Does immunity after Zika virus infection cross-protect against dengue?

Zika and dengue viruses are closely related flaviviruses, with immunological interactions and identical urban, mosquito-borne transmission.[1] Therefore, the recent introduction of Zika virus into the Americas and large-scale exposure of a uniformly previously unexposed population could affect subsequent transmission of dengue virus. This hypothesis had been untested, largely because sufficient epidemiological data were not available from affected locations. We explored this hypothesis in Salvador, the fourth largest city in Brazil (population 2·9 million), where extensive transmission of dengue viruses 1–4[2,3] occurred before the introduction and spread of Zika virus in 2015.[4]

We have done continuous enhanced surveillance of dengue among patients with acute febrile illness in a slum community of Salvador (population 76 352) since January 2009,[2] except for the periods September, 2013, to September, 2014, and August to September, 2016. The surveillance was first interrupted in 2013 with the termination of the supporting research grant; surveillance was restarted in 2014 with funding from a new award. The second interruption was due to the closing of the health centre for maintenance.

Before 2015, the frequency of RT-PCR-positivity for dengue virus followed a pattern of annual second-quarter or third-quarter peaks (figure A). By contrast, a much smaller peak occurred in 2015 during the Zika virus epidemic, and no peak occurred in 2016 and 2017, when RT-PCR positivity for dengue virus was around zero. These findings represented a significant decrease in the frequency of confirmed dengue virus among outpatients with acute febrile illness, from 484 (25%) cases of 1937 before the Zika virus outbreak from January, 2009, to March, 2015, to 43 (3%) of 1334 after the outbreak from April, 2015, to May, 2017 (p<0·0001; appendix).

In September, 2014, we augmented our dengue surveillance by adding routine testing for Zika virus and chikungunya virus. Of 1407 patients with acute febrile illness tested for Zika virus, 14 (1%) cases were confirmed by RT-PCR. The first was confirmed in May, 2015, with a peak in the number of cases during April to June, 2015 (11 [4%] of 285 tested patients). By contrast with the minimal detection of dengue in 2015, a large outbreak of chikungunya virus occurred at the surveillance site in the same year. The frequency of RT-PCR-positive or IgM-ELISA-positive cases of chikungunya virus among

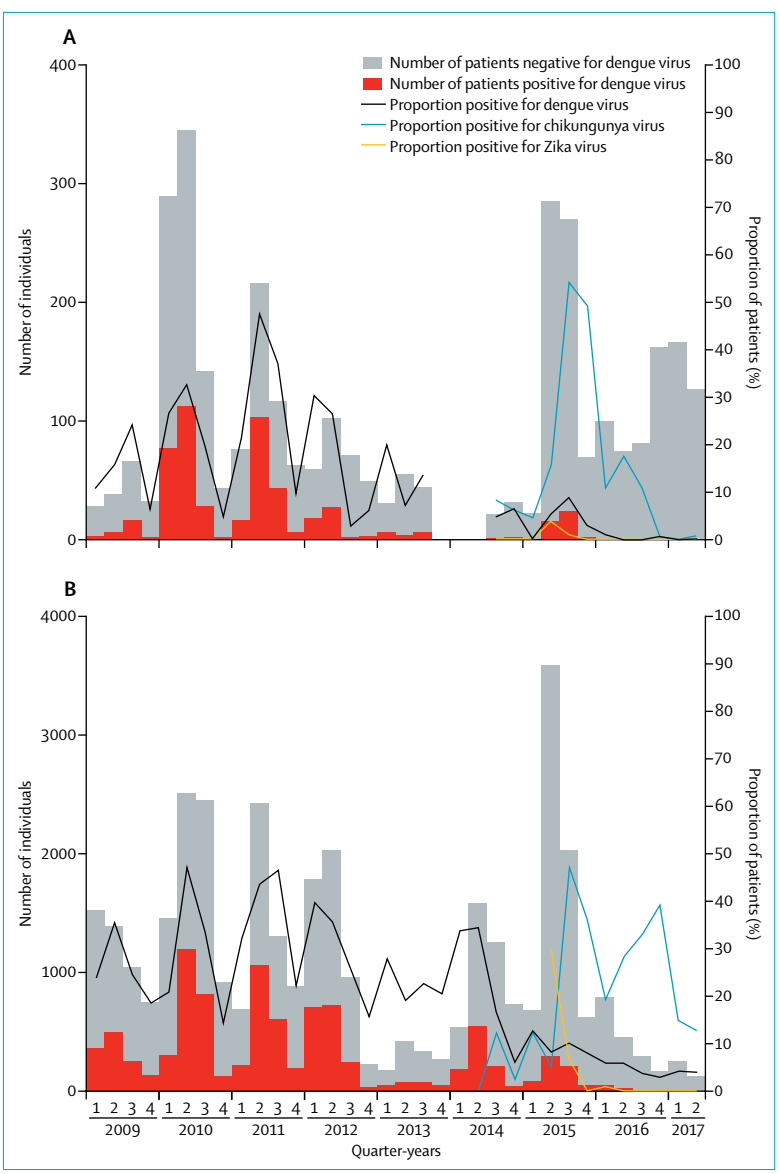

Figure: **Quarterly frequency of laboratory-confirmed dengue in Salvador, Brazil, before the appearance of Zika virus (January 2009 to March 2015) and after its appearance (April 2015 to May 2017)**
Data are (A) cases of dengue virus infection among patients with acute febrile illness enrolled during enhanced surveillance, confirmed by RT-PCR; and (B) cases of dengue virus infection confirmed by RT-PCR, IgM, or NS1 ELISA, or viral isolation, among patients with suspected dengue from Salvador. Figures also show the frequency of cases of chikungunya virus infection laboratory confirmed by RT-PCR or IgM ELISA, and of Zika virus infection confirmed by RT-PCR.

See Online for appendix

outpatients with acute febrile illness increased significantly from 7% before the Zika epidemic to 20% after; p=0·004, appendix), indicating that there were permissive environmental conditions (including mosquito vector populations) for efficient transmission of dengue virus after the Zika epidemic.

To complement the enhanced surveillance data, we analysed citywide data from 40 904 patients with suspected dengue tested between January, 2009, and May, 2017. The proportion of patients who tested positive for dengue by serological or virological methods decreased from 31% before the Zika virus outbreak to 8% after (p<0·0001; figure, appendix). Positivity for Zika virus was highest during April to June, 2015 (in three [30%] tested patients of ten), then declined to nearly zero from July, 2015, to May, 2017; figure B). Two large peaks in chikungunya virus infections occurred after the Zika-virus outbreak, during July to September, 2015 (47% confirmed as positive) and during October to December, 2016 (39% confirmed as positive).

Our findings, indicating lower frequencies of confirmed dengue virus lasting more than 2 years since early 2015—which are lower than at any other period analysed since 2009—raised the hypothesis that the 2015 outbreak of Zika virus might have inhibited transmission of dengue virus or of symptomatic infections. Because serological tests for dengue virus, especially IgM ELISA, cross-react with Zika-virus-immune serum samples,[5] it is possible that some citywide cases diagnosed as dengue since 2015 were actually cases of Zika virus. Nonetheless, a major downward trend in dengue cases occurred after the Zika virus outbreak. Few confirmed cases of Zika virus occurred during the Zika virus epidemic, probably reflecting an initial period of inadequate diagnostics. However, there is a strong consensus that northeastern Brazil (where Salvador is located) was the epicentre for the 2015 Zika virus epidemic, and a previous investigation suggested that Zika virus was the most likely cause for the acute exanthematous illness outbreak in the same year in which around 15 000 cases were reported in Salvador by mid-2015.[4]

Aside from sexual Zika virus transmission, the transmission cycles of Zika virus, dengue virus, and chikungunya virus rely on human amplification by *Aedes aegypti* mosquitoes. In our study, the proportion of patients with acute febrile illness who tested positive for chikungunya virus was highest in the second half of 2015 and throughout 2016. The prolonged and increased positivity rate for chikungunya virus, an alphavirus with no antigenic relationship to Zika virus or dengue virus, suggests that the 2015–17 reduction in dengue virus infections was unrelated to changes in vector efficiency (such as lower vector populations related to weather or control), or to other non-immunological factors.

Although temporal associations do not prove causation, the strength and consistency from both enhanced surveillance and citywide data, together with the observed maintenance of high detection of chikungunya virus after the Zika virus outbreak, suggest that Zika virus infections could induce cross-protective immunity against dengue virus. Prospective studies are needed to fully assess the subsequent risk of dengue after Zika virus exposure and determine whether the putative cross-protection is long-lasting or wanes over time. More specific serological assays, such as assays used for neutralising antibodies, might also help to show this effect more conclusively. If further studies support our hypothesis, they will have direct implications for epidemiological surveillance, immunological investigations of pathogenesis, and vaccine development and assessment.

We declare no competing interests.

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1 Dejnirattisai W, Supasa P, Wongwiwat W, et al. Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with zika virus. *Nat Immunol* 2016; **17:** 1102–08.

2 Kikuti M, Cunha GM, Paploski IA. D, et al. Spatial distribution of dengue in a Brazilian urban slum setting: role of socioeconomic gradient in disease risk. *PLoS Negl Trop Dis* 2015; **9:** e0003937.

3 Teixeira M da G, Costa M da CN, Barreto ML, Barreto FR. Epidemiologia do dengue em Salvador-Bahia, 1995–1999. *Rev Soc Bras Med Trop* 2001; **34:** 269–74.

4 Cardoso CW, Paploski IAD, Kikuti M, et al. Outbreak of exanthematous illness associated with Zika, Chikungunya, and dengue viruses, Salvador, Brazil. *Emerg Infect Dis J* 2015; **21:** 2274–76.

5 Lanciotti RS, Kosoy OL, Laven JJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis* 2008; **14:** 1232–39.