-off used to determine percent positive was calculated for each plate according to the negative controls on the same plate. The horizontal line on each graph approximates the cut-off in each assay. Raw optical density (OD) values graphed are derived from multiple ELISA plates but included in a single graph for convenience of display. b) DENV seropositivity was re-graphed after dividing the population according to CHIKV IgG serostatus, shown on the x-axis. The percentage of DENV IgG+ is included next to each CHIKV serostatus group. The *P*-value from a Chi-square test is shown at the top, indicating a significant difference in the proportion of DENV seropositivity between the two subgroups corresponding to CHIKV serostatus. c) The eFRNT50 was determined for DENV1-4 and ZIKV for the majority of the cohort (n =113), and data are displayed in a double gradient heat map, with the color scale shown at the right. Values <20 were set to 1; values >1280 were set to 2000.
Abbreviations: CHIKV, Chikungunya virus; DENV, Dengue virus; eFRNT, estimated Focus Reduction Neutralization Test; ZIKV, Zika virus.

(n = 15) was tested by conventional FRNT to determine a precise titer (Supplementary Figure S2). These experiments confirmed a good approximation between the eFRNT and true titer when determined by FRNT. The salient patterns of immunity against flavivirus represented among the cohort were categorized as follows: 1) Flavivirus naïve: Patients with negative IgG ELISA, 2) Primary DENV: eFRNT ≥200 against only one DENV serotype, 3) Primary ZIKV: eFRNT ≥200 against ZIKV and ≤50 against DENV1-4, 4) Secondary DENV: eFRNT≥200 more than one DENV serotype and ≤50 against ZIKV, and 5) Undifferentiated polyflavivirus: eFRNT ≥200 against DENV and ZIKV. Primary dengue corresponded to 11.4% of the patients (n = 13), and secondary dengue corresponded to 48.2% (n = 55). Polyflavivirus immunity corresponded to 18.4% of the cases (n = 21). And only three pregnant patients (2.63%) presented a neutralizing Ab pattern consistent with Primary ZIKV.

Variables associated with flavivirus serostatus

We found that infection rates varied across cities of residence ($P = 0.012$). Aqueduct and sewer availability were associated with flavivirus seropositive status ($P = 0.007$); however, there were only four people without aqueduct and sewer. When analyzing categories of neutralizing antibodies against flavivirus, we found an association between the region of residence (western metropolitan area of Risaralda vs. other areas) and neutralizing patterns ($P = 0.021$), as well as with the history of traveling outside Colombia ($P = 0.041$). No other factors were associated with flavivirus serostatus, including mosquito bite prevention measures.

Cluster analysis

As a complementary approach, we performed a cluster analysis to group data according to the similarity in flavivirus immune signatures and to validate serostatus definition based on eFRNT titers. Then, different groups were compared to identify the differences in demographic or epidemiological features. Based on the results of stability indices (Table 2), the best clustering strategy was k-means clustering (APN = 0.049; ADM = 0.210 for 2 clusters, and AD = 1.723; FOM = 0.614 for 6 clusters). Since the optimal Silhouette index (0.546) was also obtained with 6 k-means clusterings, this was the strategy and number of clusters selected for further analysis (Figure 3). External validation of the selected clustering strategy revealed a corrected Rand index of 0.525 when contrasted with the immune classification of the patient, 0.006 when contrasted with features of the aqueduct service, and 0.006, -0.018, -0.026 travel, region, and city of residence.

Discussion

We showed here that pregnant women living in a tropical region conducive to the transmission of ABV exhibit heterogeneous patterns of immunity to DENV, ZIKV, and CHIKV. Most (>80%) of the study participants have serologic evidence of at least one prior flavivirus infection. However, the immunity profile suggested several different flavivirus infection histories ranging from primary DENV or ZIKV to sequential infection by multiple flaviviruses. CHIKV has also clearly circulated in Risaralda based on this study

Table 2
Cluster characteristics and internal validation indexes of the two used clustering methods in the sample.

		Number of clusters				
		2	3	4	5	6
Hierarchical	Connectivity	94.091	133.865	167.349	188.806	195.139
	Dunn	0.308	0.394	0.377	0.383	0.383
	Silhouette	0.530	0.496	0.429	0.430	0.480
K-means	Connectivity	123.468	163.242	179.627	151.306	173.663
	Dunn	0.161	0.171	0.310	0.310	0.314
	Silhouette	0.534	0.501	0.481	0.516	0.546

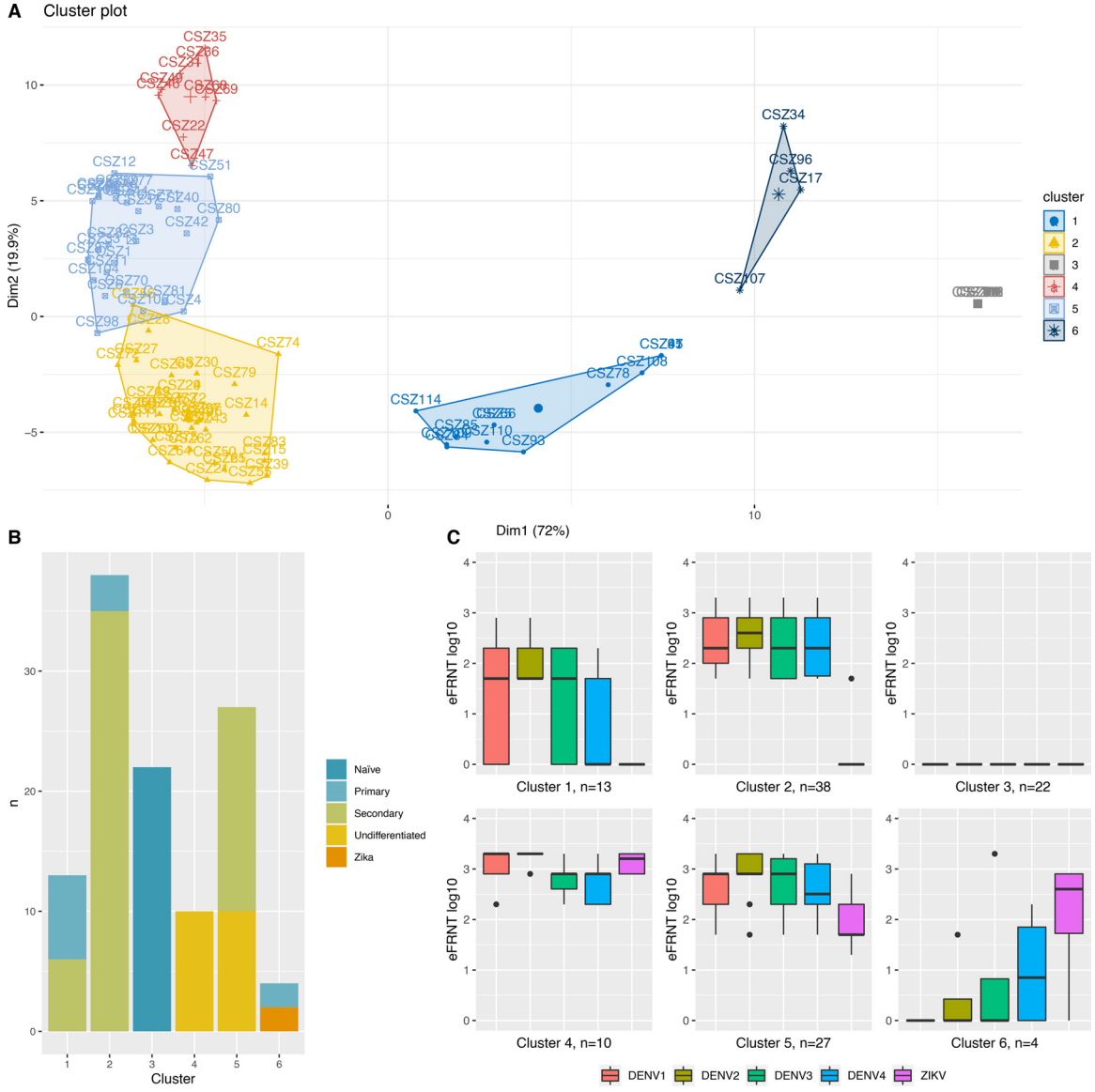


Figure 3. Clustering analysis of the participants included in our study and eFRNT profile against DENV1-4 and ZIKV per cluster. a) K-means clustering. b) The total number of participants and the breakdown by flavivirus immune profile designation are shown in a stacked bar chart. c) Median (bold line), interquartile (box), range (vertical line), and outliers (points) of the $\log_{10}$ eFRNT50 titers against DENV1-4 and ZIKV are shown for the six clusters obtained with the k-means method.
Abbreviations: DENV, Dengue virus; eFRNT, estimated Focus Reduction Neutralization Test; ZIKV, Zika virus.

and our prior work (Rodriguez-Morales, 2016). The significant association of CHIKV and DENV seroprevalence likely identifies a population living with an increased burden of mosquito exposure. This finding supports the idea that infection by one ABV partially predicts the risk for a subsequent ABV infection (Bisanzio *et al.*, 2018). A majority of pregnant women (~80%) may be susceptible to future ZIKV infection despite residing in an area where ZIKV has previously circulated. We recently found that over 50% of pregnant women living in León, Nicaragua, were likely infected by ZIKV during a single transmission season in 2016 (Collins *et al.*, 2020). Similarly, high infection rates have been noted in Managua (56%) (Zambrana *et al.*, 2018) and northeastern Brazil (61.3%) (Rodriguez-Barraquer *et al.*, 2019). Thus, ZIKV seroepidemiology varies widely in Latin America. Recent ZIKV outbreaks in Kerala India (2021) re-

mind us of the ongoing threat of ZIKV infection. We hypothesize that many places in Latin America with similar ABV transmission ecology as Risaralda are at high risk for future ZIKV epidemics, echoing concerns raised by others in the Southeast Asian setting (Densathaporn et al., 2020; Phatihattakorn et al., 2021).

Though conclusions about clinical outcomes in our cohort cannot be drawn, our data do provide evidence of transmission of multiple DENV serotypes and cases of monotypic immunity to ZIKV and DENV. Thus, this setting could foster severe dengue cases due to antibody-dependent enhancement, occurring when DENV infection follows a first DENV infection by a heterologous serotype or a first ZIKV infection (Katzelnick *et al.*, 2020). The idea that pre-existing DENV immunity may have a pathologic role in ZIKV infection, particularly congenital ZIKV infection is notable (Ausderau et al., 2021; Bardina et al., 2017; Crooks et al., 2021; Zimmerman et al., 2018). However, the impact on clinical outcomes in pregnancy is uncertain and likely complex, with one ecological study indicating that prior DENV outbreaks may be followed by increased or decreased risk for microcephaly cases (Carvalho *et al.*, 2020). Our clustering analysis identified groups of participants with high levels of neutralizing antibodies to ZIKV and multiple DENV serotypes. Other groups exhibited evidence of multitype DENV immunity with a lack of ZIKV-neutralizing antibodies. This latter group (cluster 6) may be at particular risk for future ZIKV infection given that *Aedes aegypti* exposure was likely intense over several years but immunity to ZIKV did not develop with the first wave of ZIKV infection in this region.

We did not identify demographic predictors of infection status, including the practice of mosquito bite prevention measures. It is questionable how consistently such measures were utilized, and dedicated efforts at community and stakeholder engagement are likely needed (Kolopack *et al.*, 2015). One randomized controlled trial demonstrated that community mobilization to eliminate *Aedes* breeding sites reduced the incidence of DENV infection (Andersson *et al.*, 2015). Evidence supporting the efficacy of several vector control strategies may be available in coming years (Achee, 2015; Bowman et al., 2016), providing a critical opportunity to educate and engage with communities to optimize implementations of interventions that decrease ABV transmission. To this end, our group has initiated educational efforts using audiovisual material, clowns, and theatrical presentations to promote mosquito bite prevention, particularly in pregnant patients.

Our work had some limitations. Convenience sampling enabled efficient enrollment of pregnant women presenting for peripartum care to medical facilities where it was expedient to obtain biospecimens. Thus, our sample derived from a single geographic region and only two of many regional healthcare centers may not entirely represent the ABV immune profile in the general population of Risaralda or the specific municipalities where the subjects reside. Though DENV and ZIKV are the major flaviviruses known to have recently circulated in this area, other flaviviruses may have infected some women. West Nile virus may be under-appreciated in many parts of the world. However, even in places where significant outbreaks have occurred, seroprevalence is still typically very low (Eldin et al., 2019; McDonald et al., 2019; Popescu et al., 2020), making it less likely that flavivirus cross-reactive antibodies elicited by West Nile virus confounded our analysis.

To conclude, our research highlights the potential benefit and need for serologic surveillance and immune profiling for ABV. Our methods for defining virus type-specific immunity revealed that a large proportion of the population is likely susceptible to ZIKV, which may forbode future epidemics. Novel methods to assess virus-specific immunity in broadly deployable, high throughput platforms would greatly improve public health approaches to ABV surveillance and pandemic preparedness. Finally, this study highlights the value of pregnancy cohort studies in understanding ABV epidemiology. Such studies could be expanded to efficiently assess ABV immunity in the general population and better understand the potentially under-appreciated impact of ABV on maternal-child health.

Declarations of competing interest

The authors have no competing interests to declare.

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Ethical approval statement

All research was performed consistent with the ethical standards established in the 1964 Declaration of Helsinki and the Resolution No 8430 of 1993, which governs health research in Colombia. Written informed consent was obtained from each patient in her native Spanish language. This study protocol was approved by the Bioethics Committee of Universidad Tecnológica de Pereira (Acta No. 22 Punto 03, Numeral 02, del 28 de noviembre de 2016) and Comité de Ética en Investigación, Institución Universitaria Visión de las Américas (Acta No 88 del 23 de Junio de, 2021). The study was initiated under approval by the Institutional Review Board of the University of North Carolina, Chapel Hill (17-1567) and continued under the Emory University IRB (103255 and 00106096).

Availability of data and materials

The dataset supporting the conclusions of this article is included within the article and its Supplementary files.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.07.017.

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