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## Impact of preexisting dengue immunity on Zika virus emergence in a dengue endemic region

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**Competing interests:** EJM, ETAM, and AIK are authors on a patent application, entitled "Methods and Composition for the Detection of Flavivirus Infections" related to this work (U.S. Provisional Patent Application Serial No. 62/503.201 and US Provisional Patent Application Serial No. 62/608.927). AIK received honoraria from Sanofi-Pasteur for his participation in a conference on Zika vaccines. The authors declare that they have no other competing interests.

**Data and materials availability:** All relevant data are within the paper and its Supporting Information files. Code to reproduce study findings is available at <https://doi.org/10.5281/zenodo.2528486>.

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## Abstract

The clinical outcomes associated with Zika virus (ZIKV) in the Americas have been well documented but other aspects of the pandemic, such as attack rates and risk factors, are poorly understood. We prospectively followed a cohort of 1453 urban residents in Salvador, Brazil and, using a novel assay measuring IgG3 responses against ZIKV NS1 antigen, estimated that 73% (95% CI 70%-76%) of individuals were infected during the 2015 outbreak. Attack rates were spatially heterogeneous, varying three-fold within a community spanning 0.17km<sup>2</sup>. Pre-existing high antibody titers to dengue virus (DENV) were associated with reduced risk of ZIKV infection (OR 0.56, 95% CI 0.37-0.86) and symptoms (OR 0.52, 95% CI 0.30-0.90). The landscape of ZIKV immunity that now exists may affect the risk for future transmission.

## One Sentence Summary:

Within a Brazilian urban community, pre-existing immunity to dengue virus and spatial variation influenced Zika virus transmission.

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There are many uncertainties about the dynamics of Zika virus (ZIKV) outbreaks in large, immunologically naive populations. There is also continued uncertainty surrounding the circumstances that allowed ZIKV to emerge in American populations and the future of ZIKV transmission in the Americas and elsewhere (1). The association between ZIKV infection and microcephaly cases makes it important to determine whether future cases can be expected or whether immunity levels in a population are likely to prevent significant future transmission. These uncertainties underscore the need to characterize infection rates of the 2015/2016 epidemic (1, 2).

One key, unanswered question is the role of pre-existing immunity to dengue virus (DENV), an endemic pathogen that is genetically and antigenically similar to ZIKV, and has been hypothesized both to promote (3) and suppress ZIKV emergence (4). Numerous *in vitro* studies have shown enhanced ZIKV infection in the presence of DENV antibodies, but results from *in vivo* studies have been less clear (3, 5-8). Immunity to DENV has been shown experimentally to neutralize and protect against ZIKV infection (9-11) and may therefore reduce the probability of successful establishment of ZIKV in a DENV immune

population (12). Yet to date, the influence of pre-existing DENV immunity on susceptibility or resistance to ZIKV infection has not been rigorously evaluated in human populations.

Ideally, to characterize the interactions between ZIKV and DENV requires prospectively monitoring individuals of different DENV serostatus and measuring any ZIKV infection. However, a large proportion of ZIKV and DENV infections are asymptomatic, and symptomatic infections have a nonspecific clinical presentation, making diagnosis challenging. Therefore, infection rates in populations can only be accurately quantified using serological measures. At present, there is a lack of commercially available serological tests that can measure antibody responses to DENV and ZIKV with sufficient sensitivity and specificity as a result of cross-reactivity between the two related viruses (13-16). These limitations have hampered efforts to quantify the extent of the American epidemic and evaluate interactions between DENV and ZIKV.

### High ZIKV attack rates in an urban population

We had the opportunity to prospectively characterize the dynamics of ZIKV transmission in Pau da Lima, a community in the city of Salvador, where we have been conducting long-term cohort investigations of urban slum health problems (17-19). Northeast Brazil has been known to be hyperendemic for DENV for over 30 years. It was also the epicenter of the 2015 Zika epidemic (20-22). Multiple rounds of sample collection occurred in our cohort since 2013, before and after the majority of clinical Zika cases were detected in this community and the city (23). Here, we quantified the attack rate of ZIKV during the 2015 outbreak using data from 1,453 individuals in this cohort (Fig. 1, Tables S1 and S2). In a subset of 642 individuals for whom dengue immunity data was available (Table S1), we investigated the impact of prior DENV immunity on the risk of ZIKV. Figure 2 shows the timing of sample collection before and after the period of the Zika outbreak.

We identified ZIKV infections with an assay that measures IgG3 responses to the ZIKV NS1 protein (anti-ZIKV NS1 IgG3). The sensitivity and specificity of the assay were estimated to be 85% and 97%, respectively, by testing a random subset of samples using plaque-reduction neutralization tests (PRNT) (Fig. 2). We performed additional validation of this assay to ensure that cross-reactivity did not bias our inferences, using samples from before and after ZIKV emergence in the study population (Fig. 2), as well as external validation using patient samples from PCR-confirmed infections from multiple locations (Figs. S2 and S3, Table S3).

Samples collected in October 2014 and March 2015, prior to the epidemic in Salvador (Fig. S1), were mostly seronegative by the anti-ZIKV NS1 IgG3 assay (7%, 95% CI 4-10%, n=249; and 8%, 95% CI 6-10%, n=675; respectively), as well as by PRNT (1%, 95% CI 0%-4%, n=101), suggesting little or no transmission before this date. In contrast, 63% (95% CI 60%-65%, n=1453) of samples collected in October 2015, five months after recognized clinical cases peaked, were positive, indicating infection of the majority of individuals in the cohort (Fig. 3A). Adjusting for the sensitivity and specificity of the IgG3 assay (Fig. 2C, Table S3), this level of seropositivity corresponds to an attack rate of 73% (95% CI

70%-76%) and is consistent with the high seropositivity rate found in non-random subsets of individuals previously tested in Salvador (24).

## Heterogeneity in attack rates at fine spatial scales

Interestingly, despite the high seropositivity rates across all age-groups (Fig. 3A), attack rates were not spatially uniform across the population that resided within the 0.17km<sup>2</sup> study site (Fig. 1C). The attack rate among individuals living in the valley located in the northeast sector of the study area was 29% (95% CI 19%-41%, n=85) as compared to 83% (95% CI 76%-90%, n=241) among individuals living in the southeast corner (Area B, Fig. 1C). Similar spatial heterogeneity in DENV transmission has been reported within this community and elsewhere (21, 25). This variation could be due to differences in populations of mosquito vectors or in exposure of people living under different conditions.

Based on the attack rate, we estimated that the basic reproductive number ( $R_0$ ) of ZIKV in this setting was 1.8 (95% CI 1.7 - 1.9) but ranged between 1.2 and 2.1 between areas of the study site. This result is consistent with estimates obtained using case reports from the early epidemic which suggest that reproductive numbers fell from 2.2 to 0.7 from April 2015 to July 2015 (average of 2.0 (95% CI 1.9-2.1) (Fig. S1) (23). These results are also within the range of previously published estimates for ZIKV (2, 24, 26-28), as well as the basic reproductive number for DENV (2.3 95% CI 2.1-2.4) which was estimated using age-stratified serological data collected prior to the ZIKV outbreak (Fig. 3B, Supplementary material). Altogether, the reduction in reproductive numbers and the large reduction in case numbers reported in the city of Salvador in 2016 and 2017 compared with 2015 (29) is consistent with acquired immunity blunting the efficiency of transmission, as suggested by Netto *et al.* (24).

## Influence of dengue immunity on ZIKV infection

We investigated potential associations between DENV antibody titers measured before the ZIKV epidemic and the subsequent risk of ZIKV infection. A total of 642 samples collected in March 2015 was tested for total IgG against DENV NS1 protein, a marker of prior DENV infection, of which 553 (86%) were positive (30). Age-specific seropositivity patterns were consistent with high levels of endemic DENV transmission in this population (Fig. 3B). Among individuals who had prior immunity to DENV, each two-fold increase in total IgG titers against DENV NS1 was associated with a 9% (95%CI 1%-17%) reduction in the risk of ZIKV infection (Table S5). A similar trend was observed in a subset of 96 individuals that was tested for DENV neutralizing antibodies using the PRNT (26% reduction, 95%CI -18%-54%).

To investigate a possible non-linear effect of DENV IgG titers on the risk of ZIKV infection, we estimated the risk of ZIKV infection among individuals in different tertiles of pre-outbreak IgG titers. We found that individuals in the middle and highest tertiles experienced a 38% (95% CI 8%-59%) and 44% (95%CI 14%-63%) reduction in the odds of being ZIKV-positive after the outbreak, respectively, compared with individuals in the lowest tertile with no or low titers of DENV IgG (Table S4). We did not find any association between age and

risk of ZIKV infection, despite the positive association between DENV IgG levels and age that is characteristic of endemic settings (31, 32). This contrasts with a study in Nicaragua that found a positive association between age and ZIKV infection risk (33).

We also explored the association between pre-outbreak IgG3 antibodies against DENV NS1 and the risk of subsequent ZIKV infection. This subclass of IgG antibodies has been previously shown to be a marker of recent exposure to DENV infection and to be detectable for up to 4-6 months after infection (30). In contrast to total pre-outbreak IgG titers against DENV NS1, IgG3 levels were positively associated with risk of infection by ZIKV. A twofold increase in IgG3 was associated with a 23% (95%CI 7%-43%) increase in the odds of being ZIKV positive after the outbreak, in models adjusted for age and total IgG NS1 titers. To assess whether the observed results could be due to cross-reactivity between pre-outbreak ZIKV IgG3 and DENV IgG3 responses, we performed sensitivity analyses where we adjusted for ZIKV IgG3 levels measured in March 2015 (Table S6). This additional adjustment did not affect the results for the positive association between DENV IgG3 and ZIKV seropositivity. This positive association might reflect an immune profile, among individuals who have experienced a recent DENV infection, that is associated with having a greater risk of a subsequent ZIKV infection. Alternatively, it is also possible that higher levels of IgG3 are a proxy for frequent DENV exposure, and thus greater risk of infection by *Aedes aegypti* transmitted viruses.

To further investigate the association between prior DENV exposure and ZIKV infection, we fit a generalized additive model including DENV NS1 total IgG, DENV NS1 IgG3 and age as covariates (Fig. 4A). Consistent with results from logistic models, these analyses showed a negative linear association between pre-outbreak anti-DENV NS1 total IgG (on the log scale) and subsequent ZIKV infection, and positive linear association with pre-outbreak anti-DENV NS1 IgG3. While age was not associated with risk of ZIKV infection when modeled using logistic regression or GAMS, we did find a non-linear effect of age on the ratio of ZIKV IgG3 levels measured after and before the outbreak (Fig. 4b). The average ratio was 3.8 (95%CI 3.0-4.6) in children aged 5-10 years but was 2.4 (95%CI 2.1-2.8) in individuals older than 20 years of age. This difference remained significant in models adjusted for ZIKV IgG3 levels prior to the outbreak.

We also investigated associations between prior DENV immunity and self-reported Zika symptoms recorded from the cohort. Among 642 cohort individuals, 29 (5%), 206 (32%) and 11 (2%) reported experiencing an acute episode of skin rash, fever, or both when interviewed after the Zika epidemic during the October 2015 survey, consistent with at most 36% of individuals presenting any symptoms. ZIKV seropositivity at the time of interview was associated with reporting rash (OR 3.17, 95% CI 1.19-8.43) but not with fever alone (OR 0.99, 95%CI 0.71-1.40). All 11 individuals who reported both rash and fever during this visit were ZIKV seropositive. In addition, among individuals who were infected by ZIKV, the presence of high anti-DENV NS1 total IgG titers prior to the outbreak was associated with 47% (95% 0.10-0.69) reduction in the odds of fever, but no reduction in the odds of rash (Tables S8 and S9). Pre-outbreak anti-DENV NS1 IgG3 levels were not associated with decreased odds of fever or rash.

## Conclusions

In summary, we found that our cohort of individuals residing at the epicenter of the Zika epidemic in Brazil was heavily affected in 2015, with an estimated attack rate of 73% (95% CI 70%-76%). The pattern of cases observed is consistent with the emergence of a highly transmissible and mostly asymptomatic pathogen, followed by reduced transmission once a large proportion of individuals became immune. These findings provide empirical support for the hypothesis that accumulated immunity drove ZIKV to local extinction by reducing the efficiency of transmission. Spatial heterogeneity in ZIKV attack rates show that focal pockets of susceptible individuals may remain after the apparent end of the epidemic. While these populations could sustain future ZIKV transmission and may represent an opportunity for vaccine trials, the overall high rates of immunity will present a barrier for significant ZIKV incidence in this community.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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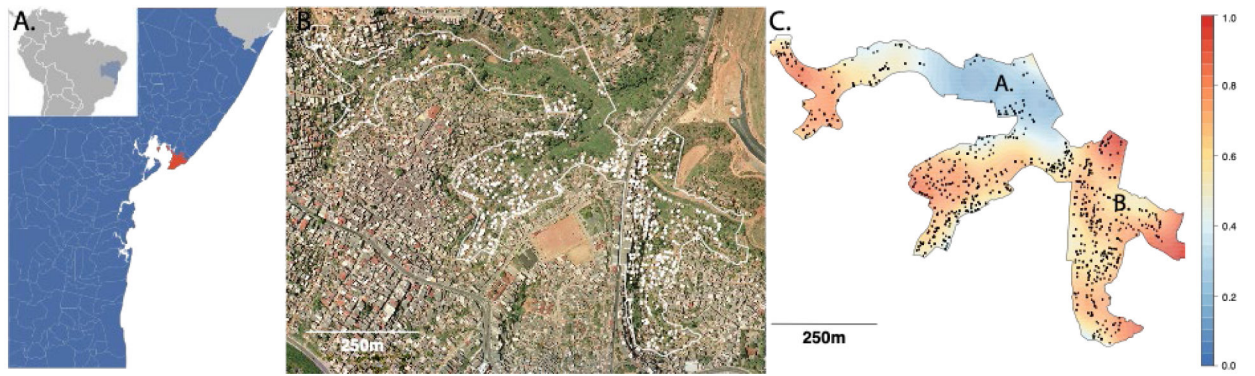
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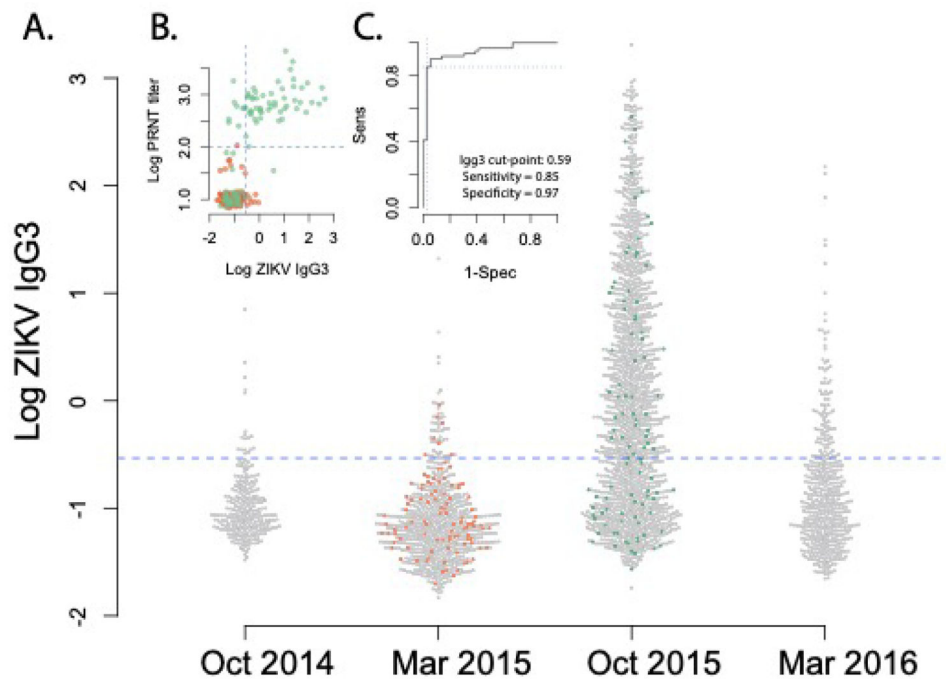
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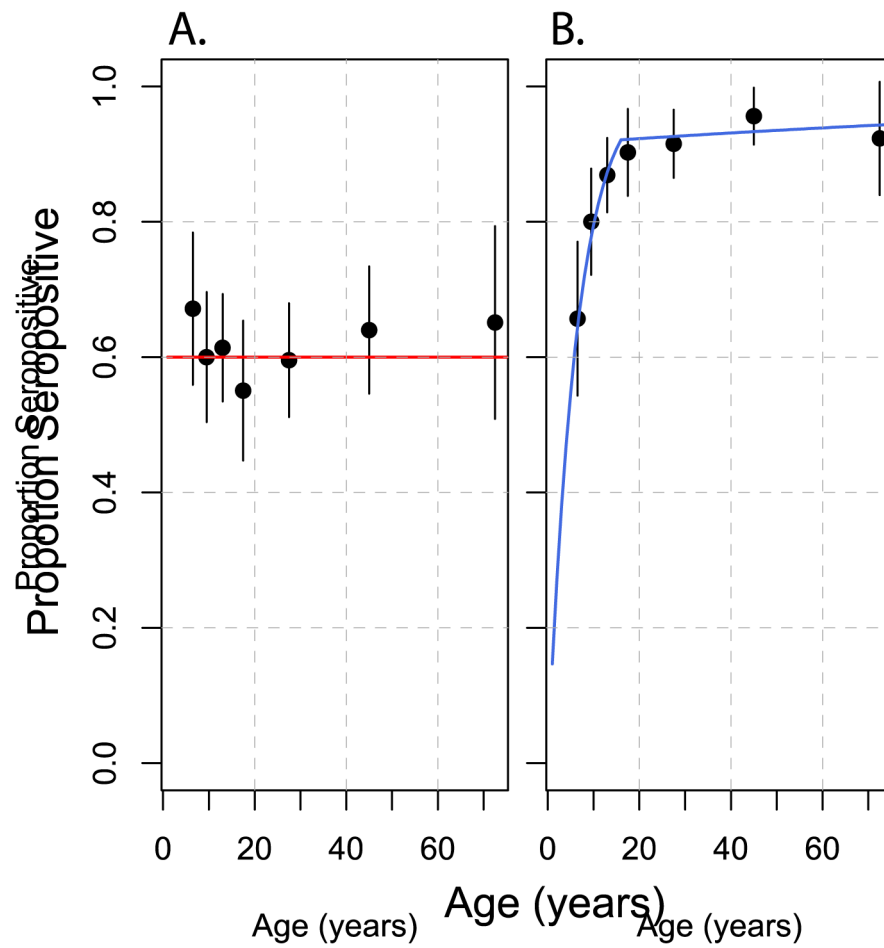
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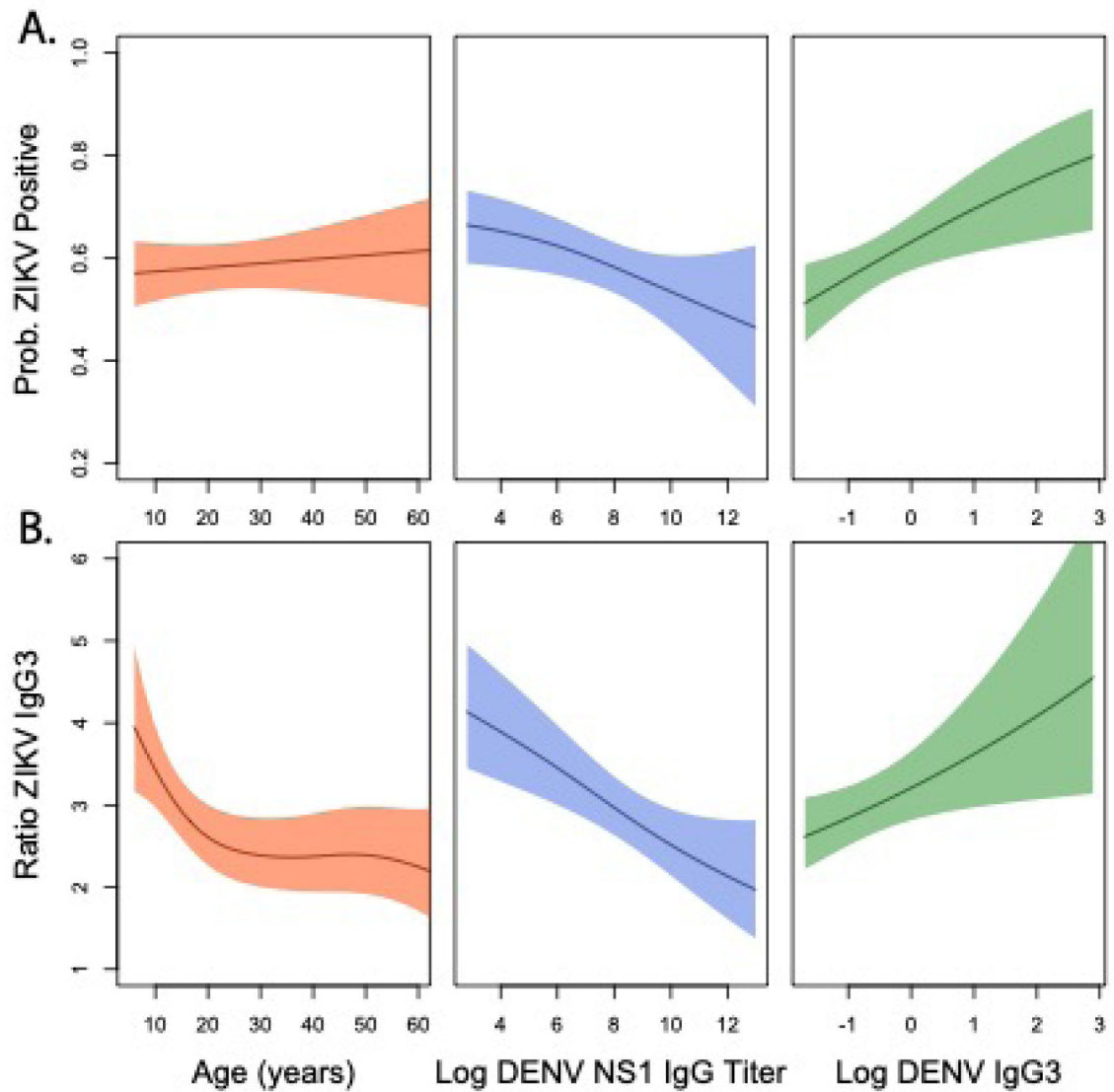
**Fig. 1.** Location of the study. **A.** Location of the city of Salvador in the state of Bahia and Brazil. **B.** Location of households enrolled at the Pau de Lima community study site in Salvador. **C.** Spatial heterogeneity in the proportion of the samples that were found to be ZIKV IgG3 positive (indicated by colors) in October 2015.. The smoothed surface was generated using a generalized additive model (GAM) that included latitude, longitude and altitude as predictors.



**Fig. 2.** Results of serological testing conducted prior to and after the ZIKV outbreak among Pau da Lima cohort participants (A). Each dot shows the result of an individual participant sample using our ZIKV IgG3 assay (see supplementary material). ZIKV PRNT was performed on a random subset of samples collected in March 2015 (orange) and October 2015 (green) (B). Dashed line indicates the cutoff established, using the PRNT results as the gold-standard, to achieve a sensitivity of 85% and a specificity of 97% (C).



**Fig. 3.** Age-stratified seropositivity to ZIKV (**A**) and DENV (**B**) measured in October 2015 (post Zika outbreak) and March 2015 (pre-Zika outbreak) respectively. Red line (**A**) shows the mean seropositivity in this population. Blue line (**B**) shows the fit of the best model estimating the historical DENV force of infection in this population (see supplementary methods).



**Fig. 4.** Results of generalized additive models looking at two metrics of ZIKV infection as a function of age and prior DENV exposure (DENV NS1 IgG titer and DENV NS1 IgG3 OD value measured in March 2015). **A.** Proportion ZIKV positive in October 2015 according to our ZIKV IgG3 assay and **B.** Ratio of IgG3 levels in October 2015 vs. March 2015.