Title

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Journal

The Journal of Infectious Diseases, 230(5)

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Publication Date

2024-11-15

DOI

10.1093/infdis/jiae384

Peer reviewed

The Journal of Infectious Diseases

MAJOR ARTICLE

The Impact of Zika Emergence in Remote Communities in Northwestern Ecuador

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The Zika virus (ZIKV) epidemic in Latin America (2015–2016) has primarily been studied in urban centers, with less understanding of its impact on smaller rural communities. To address this gap, we analyzed ZIKV seroepidemiology in 6 rural Ecuadorian communities (2018–2019) with varying access to a commercial hub. Seroprevalence ranged from 19% to 54%, measured by nonstructural protein 1 blockade of binding enzyme-linked immunosorbent assay. We observed a decline in ZIKV seroprevalence between 2018 and 2019 that was greater among younger populations, suggesting that the attack rates in the 2015–2016 epidemic were significantly higher than our 2018 observations. These data indicate that the 2015–2016 epidemic included significant transmission in rural and more remote settings. Our observations of high seroprevalence in our area of study highlights the importance of surveillance and research in rural areas lacking robust health systems to manage future Zika outbreaks and vaccine initiatives.

Keywords. Zika virus; rural communities; seroprevalence; waning; community cohort study.

Much uncertainty exists about the emergence of the Zika virus (ZIKV) in the Americas during 2015–2016 and the risk of new transmission and epidemics. Like dengue virus (DENV), ZIKV can spread beyond the traditional "urban" settings once thought to be the foci of arbovirus transmission [1, 2]. However, the specific environmental, biological and social factors that promote ZIKV spread in rural populations remain unclear. Moreover, many such areas have underresourced health services, surveillance infrastructure, and vector control, leading to underreporting of cases and outbreaks.

Although the number of Zika cases declined abruptly by the end of 2016 [3], ZIKV still circulates at low levels in Latin America [4]. Studies revealed ZIKV seroprevalence ranging from 21% to 73% in various Latin American cities [5–9], with limited data in rural centers [10]. Ecuador lacks published data on ZIKV seroprevalence, relying instead on the Ministry of Health's passive case surveillance system. Between 2016 and 2018, the country recorded 3753 suspected and 3058 confirmed Zika cases. The coastal provinces, particularly Esmeraldas in the Northwest of the country, faced the highest incidence rates (23.9–201.8 per 100 000) [11]. Esmeraldas, one of Ecuador's poorest region [12], underwent rapid socioecological changes due to deforestation and highway construction [13]. These changes led to a shift from malaria to arboviral infections [14]; however, given the lack of awareness of dengue transmission many dengue outbreaks went unreported [15]. Official reports of ZIKV infection are limited to Esmeraldas city, with no data on transmission in nearby rural areas.

Understanding the extent of previous ZIKV infections and current immunity is key to assessing future outbreak risks, especially in rural areas with suitable conditions for ZIKV but under resourced surveillance [1, 2]. Moreover, the interaction between ZIKV and DENV immunity [6, 16], alongside the endemic circulation and recent expansion of DENV in these Ecuadorian rural areas [14, 15], elevates the importance of determining ZIKV seroprevalence.

Here, we evaluate the age-stratified seroprevalence and risk factors associated with ZIKV seropositivity across 6 rural communities that differ in their degree of accessibility to the commercial center, the town of Borbón, in Esmeraldas province. One difficulty in ZIKV serosurveillance is the potential cross-reactivity with other flaviviruses such as DENV. To address

Received 11 June 2023; editorial decision 22 July 2024; accepted 29 July 2024; published online 31 July 2024

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The Journal of Infectious Diseases® 2024;230:e1058–66

https://doi.org/10.1093/infdis/jiae384

this problem, we used a ZIKV nonstructural protein 1 (NS1) blockade of binding (BOB) enzyme-linked immunosorbent assay (ELISA) that can distinguish between ZIKV and DENV antibody responses [17, 18]. In addition, to overcome sample preservation issues in remote areas, we adapted this assay for dried blood spot (DBS) samples, validated against matched serum samples. We also investigated ZIKV NS1 antibody seroprevalence decline and the impact of DENV antibody status on ZIKV seroprevalence, by comparing antibody levels from 2018 and 2019 serosurveys in a community cohort study.

METHODS

Study Site

Located in the northwestern Ecuadorian province of Esmeraldas, in Eloy Alfaro County, 5 of our study communities (Borbón, Maldonado, Timbiré, Santa Maria, and Santo Domingo) lie along 3 rivers that flow toward the Pacific Ocean. Our sixth community, Colon Eloy, resides along the Santa Maria tributary between Maldonado and Timbiré. Five of the 6 communities were selected to represent different levels of accessibility to the sixth, the town of Borbón. Borbón (population 7000) is the regional commercial center serving many communities in the county. Maldonado, Colon Eloy, and Timbiré are towns with secondary (unpaved) road access (population 1000–2000). Santa Maria and Santo Domingo are accessible only through rivers (population <1000). The population primarily self-identifies as Afro-Ecuadorian (majority group), Indigenous Chachi, or Mestizo [19].

Study Design and Sampling

From July to October 2018, we enrolled 5000 participants aged 2–60 years, collecting 3–6 DBS samples per person using Whatman 903 filter paper or TropBio Disks (CellLabs). Participants included all residents from the 5 smaller villages and those that reside within Borbón's town center. In Borbón, 120 participants also provided a serum sample for assay validation. A year later, from August to October 2019, we enrolled a longitudinal cohort of 1200 participants from all study communities, using stratified sampling, oversampling younger ages (2–14 years) for better detection of primary flavivirus immune status. Because we did not conduct a random sample, we used census data, which we collected just before the serosurvey, to create appropriate weights that ensured our sample was representative of the age and sex distribution for the community. The study received approval from the bioethics committees of Universidad San Francisco de Quito, the University of Michigan, and the Ecuadorian Ministry of Health.

Serological Assays

All DBS and serum samples were processed, preserved and stored in the field and then transported in a container with silica bags (DBS samples) or in liquid nitrogen (serum samples) to the Laboratorio de Biomedicina at the Universidad Central del Ecuador for biobanking and serological assays.

ZIKV NS1 BOB ELISA

The ZIKV BOB ELISA quantifies anti-ZIKV antibodies [17] against ZIKV NS1 via competition with a specific human anti-ZIKV NS1 monoclonal antibody [20], regardless of past DENV exposure [18]. We adapted this technique for the analysis of the DBS samples from the baseline year and we also used it to analyze serum samples. Briefly, antibodies were extracted by eluting two 5-mm DBS punches in 250 µL of phosphate-buffered saline (PBS) with 5% bovine serum albumin (BSA), overnight at 4°C. For the ELISA, 1 µg/mL ZIKV NS1 was coated on Maxisorp plates overnight at 4°C, blocked with 1% BSA in PBS, and washed with PBS–0.05% Tween 20. Samples were added, followed by 20 ng/mL horseradish peroxidase (HRP)–conjugated anti-NS1 ZKA35, incubated at room temperature. After washes, 3,3′,5,5′-tetramethylbenzidine (TMB) was added, and the reaction was stopped with 2N sulfuric acid. Optical density (OD) was read at 450 nm. The percentage of BOB value (% BOB) was calculated using the following formula:

$$\%\,\mathrm{BOB} = [1 - (\mathrm{OD}_{\mathrm{sample}} - \mathrm{OD}_{\mathrm{min}}/\delta)],$$

where δ represents the difference between maximum OD and minimum OD ($\mathrm{OD}_{\mathrm{min}}$) values. A sample was considered positive if the percentage of BOB value was ≧50% for serum samples [17] and ≧51% for DBS samples (see Supplementary Materials for detail).

DENV Immunoglobulin G Capture ELISA

Serum samples from the 2019 serosurvey were also analyzed for DENV antibodies with a DENV immunoglobulin (Ig) G capture ELISA. Briefly, Maxisorp plates were coated with 4G2 antibody and blocked with PBS-BSA. DENV antigens and diluted serum samples were sequentially incubated. An HRP-conjugated anti-human IgG was used for detection. After washes, TMB was added, and the reaction stopped with 2N sulfuric acid. OD was measured at 450 nm. DENV positivity was assessed by means of receiver operating characteristic analysis, with an OD cutoff of >0.9 (see Supplementary Materials for details).

Statistical Methods

Comparing DBS and Serum Seropositivity

We used paired data from participants who had both DBS and serum samples collected in 2018. We calculated the κ coefficient to estimate the degree of agreement when determining whether a test result was positive using DBS and serum samples. The κ coefficient ranges from −1 to 1, where higher values indicate higher agreement.

ZIKV Seroprevalence

Seroprevalence was estimated through intercept-only logistic regression models using generalized estimating equations. We incorporated clustering at the household level, using an exchangeable correlation structure. We chose not to cluster at the community level because we wanted to use community as a covariate in our models. As our study was cross-sectional, we did not need to include clustering at the individual level. Log-odds point estimates and their respective confidence intervals (CIs) were transformed to reflect ZIKV seroprevalence [9]. ZIKV seroprevalence among participants was compared by sex and age group. We also plotted age-dependent seroprevalence curves using generalized additive models with thin-plate regression splines [9, 21].

Risk Factors for ZIKV Seroprevalence

We used bivariate logistic regression models to assess predictors of ZIKV seroprevalence in population-weighted samples. We explored the effects of variables thought to be potentially important determinants of flavivirus risk based on prior knowledge, such as age (2–10, 11–20, 21–40, or ≥41 years), community of residence (Borbón, Maldonado, Colon Eloy, Timbiré, Santo Domingo, or Santa Maria, respectively), sex (female or male), race (Afro-Ecuadorian, Chachi, or Mestizo/other), highest education level achieved (preschool, primary school, school, high school, or higher education) and years of residence in the community of study. For years of residence, we selected a cutoff of 2 years to account for people living in the community during the Zika epidemic, which in Ecuador occurred from 2016 to 2017 [10]. Variables with P values <.05 were added to multivariable logistic regression models.

Table 1. General Characteristics of the Study Population in 2018 and 2019

Characteristic	Participants, No. (Weighted %)[a]	
	2018 (N = 1192)	2019 (N = 1126)
Female sex	664 (51)	616 (52)
Age, median (IQR), y	19 (12–34)	12 (8–19)
Age category, y		
0–10	227 (22)	471 (21)
11–20	407 (25)	391 (26)
21–40	361 (28)	154 (26)
≥41	197 (25)	110 (27)
Race/ethnicity		
Afro Ecuadorian	900 (76)	858 (74)
Mestizo/other	220 (18)	126 (16)
Chachi	72 (6)	142 (10)
Educational level		
Preschool/unknown	343 (33)	594 (38)
Primary school	534 (40)	394 (38)
High school	247 (20)	112 (18)
Higher education	68 (7)	26 (6)
Community		
Borbón	329 (27)	207 (14)
Maldonado	210 (18)	201 (17)
Colon Eloy	160 (13)	200 (16)
Timbiré	181 (15)	187 (14)
Santo Domingo	148 (12)	156 (16)
Santa María	164 (14)	175 (22)

Abbreviation: IQR, interquartile range.

[a]Data represent no. (weighted %) of participants unless otherwise specified.

Decrease in ZIKV Seroprevalence

We compared age-stratified population-weighted seroprevalence from those in 2018 to those in 2019 to measure the decrease in ZIKV seroprevalence over time using a z test. In addition, the waning of ZIKV seroprevalence among participants whose samples were collected in 2018 and 2019 was assessed by paired analysis using McNemar test. Risk factors associated with ZIKV antibody positivity in 2019 were assessed to investigate whether waning of antibodies influenced our perceptions of which populations were at risk.

Sensitivity Analysis

Samples whose readings were close to the cutoff point in the NS1 BOB ELISA are the most uncertain with respect to a positive or negative outcome. Therefore, we conducted a sensitivity analysis by eliminating samples close to the cutoff (based on our κ analysis) and repeated the analysis. All data were analyzed using R software, version 4.2.1 (R Core Team, 2021).

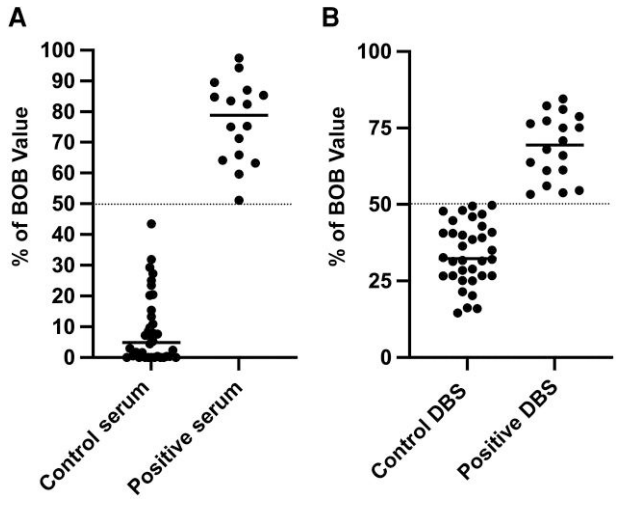

Figure 1. Adaptation of nonstructural protein 1 (NS1) blockade-of-binding (BOB) enzyme-linked immunosorbent assay (ELISA) in serum (*A*) and dried blood spot (DBS) (*B*) samples in individuals from the community of Borbón (n = 52). Dots represent percentage of BOB. The dark horizontal lines represent the median value. Dotted horizontal lines represent cutoffs between negative and positive results. Controls consisted of Zika virus (ZIKV)–negative DBS and serum samples with or without preexisting immunity to dengue virus antibodies that resulted in a BOB value of <50% in the NS1 BOB ELISA. Positive samples were those that resulted in BOB values >50% in serum and >51% in DBS samples in the ZIKV NS1 BOB ELISA.

RESULTS

Participants Characteristics

The main difference between our 2018 and 2019 samples (N = 1192 and N = 1126. respectively) is that there is a lower median age in 2019 because we oversampled 2–14-year-olds when enrolling the cohort (Table 1). Participants' characteristics by community of residence for 2018 can be found in Supplementary Table 1.

Comparison DBS and Serum Seropositivity

In our side-by-side comparison of the NS1 BOB ELISA (Figure 1) our κ coefficient analysis (Supplementary Table 2) found strong agreement (0.83 [95% CI, .66–.99]) between serum and DBS samples.

ZIKV Seroprevalence

Using the 2018 population-weighted baseline samples, as they were closer to the 2016–2017 Zika outbreak in Ecuador, we found an overall ZIKV seroprevalence of 42% (95% CI, 39%–45%). Towns with road access (Maldonado, Colon Eloy, and Timbiré) had the highest seroprevalence (53%, 56%, and 47%, respectively), followed by the commercial center of Borbón (39%) and the river-accessible villages of Santo Domingo (34%) and Santa Maria (19%) (Figure 2 and Supplementary Table 3).

Trend Analysis of ZIKV Seroprevalence by Age

Aggregating across all communities, ZIKV seroprevalence in 2018 increased from 32% among 2-year-olds to 54% among 60-year-olds (69% increase in seroprevalence) (Figure 3*A*). When we stratified by community, we found an increase in ZIKV seroprevalence by age in all the communities except for Santa Maria (Figure 3*B*). Borbón, Colon Eloy, and Maldonado exhibited a biphasic trend, with seroprevalence increasing sharply in younger ages (to approximately 40, 30, and 30 years of age, respectively), followed by a more gradual increase. Seroprevalence tended to saturate at 50%–70% in the older age groups. Colon Eloy had the steepest increase, from approximately 35% at age 2 to approximately 60% by age 30 years, while Maldonado had a shallower increase, from approximately 40% at age 2 to approximately 55% at age 30 years. In contrast, the seroprevalence in Santo Domingo, Timbiré and Santa Maria increased linearly with age. The linear increase between the ages of 2 and 30 years was from 30% to 38% in Santo Domingo and from 42% to 48% in Timbiré; Santa Maria showed minimal change in seroprevalence with age.

Trend Analysis of ZIKV Seroprevalence by Sex

Across all ages, ZIKV seroprevalence was higher in female than in male participants (Supplementary Figure 1*A*), although the difference was not significant. When these data were stratified by community, the small sample sizes made interpretation difficult (Supplementary Figure 1*B*). When analyzing the ZIKV seroprevalence by age group and sex we found the highest seroprevalence levels in women aged 21–40 (45%) or >41 (44%) years. In male participants, the >41-year age group had the highest seroprevalence (55.2%) (Supplementary Table 4).

Sociodemographic Risk Factors

Age, race, community of residence, and education level were associated with ZIKV seroprevalence in 2018 in our bivariate models (Supplementary Table 5). We found that the distribution of these variables varied significantly across some communities (Supplementary Table 1). Only age and community of residence remained significant in our multivariable regression model (Table 2). Maldonado and Colon Eloy, road-accessible communities, had higher ZIKV seroprevalence odds compared with Borbón (odds ratio [OR] [95% CI], 1.85 [1.27–2.68] for Maldonado and 1.80 [1.19–2.72] for Colon Eloy), whereas the river-accessible community of Santa Maria had lower ZIKV seroprevalence odds compared with Borbón (0.37 [.21–.64]).

In our 2019 samples, age, community of residence and education level were also associated with ZIKV seroprevalence in our multivariable regression model (Supplementary Table 6). Similarly, we found that ZIKV seroprevalence increased with age. Contrary to the 2018 results, residents of the road-accessible communities (Maldonado and Timbiré) had lower odds of ZIKV seropositivity compared with residents of Borbón (OR [95% CI], 0.63 [.39–1.03] for Maldonado and 0.40 [.23–.68] for Timbiré), whereas Colon Eloy remained higher in ZIKV seroprevalence (1.44 [.91–2.31]). The riverine communities (Santa Maria and Santo Domingo) also remained lower in ZIKV seroprevalence compared with Borbón (OR [95% CI], 0.56 [0.33–0.95] for Santa Maria and 0.40 [0.23–0.69] for Santo Domingo).

Sensitivity Analysis

Ninety DBS samples in 2018 and 11 serum samples in 2019 had a percentage of ZIKA NS1 antibody inhibition close to the established cutoff, between 51% and 53%. After excluding those samples, results matched those of the main analysis, except for small decreases in seroprevalence in Santo Domingo and Santa Maria (5% and 7%, respectively) (Supplementary Table 7).

Waning of ZIKV Seroprevalence

ZIKV seroprevalence during 2018 was almost twice that found in the samples from 2019 (42 per 100 [95% CI, 39–45] vs 29 per 100 [25–33], respectively). This decrease in seroprevalence was consistent across all communities except Borbón and Santa Maria (Figure 4). Among the 231 participants who had matched samples from both 2018 and 2019, we found a similar reduction in seroprevalence (from 32 [95% CI, 26–38] to 18

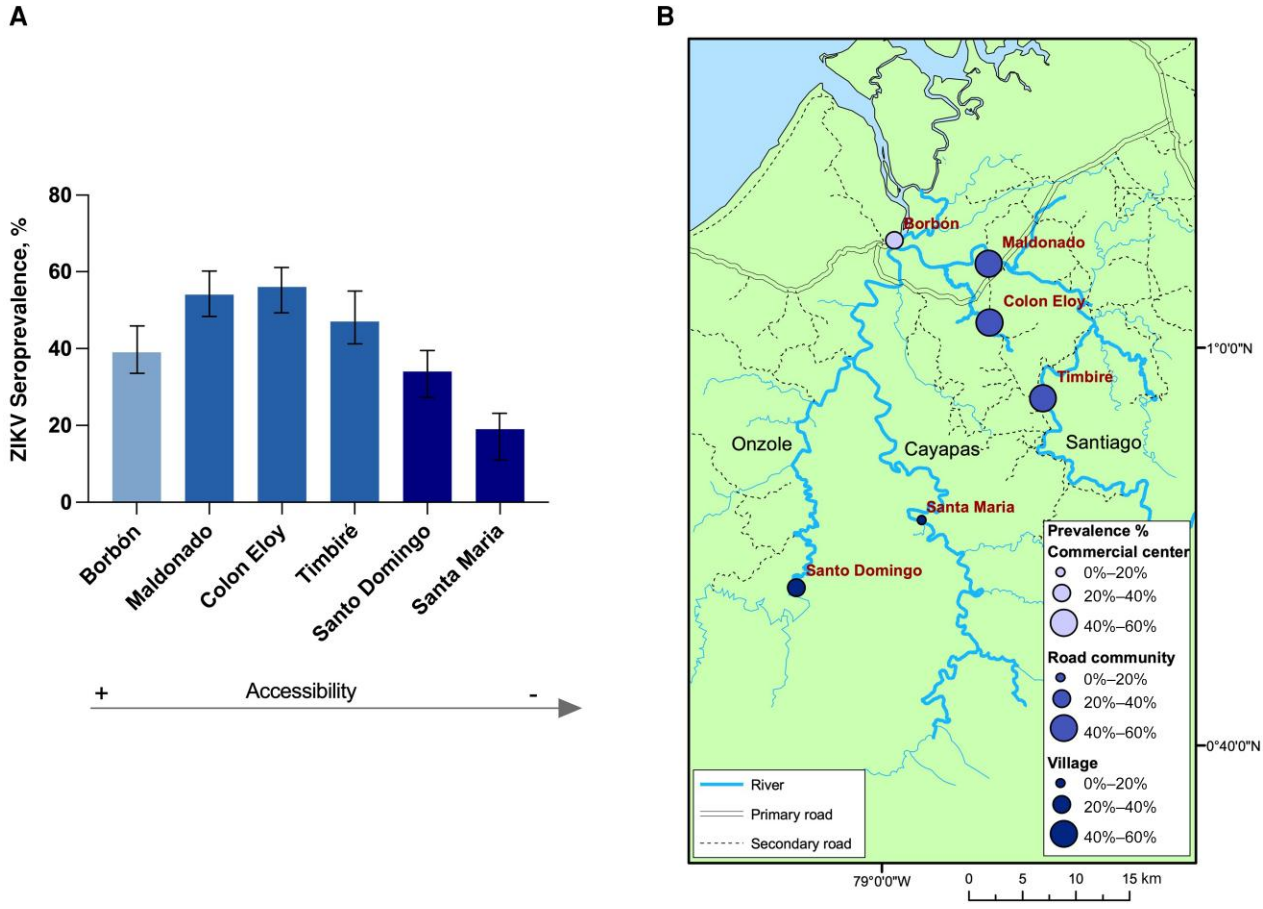

Figure 2. *A,* Zika virus (ZIKV) seroprevalence by community and gradient of remoteness in a cross-sectional study. *B,* Map of study setting. Borbón represents the commercial center; Maldonado, Colon Eloy and Timbiré, the road communities; and Santo Domingo and Santa Maria, the river communities. Sizes of the circles represent seroprevalence groupings (0%–20%, 20%–40%, and 40%–60%). Seroprevalence was estimated through intercept-only logistic regression models using generalized estimating equations. Log-odds point estimates and their respective confidence intervals were transformed to reflect ZIKV seroprevalence.

[14–24] per 100). After stratification by age, only participants <11 years of age had a significant decrease in seroprevalence (from 32 [95% CI, 22–43] to 9 [5–18] per 100). Changes in ZIKV-positive antibody levels from 2018 to 2019 were heterogeneous at the individual level; while 63% of the participants exhibited a decrease, 21% exhibited no change, and 16% exhibited an increase (Supplementary Figure 2). ZIKV seroprevalence rates also varied by community and age when comparing 2018 and 2019. Maldonado, Timbiré, and Santo Domingo experienced similar declines in seroprevalence among all age groups, while Borbón, Colon Eloy, and Timbiré experienced increased declines among certain age groups (Supplementary Table 8).

Effect of DENV Antibody Status on ZIKV Seroprevalence

We added a DENV variable to our models to examine the role of DENV antibody status on ZIKV seroprevalence during 2019. We found that a sample that is positive for DENV antibodies based on an IgG ELISA has increased odds of ZIKV seropositivity (odds ratio, 7.03 [95% CI, 3.94–12.53]; $P < .001$). After stratification of participants by DENV seropositivity, the average decline in the percentage of ZIKV inhibition (BOB) was approximately 15% for DENV-positive individuals, compared with 23% for those who were DENV negative ($P = .55$ using a t statistic) (Supplementary Figure 3).

DISCUSSION

In characterizing the post–Zika outbreak landscape in both rural and more remote settings, we describe an important dimension of arboviral epidemiology. We found high ZIKV seroprevalence, ranging from 39% in the commercial center (Borbón) to 47%–56% in road-accessible communities (Maldonado, Colon Eloy, and Timbiré). All of these communities are considered rural. In our more remote river-accessible communities (Santo Domingo and Santa Maria) we found 19%–35% seroprevalence levels. A significant decline in

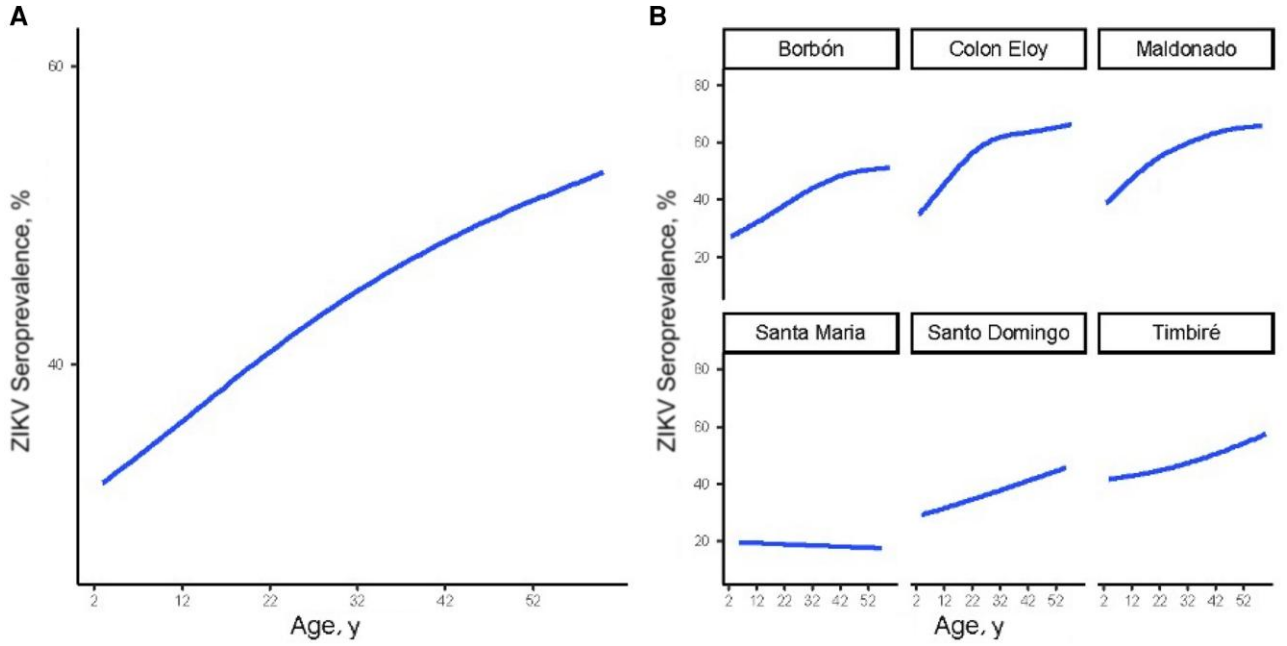

Figure 3. Zika virus (ZIKV) age-dependent seroprevalence curves using generalized additive models. *A,* Overall ZIKV seroprevalence among all communities. *B,* ZIKV seroprevalence by community. ZIKV seroprevalence increased gradually with age in all communities except Santa Maria.

Table 2. Multivariable Logistic Regression Assessing Risk Factors for Zika Virus Seroprevalence

Variable	OR for ZIKV Seroprevalence (95% CI)[a]	*P* Value
Age, y		
2–10	Reference	Reference
11–20	1.13 (.72–1.78)	.60
21–40	1.56 (1.00–2.43)	.05
41	1.86 (1.19–2.91)	.007
Race ethnicity		
Afro-Ecuadorian	Reference	Reference
Chachi	0.72 (.34–1.50)	.38
Mestizo/other	0.74 (.53–1.03)	.07
Community		
Borbón	Reference	Reference
Maldonado	1.85 (1.27–2.68)	.001
Colon Eloy	1.80 (1.19–2.72)	.005
Timbiré	1.28 (.88–1.88)	.20
Santo Domingo	0.82 (.52–1.29)	.39
Santa Maria	0.37 (.21–.64)	<.001
Educational level		
Preschool	Reference	Reference
Primary school	1.05 (.73– 1.52)	.78
High school	1.16 (.78–1.74)	.46
Higher education	1.14 (.59–2.20)	.70

Abbreviations: CI, confidence interval; OR, odds ratio; ZIKV, Zika virus.
[a]Based on adjusted model from 2018 data set (N = 1192).

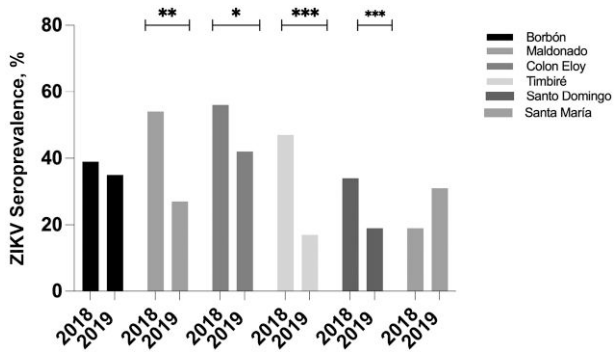

Figure 4. Analysis of Zika virus (ZIKV) seroprevalence decline from 2018 to 2019 in each community; χ^2 tests were performed to compare of seroprevalence in 2018 versus 2019. $^*P < .05$; $^{**}P < .01$; $^{***}P < .001$.

ZIKV seroprevalence from 2018 to 2019, especially among younger participants, suggests a higher attack rate during the 2016–2017 outbreak in Ecuador than our 2018 seroprevalence data would indicate. Finally, we demonstrated that DENV antibody status was an important determinant of ZIKV seroprevalence decline. We elaborate on these 3 findings below.

The ZIKV seroprevalence in our rural communities (21%–63%) obtained 2 years after the Zika epidemic, are comparable with those reported in urban centers across the Americas. For example, Salvador, Brazil, estimated a 63% seroprevalence [5, 6], while Managua, Nicaragua, estimated rates between 36% and 56% [9]. In Beni and Santa Cruz de la Sierra, Bolivia, the estimated rates were 39% and 21.5%, respectively [7]. Notably, in one of the few studies besides ours reporting seroprevalence in a remote village, our most remote communities had seroprevalence levels comparable to that in a remote village in Suriname (19%–35% vs 24.5%) [10].

Given these high seroprevalence estimates, many have asked whether and when ZIKV will reemerge in Latin America [22]. Our data reveal a notable decline in ZIKV seroprevalence related to anti-NS1 antibodies between 2018 and 2019. This significant decline, about 2–3 years after the Zika outbreak in the region, suggest that our seroprevalence measures underestimate the actual ZIKV attack rate during the 2016–2017 epidemic. This decline in seroprevalence aligns with findings from previous studies in French Polynesia and Fiji, which described decreases in ZIKV seroprevalence and neutralizing antibodies [23, 24]. Future longitudinal studies are essential for estimating the decline of ZIKV antibodies over time and age, understanding historical incidence patterns, and assessing the attack rate of the previous Latin American outbreak [22, 25]. In addition, research using validated assays as a correlate of protection is necessary to determine how the waning of ZIKV antibodies affects future infection risks.

In our analysis of 231 participants present in both the 2018 and 2019 serosurveys, we found that ZIKV NS1 antibodies decreased more rapidly among those <11 years of age compared with older age groups. This explains why we observed ZIKV seroprevalence increasing with age in most communities and is consistent with previous reports indicating a faster waning of antibodies in children compared with adults [26].

Although some communities had a higher proportion of younger participants, this alone could not account for the differences in the decline of ZIKV seroprevalence among communities. We showed that DENV antibody status also influences the decline of ZIKV seroprevalence. DENV exposures before or after the Zika outbreak [6] may have modulated the rate of decay of ZIKV NS1-specific antibodies and therefore might explain the differential decline of ZIKV seroprevalence by community. Notably, in Borbón, where DENV has been endemic for several years [14], and in Santa Maria, which recently experienced a large mostly primary DENV-1 outbreak [15], we did not observe significant waning of ZIKV seroprevalence. Further research is warranted to investigate and clarify the influence of dengue antibody status on declines in ZIKV antibodies [27].

The potential drivers behind our finding that ZIKV seroprevalence varies by community, even after adjusting for age, are numerous. For example, a higher ZIKV seroprevalence in communities with better road access suggests that large- and medium-scale human movement via transportation networks plays a significant role in the spread of flavivirus infections, as shown before [28–31]. Communities with road connectivity frequently interact with larger coastal population centers, notably the city of Esmeraldas, which experienced a substantial number of Zika cases during the epidemic [11]. In addition, the geographic location of Esmeraldas province, on the northern Ecuadorian border, facilitates extensive commercial activity with Colombia's southern region, similarly affected by the ZIKV outbreak [32]. This cross-border trade and travel, alongside the increased human mobility facilitated by transportation networks, likely contributed to the accelerated transmission of ZIKV to these regions.

Another important determinant of ZIKV seroprevalence is sex. Previous reports on seroprevalence [9, 33] and incidence of Zika [34, 35] found that women were at higher risk of ZIKV infection due to increased mosquito exposure within the household and sexual transmission from men to women. In our analysis, while we observed an increased ZIKV seropositivity among women relative to men, this difference did not reach statistical significance. This absence of significance might stem from our limited sample size and/or differences in social structures and behaviors in our study location compared with other sites [36].

Our assay deployment distinguished between ZIKV and DENV seroprevalence. To minimize cross-reactivity with other flaviviruses such as DENV, we used a highly sensitive and specific assay, the ZIKV NS1 BOB ELISA, which has performed well in dengue-endemic regions soon after Zika outbreaks [18]. We then adapted the assay for the analysis of antibodies eluted from fingerpick blood spots on filter paper (DBS samples) because it is easier to preserve filter paper samples in remote settings and we had a large sample set from our baseline serosurvey. Our validation of DBS samples (κ test, 0.83 [95% CI, .66–.99]) indicates for the first time that antibodies eluted from DBS samples collected from remote field conditions performs as well as serum antibodies in the ZIKV NS1 BOB ELISA.

It is important to understand ZIKV serostatus and, more broadly, the transmission dynamics of DENV across communities with differing degrees of remoteness; we observed endemic circulation in the commercial center and neighboring towns and recent expansion of DENV in the more remote communities [14, 15]. These are all marginalized communities that receive few resources from the government to address these public health concerns [37] and, as such, are more vulnerable to outbreaks. Our findings that there may have been significant ZIKV transmission in rural areas during the 2016–2017 epidemic highlight the need for enhanced epidemiological surveillance, cohort studies, and targeted prevention and mosquito control measures in rural communities throughout Latin America, as a new Zika epidemic wave is likely to re-emerge in the near future [4, 38].

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data

are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank the Ecología, Desarrollo, Salud, y Sociedad (EcoDeSS) project field team for their invaluable contribution collecting samples and data and the Instituto de Microbiología at Universidad San Francisco de Quito for their support with logistics, student support, and training. We are also grateful to the study participants and their families. Finally, we thank Eva Harris, PhD, Magelda Montoya, and Angel Balmaseda, MD (Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministry of Health, Managua, Nicaragua) for providing us with training and reagents to establish the NS1 BOB ELISA in our laboratory.

Author contributions. Data analysis: P. A., A. S. M., and G. O. L. Establishment of the NS1 BOB ELISA and laboratory data production: S. V. and V N. Database management: P. A. and G. O. L. Supervision of laboratory data production: W. C. and J. C. Supervision of studies: J. N. S. E. and J. C. Manuscript writing: P. A., A. S. M., J. N. S. E., and J. C.

Data availability. Data not publicly available

Financial support. This work was supported by the National Institute of Allergy and Infectious Diseases of the National Institute of Health (grants 1AI132372 and U01AI151788).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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