

Supplementary Information for

Seroprevalence, risk factor, and spatial analyses of Zika virus infection after the 2016 epidemic in Managua, Nicaragua

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Survey data: All surveys were administered orally, and answers were recorded on smartphones or tablet computers using custom-built software (1). Two questionnaires were administered per study, one for every household and one for every participant. The participant questionnaires covered demographic information such as sex, age, and level of education. The household questionnaires for both studies were similar and included questions on assets and conditions of the home, which served to determine a household's asset-based wealth index. The wealth index was based on: household construction material; number of fans, TVs, and refrigerators; ownership of motorcycles and/or cars; and use of firewood for cooking. We used principal components analysis to determine the weights for an index of the asset variables based on the score of the first principal component (PC1). The index was divided into terciles using quantile regression with the number of people living in each house used as a vector of observation weights for minimizing the sum of the weights multiplied into the absolute residuals. The wealth index was constructed using a principal component analysis (PCA) in R (2-5). PCA analyses were run separately for both study cohorts. To reflect the SES level of the study area, households were categorized as not poor, poor, and very poor according to their tercile along the first principal component. Individual participants were then assigned the SES proxy category of their household. Each participant's height and weight were also measured (6). The questionnaires, administered several months after the end of the epidemic, did not retrospectively ask about symptoms during the timeframe of the epidemic.

Zika NS1 BOB ELISA: As described previously (7, 8), 1 $\mu\text{g/ml}$ of ZIKV NS1 (MR766 strain, Native Antigen Company, Inc.) diluted in Phosphate-buffered Saline (PBS) was coated overnight at 4°C onto polystyrene Maxisorp plates (Nunc Thermo Scientific). Plates were blocked for 1 hour with PBS containing 1% Bovine Serum Albumin (PBS-BSA) and then washed 2 times with PBS plus 0.05% Tween 20 (PBS-T). Sera and internal controls (known positive and negative samples)

were added at a 1:10 dilution in PBS-BSA to the plates. Four wells were designated for the negative (maximum optical density (OD) value) controls and four wells for the positive (minimum OD value) controls. After 1 hour, 20 ng/ml biotinylated anti-NS1 ZKA35 (Humabs Biomed S.A.) was added 1:1 and incubated for 15 minutes. Following this, plates were washed with PBS-T four times, and alkaline phosphatase-conjugated streptavidin (Jackson ImmunoResearch) was added for 1 hour. Plates were washed with PBS-T 5 times, and para-nitrophenylphosphate (*p*NPP, Sigma) diluted in carbonate-bicarbonate buffer was added for 20 minutes. All incubation steps were performed at room temperature except for the coating, which was done at 4°C. OD was then measured using a Microplate Photometer at 405 nm. The percentage of blockade-of-binding value (%BOB) was calculated as follows:

$$\%BOB = \left[1 - \left(\frac{OD_{sample} - OD_{minimum\ value}}{\delta} \right) \right] \times 100\%, \text{ where}$$

$\delta = OD_{\text{maximum value}} - OD_{\text{minimum value}}$. The positivity cut-off was set at 50%, where readings of 50% or above were considered BOB-positive.

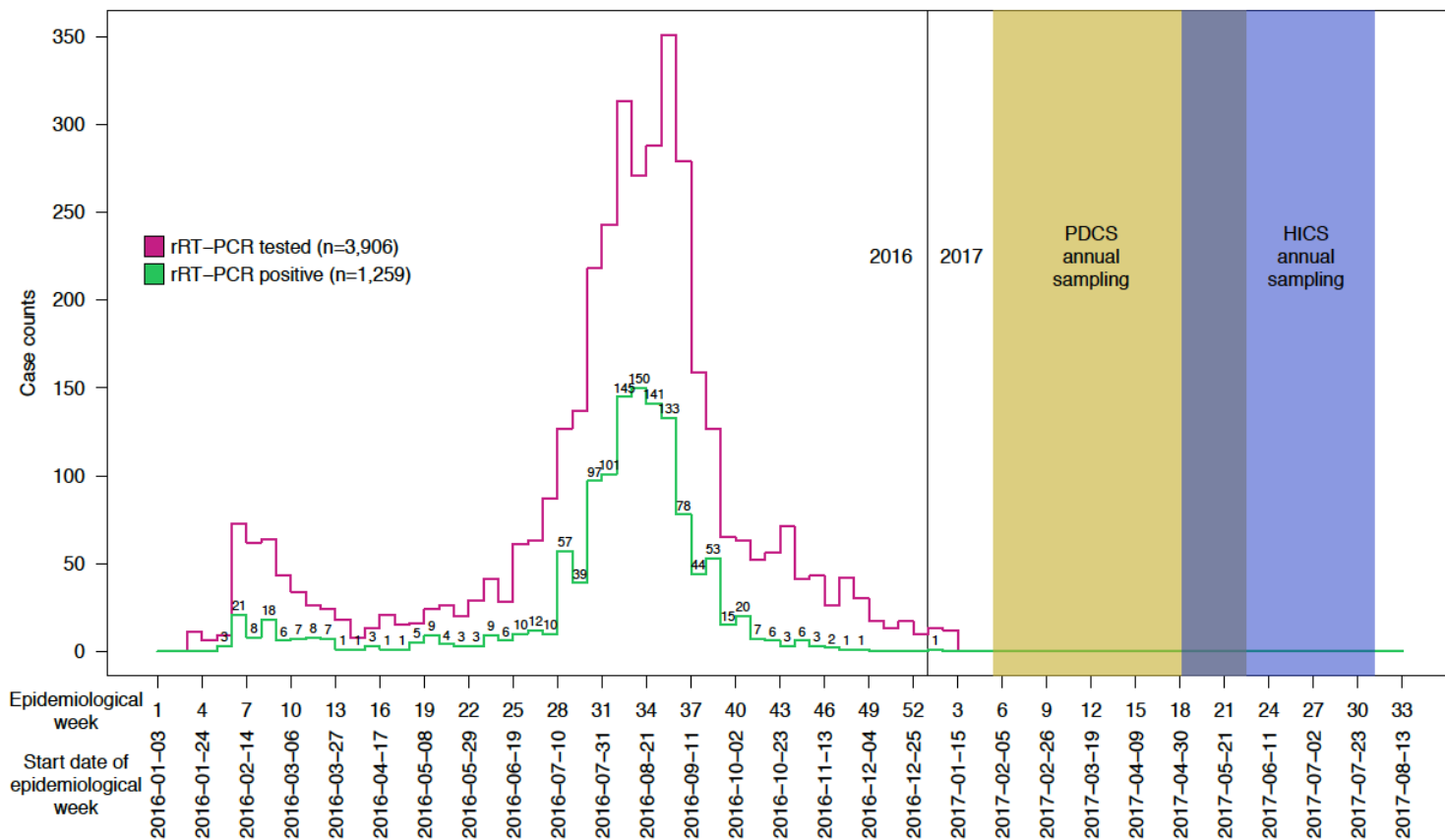


Fig. S1. Zika epidemic curve for Managua, Nicaragua. National surveillance data from the Nicaraguan Ministry of Health, restricted to Managua, is shown. National surveillance was resource-limited, and the data mostly reflects testing of pregnant women in Managua. However, the epidemic curve mirrors the epidemic as seen in the Pediatric Dengue Cohort Study (PDCS). Total individuals tested and rRT-PCR-confirmed cases are shown in pink and green, respectively. The duration of the annual sampling for the PDCS and the Household Influenza Cohort Study (HICS) is shown in yellow and blue, respectively.

Table S1. Characteristics of the pediatric, adult, and household groups who participated in the annual sampling from February to May 2017.

	Pediatric group (2-15 years old)	Adult group (15-80 years old)	Household group (2-80 years old)
Participants - N	3740	1074	2147
Households - N	2127	433	433
Persons per household - mean (standard deviation)	1.76 (1.13)	2.48 (1.49)	4.96 (2.49)
Female sex - N (%)	1850 (49.47)	770 (71.69)	1306 (60.83)
Age - median (IQR*)	7 (4-10)	32 (24-42)	15 (7-32)
Age Groups (years) - N (%)			
2-4	964 (25.78)	-	287 (13.37)
5-9	1545 (41.31)	-	433 (20.17)
10-14	1231 (32.91)	-	383 (17.84)
15-29	-	442 (41.15)	412 (19.19)
30-44	-	402 (37.43)	402 (18.72)
45-59	-	140 (13.04)	140 (6.52)
60-74	-	77 (7.17)	77 (3.59)
>75	-	13 (1.21)	13 (0.61)
Body surface area (m²) - median (IQR*)	0.91 (0.70 - 1.20)	1.75 (1.60 - 1.92)	1.45 (0.90 - 1.76)
Socioeconomic status - N (%)			
Not poor	1384 (37.69)	391 (36.41)	807 (40.01)
Poor	1229 (33.47)	355 (33.05)	644 (31.93)
Poorest	1059 (28.84)	328 (30.54)	566 (28.06)
Daily Hours Without Water - N (%)			
0	2464 (67.1)	660 (61.45)	1261 (62.52)
1-7	544 (14.81)	188 (17.5)	346 (17.15)
> 7	664 (18.08)	226 (21.04)	410 (20.33)
Faucet outside the home - N (%)			
No	1764 (48.04)	359 (33.43)	817 (40.51)
Yes	1908 (51.96)	715 (66.57)	1200 (59.49)
School shift - N (%)			
Morning	2512 (67.33)	-	-
Afternoon	647 (17.34)	-	-
Not attending school	572 (15.33)	-	-
Store water - N (%)			
No	-	629 (58.57)	-
Yes	-	445 (41.43)	-

*Inter-quartile range

Table S2. Breakdown of the pediatric group (from the PDCS cohort) by BMI after considering participants' age and sex.

Pediatric group sub-populations by BMI for age and sex	N	%
Underweight: 0-5th percentile	128	3.43
Healthy: 6-84th percentile	2398	64.31
Overweight: 85-94th percentile	502	13.46
Obese: 95-100th percentile	701	18.80

Table S3. Breakdown of the adult group (from the HICS cohort) by BMI.

Adult group sub-populations by BMI	N	%
Underweight: 14.6-18.5	39	3.72
Healthy: 18.5-25.0	258	24.59
Overweight: 25.1-30.0	308	29.36
Obese: 30.1-40.0	362	34.51
Morbidly obese: 40.1-54.3	82	7.82

Table S4. QIC_u comparisons in binomial GEE models adjusted for SES, hours without household access to water, and location of faucet.

QIC_u	BSA	BMI percentiles for age and sex	BMI	Height
Pediatric group	4394.9	4560.0	4460.9	4420.2
Adult group	1448.4	NA	1445.6	1443.0

Note: A smaller QIC_u statistic indicates a better fit to the data.

Table S5. Risk factor analysis of the pediatric group (n=3,740)

	N (%)	Prevalence (95% CI)	Prevalence ratio (95% CI)	p-value	Adjusted prevalence ratio (95% CI)	p-value
Total	3740 (100)	36.11 (34.45-37.8)				
Male	1890 (50.53)	33.6 (31.39-35.88)	1		1	
Female	1850 (49.47)	38.54 (36.28-40.86)	1.15 (1.06-1.25)	0.0013	1.11 (1.02-1.21)	0.014
Age Groups (years)						
2-4	964 (25.78)	24.26 (21.65-27.08)	1		1	
5-9	1545 (41.31)	32.32 (29.95-34.79)	1.34 (1.18-1.53)	< 0.001	1.02 (0.81-1.28)	0.873
10-14	1231 (32.91)	49.85 (46.98-52.72)	2.07 (1.83-2.33)	< 0.001	1.24 (0.95-1.63)	0.117
Body surface area (m²)						
1st quintile	714 (20.01)	24.01 (21.03-27.26)	0.69 (0.59-0.81)	< 0.001	0.73 (0.55-0.97)	0.03
2nd quintile	713 (19.98)	27.46 (24.26-30.91)	0.8 (0.69-0.93)	0.0044	0.83 (0.7-0.98)	0.032
3rd quintile	714 (20.01)	34.68 (31.24-38.29)	1		1	
4th quintile	713 (19.98)	41.43 (37.77-45.2)	1.21 (1.06-1.37)	0.0038	1.08 (0.93-1.27)	0.312
5th quintile	714 (20.01)	53.28 (49.57-56.95)	1.55 (1.38-1.74)	< 0.001	1.28 (1.07-1.52)	0.006
Socioeconomic status						
Not poor	1384 (37.69)	34.46 (31.71-37.31)	1		1	
Poor	1229 (33.47)	35.98 (33.16-38.9)	1.05 (0.93-1.17)	0.447	1.04 (0.93-1.17)	0.495
Poorest	1059 (28.84)	38.55 (35.46-41.74)	1.12 (1-1.26)	0.0558	1.10 (0.98-1.24)	0.094
Daily Hours Without Water						
0	2464 (67.1)	35.79 (33.73-37.9)	1		1	
1 - 7	544 (14.81)	36.21 (32.23-40.4)	1.01 (0.89-1.15)	0.8235	1.02 (0.90-1.16)	0.743
> 7	664 (18.08)	37.78 (33.82-41.91)	1.06 (0.93-1.19)	0.3892	1.08 (0.96-1.22)	0.216
Faucet outside the home						
No	1764 (48.04)	36.14 (33.74-38.61)	1		1	
Yes	1908 (51.96)	36.25 (33.93-38.64)	1.00 (0.91-1.10)	0.957	1.00 (0.91-1.10)	0.985
School attendance period						
Morning	2512 (67.17)	36.43 (34.45-38.46)	1		1	
Afternoon	647 (17.3)	44.89 (41-48.84)	1.24 (1.13-1.37)	< 0.001	1.09 (0.99-1.21)	0.093
Not attending school	572 (15.33)	23.95 (20.64-27.6)	0.66 (0.57-0.76)	< 0.001	0.99 (0.8-1.22)	0.932

Table S6. Risk factor analysis of the adult group (n=1,074)

	N (%)	Prevalence (95% CI)	Prevalence ratio (95% CI)	p-value	Adjusted prevalence ratio (95% CI)	p-value
Total	1074	56.36 (53.1-59.56)				
Male	304 (28.31)	51.57 (46.01-57.09)	1		1	
Female	770 (71.69)	57.87 (54.21-61.45)	1.11 (0.99-1.25)	0.07	1.14 (1.01-1.28)	0.041
Age Groups (years)						
15-29	442 (41.15)	51.55 (46.72-56.35)	0.92 (0.82-1.04)	0.19	0.94 (0.82-1.07)	0.328
30-44	402 (37.43)	55.97 (51.13-60.69)	1		1	
45-59	140 (13.04)	61.37 (52.75-69.32)	1.08 (0.92-1.26)	0.33	1.08 (0.92-1.27)	0.335
60-74	77 (7.17)	69.12 (57.58-78.69)	1.21 (1.02-1.45)	0.03	1.24 (1.03-1.49)	0.024
>75	13 (1.21)	69.23 (40.93-87.96)	1.22 (0.84-1.78)	0.31	1.39 (0.99-1.96)	0.059
Body surface area (m²)						
1st quintile	210 (20.02)	54.68 (47.99-61.21)	0.96 (0.8-1.14)	0.615	0.97 (0.82-1.16)	0.772
2nd quintile	210 (20.02)	52.91 (45.76-59.94)	1.11 (0.95-1.29)	0.19	1.1 (0.95-1.29)	0.201
3rd quintile	209 (19.92)	60.9 (54.57-66.88)	1		1	
4th quintile	210 (20.02)	54.49 (47.93-60.91)	1.00 (0.85-1.17)	0.97	1.01 (0.86-1.19)	0.907
5th quintile	210 (20.02)	58.96 (52.04-65.53)	1.08 (0.91-1.27)	0.39	1.11 (0.94-1.31)	0.208
Socioeconomic status						
Not poor	391 (36.41)	52.74 (47.25-58.17)	1		1	
Poor	355 (33.05)	57.18 (51.71-62.47)	1.09 (0.94-1.25)	0.25	1.06 (0.92-1.22)	0.435
Poorest	328 (30.54)	59.29 (53.35-64.97)	1.12 (0.97-1.29)	0.12	1.11 (0.96-1.28)	0.167
Daily Hours Without Water						
0	660 (61.45)	56.02 (51.95-60.02)	1		1	
1 - 7	188 (17.5)	57.62 (49.57-65.28)	1.03 (0.88-1.2)	0.745	1.03 (0.87-1.21)	0.764
> 7	226 (21.04)	56.69 (49.37-63.74)	1.00 (0.86-1.16)	1.00	1.04 (0.88-1.22)	0.649
Faucet outside the home						
No	359 (33.43)	54.18 (48.89-59.38)	1		1	
Yes	715 (66.57)	57.48 (53.36-61.49)	1.05 (0.93-1.19)	0.40	1.02 (0.9-1.15)	0.768
Store water						
No	629 (58.57)	56.78 (52.7-60.78)	1		1	
Yes	445 (41.43)	56.07 (50.78-61.22)	0.98 (0.87-1.1)	0.69	0.96 (0.84-1.09)	0.526

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