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# Chikungunya Outbreak Risks after the 2014 Outbreak, Dominican Republic

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The 2014 chikungunya outbreak in the Dominican Republic resulted in intense local transmission, with high postoutbreak seroprevalence. The resulting population immunity will likely minimize risk for another large outbreak through 2035, but changes in population behavior or environmental conditions or emergence of different virus strains could lead to increased transmission.

In early 2023, a substantial increase in chikungunya disease cases in South America prompted an alert from the Pan American Health Organization (1). Although most chikungunya virus (CHIKV) transmission has occurred in Paraguay and Brazil, the proximity of nearby regions, including the Caribbean, with histories of intense arboviral transmission raises the prospect of epidemic spread. The Caribbean is an ecologically receptive setting for CHIKV and was heavily impacted by a 2014 epidemic (2). The epidemic was particularly severe in the Dominican Republic, where >539,000 cases and more deaths per capita than any other country in the Americas were reported (3,4). Given that vulnerability, we conducted a study to assess the risks for future CHIKV outbreaks in the Dominican

Republic by evaluating post-2014 CHIKV transmission, estimating current population-level immune protection, and modeling future epidemic risks. Our research aims to inform public health interventions and preparedness strategies in anticipation of potential regional CHIKV resurgence.

Data and specimens were collected as part of a US Centers for Disease Control and Prevention–funded acute febrile illness (AFI) research program. The studies were approved by the Dominican Republic National Council of Bioethics in Health (#013-2019); the institutional review board of Pedro Henríquez Ureña National University (Santo Domingo, Dominican Republic), and the Massachusetts General Brigham Human Research Committee (Boston, MA, USA) (#2019P000094).

## The Study

To understand local CHIKV epidemiology and transmission after the 2014 outbreak, we first performed reverse transcription PCR (RT-PCR) on acute-phase serum samples from patients enrolled as part of a prospective AFI surveillance program at Dr. Toribio Benosme Hospital in Espailat Province in northwestern

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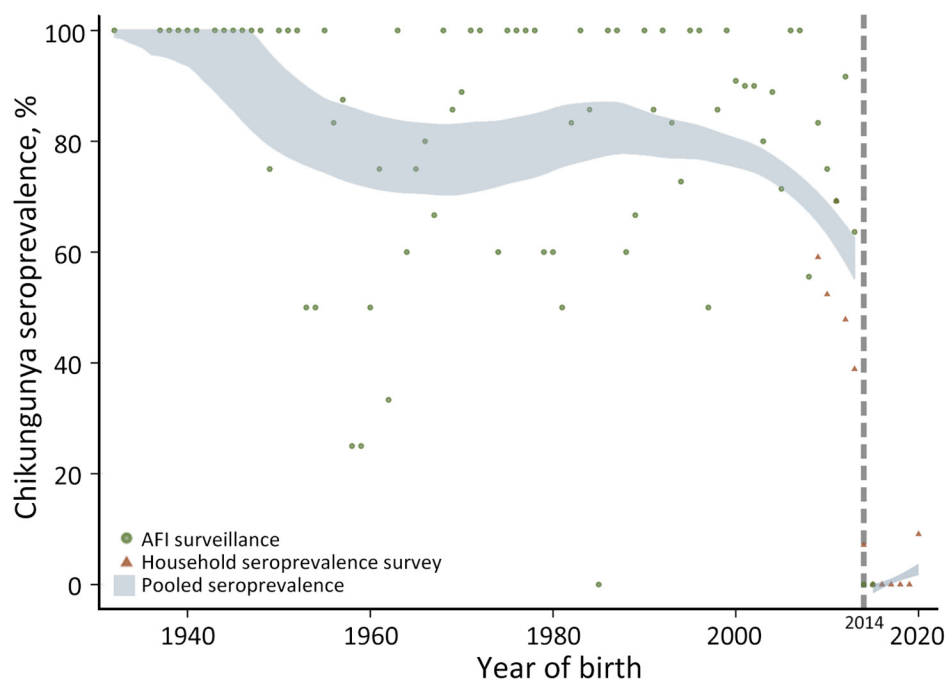
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Dominican Republic and Dr. Antonio Musa Hospital in San Pedro de Macorís Province in southeastern Dominican Republic, using study processes described elsewhere (5). We invited patients  $\geq 2$  years of age with measured ( $\geq 38^{\circ}\text{C}$ ) or reported undifferentiated fever to participate. All adult participants and parents or legal guardians of child participants provided written consent; children 7–17 years of age provided assent. During November 2019–June 2023, we enrolled and tested 2,792 persons for a range of pathogens, including CHIKV, by RT-PCR (Appendix Table 1, <https://wwwnc.cdc.gov/EID/article/30/12/24-0824-App1.pdf>). We detected no cases of acute CHIKV infection, which aligns with national reported surveillance data that suggest minimal post-2014 transmission (Appendix Figure 1).

Next, to estimate population-level chikungunya seroprevalence, we conducted serologic screening among asymptomatic persons enrolled in a cross-sectional household cluster survey during July–October 2021 that included San Pedro de Macoris and Espaillat Provinces, which aligned with locations from the AFI surveillance program. The 3-stage cross-sectional sampling strategy, which enrolled household members  $\geq 5$  years of age, has been described elsewhere (6). We screened 201 serum samples using plaque reduction neutralization tests (PRNT) and 196 using ELISA IgG tests (Appendix). Of 397 persons enrolled from the 2 provinces, 319 (80.4%) were seropositive (Table). After adjusting for sex, age, and urban/rural setting, the estimated population sero-

prevalence across all age groups was 69.6% (95% CI 64.5%–74.8%) in 2021. That estimate based on national surveillance data assumed that persons born after 2014 were seronegative (Appendix Figure 1). To further assess that assumption, and because few participants enrolled in the serologic survey were born after the 2014 outbreak, we conducted serologic screening on serum samples from children enrolled through the prospective AFI surveillance platform (5). That testing enabled us to assess seropositivity derived from infection after the 2014 outbreak. Date of birth relative to the 2014 outbreak strongly predicted serostatus. Of 275 children screened using PRNT, 110 were born during 2009–2013 (60 [55%] seropositive), 14 in 2014 (1 [7%] seropositive), and 151 after 2014 (1 [0.7%] seropositive) (Table; Figure 1).

We then used our seroprevalence estimates to model population-level immune protection from 2012 through 2045 using methods reported elsewhere (7). We considered that infection generates lifelong immune protection and adjusted population immune protection over time to account for new births adding susceptible persons to the population and deaths reducing the pool of immune persons (Figure 2). Given minimal post-2014 transmission, we assumed no additional population immunity was generated after 2014. Based on our seroprevalence values, we calculated a basic reproduction number ( $R_0$ ) of 2.0 (95% CI 1.84–2.33). We calculated the effective reproduction number ( $R_{\text{eff}}$ ) over time using the  $R_0$  and population immune protection (Figure



**Figure 1.** Chikungunya seroprevalence by year of birth in study of chikungunya outbreak risks after the 2014 outbreak, Dominican Republic. Blue band represents the 95% CI for pooled seroprevalence, which combined data from acute febrile illness and serosurvey cohorts. Estimates were obtained using kernel-weighted local polynomial smoothing weighted by the size of each birth cohort. AFI, acute febrile illness.

2, panel A). Our findings suggest that the  $R_{\text{eff}}$  will remain  $<1.0$  through 2035, indicating that, although clusters of CHIKV infection may occur, widespread and intense community transmission is unlikely. Finally, for comparison, we performed similar analyses for other Caribbean settings, including Jamaica and Puerto Rico, that have reported on CHIKV seroprevalence since the 2014 outbreak. Those analyses suggest that although postoutbreak seroprevalence and  $R_0$  values differ between settings (Appendix Table 2), the risk of widespread regional transmission will remain low through 2035 (Appendix, Appendix Figure 2, panels A, B).

## Conclusion

We found serologic evidence of intense CHIKV transmission in study sites in northwestern and southeastern Dominican Republic during the 2014 outbreak but little subsequent transmission. Those findings were corroborated across a range of sources, including national surveillance, sentinel AFI surveillance, and a cross-sectional serologic survey. Our postoutbreak seroprevalence estimates provide key public health data for the Dominican Republic; the estimate (69.6%) was slightly lower than estimates from Jamaica (83.6%) and Haiti (78.7%) but higher than in other countries in the region (8–11). However, when

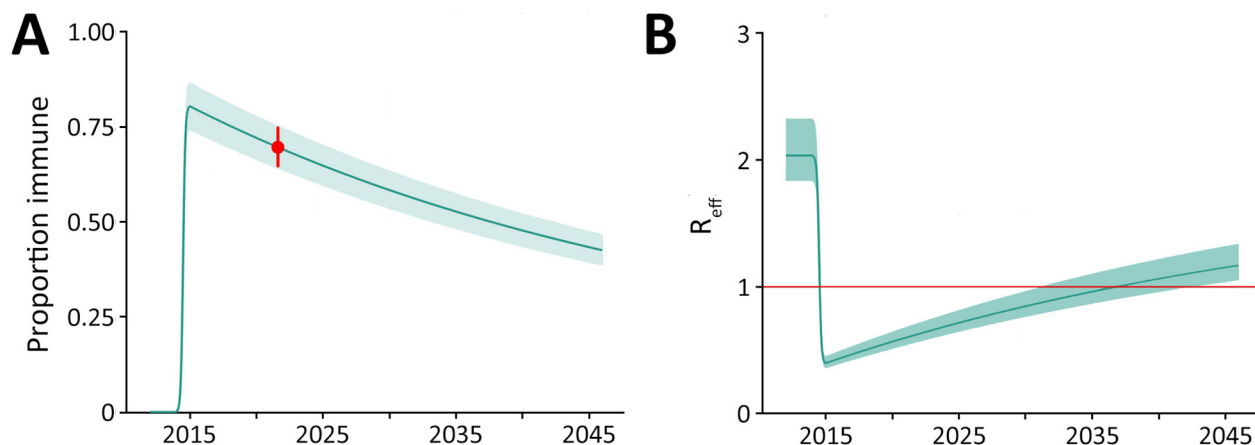
**Table.** Population characteristics and prevalence information from study of future risks for chikungunya outbreaks in the Dominican Republic\*

Category	Household serologic survey†			Acute febrile illness‡		
	All study participants, n = 397	CHIKV seropositive, n = 319	CHIKV seronegative, n = 78	All study participants, n = 275	CHIKV seropositive, n = 62	CHIKV seronegative, n = 213
Province						
San Pedro de Macoris	311	260 (84)	51 (16)	131	34 (26)	97 (74)
Española	86	59 (69)	27 (31)	135	25 (19)	110 (81)
Other	0	0	0	9	3 (33)	6 (67)
Year of birth						
Before 2014	392	319 (81)	73 (19)	110	60 (55)	50 (45)
2014	3	0	3 (100)	14	1 (7)	13 (93)
After 2014	2	0	2 (100)	151	1 (1)	150 (99)
Median age, y (IQR)	32 (17.4–55.5)	31.9 (18–55.5)	32.7 (13–56.3)	6.7 (4.0–10.0)	10.7 (9.5–11.4)	5.3 (3.7–7.5)
Age group, y						
1–5	0	0	0	118	1 (1)	117 (99)
6–10	43	28 (65)	15 (35)	114	37 (32)	77 (68)
11–20	95	81 (85)	14 (15)	43	24 (56)	19 (44)
21–40	99	81 (82)	18 (18)	0	0	0
41–60	87	68 (78)	19 (22)	0	0	0
≥61	73	61 (84)	12 (16)	0	0	0
Sex						
F	250	206 (82)	44 (18)	131	37 (28)	94 (72)
M	147	113 (77)	34 (23)	144	25 (17)	119 (83)
Place of birth						
Dominican Republic	384	309 (80)	75 (20)	270	61 (23)	209 (77)
Other	13	10 (77)	3 (23)	5	1 (20)	4 (80)
Educational attainment						
No formal education	23	17 (74)	6 (26)	153	12 (8)	141 (92)
Primary	112	95 (85)	17 (15)	119	49 (41)	70 (59)
Secondary	123	104 (85)	19 (15)	2	1 (50)	1 (50)
Technical	13	11 (85)	2 (15)	0	0	0
University	37	26 (70)	11 (30)	0	0	0
Missing or not available	89	66 (74)	23 (26)	1	0	1 (100)
Occupation						
Active worker	91	67 (74)	24 (26)	1	1 (100)	0
Houseperson	82	72 (88)	10 (12)	0	0	0
Student	130	102 (78)	28 (22)	161	58 (36)	103 (64)
Retired	12	10 (83)	2 (17)	0	0	0
Unemployed	79	67 (85)	12 (15)	3	0	3 (100)
Preschool	0	0	0	110	3 (3)	107 (97)
Other	3	2 (67)	2 (67)	0	0	0
Self-reported prior infection						
Chikungunya	108	97 (90)	11 (10)	NA	NA	NA
Dengue	14	14 (100)	0	NA	NA	NA
Zika	3	3 (100)	0	NA	NA	NA

\*Values are no. (%) except as indicated. CHIKV, chikungunya virus; NA, not applicable.

†Serologic status was assessed for household serologic survey participants using chikungunya plaque reduction neutralization test (n = 202) and ELISA to detect CHIKV IgG (n = 195).

‡Serologic status for all acute febrile illness surveillance participants was assessed using chikungunya plaque reduction neutralization test.



**Figure 2.** Projected chikungunya population immunity and  $R_{\text{eff}}$  in study of chikungunya outbreak risks after the 2014 outbreak, Dominican Republic. A) Estimated population immunity from 2012 through 2045 using a simulated population parameterized to the current population seroprevalence (red dot). Solid line represent point estimates and shading 95% CIs. Changes in population immunity over time reflect the introduction of new susceptible persons through births and decrease in immune persons through deaths. B) Projected changes in effective reproduction number over time calculated from the basic reproduction number  $R_0$  and population immunity. Solid line represents change in  $R_{\text{eff}}$  and shading 95% CIs, based on the simulated proportion of the immune population. The solid red horizontal line at  $R_{\text{eff}} = 1$  represents the threshold for sustained transmission; values above this line indicate  $R_{\text{eff}} > 1$ , suggesting potential for ongoing transmission, whereas values below this line indicate  $R_{\text{eff}} < 1$ , suggesting a decline in transmission.  $R_{\text{eff}}$  effective reproduction number.

differences in the timing of the serological surveys in relation to the 2014 outbreak are considered, and interval decreases in seroprevalence accounted for, post-2014 outbreak seroprevalence estimates across the 3 countries were broadly similar, and all were  $>80\%$ .

We concluded that the 2014 outbreak generated high levels of population immune protection. However, in the absence of meaningful ongoing transmission, population protection will decrease. Because new births add susceptible persons to the population and deaths reduce the pool of immune persons, the ratio of susceptible to immune persons increases over time. As this ratio increases, so does the risk for a sustained outbreak. Awareness of when population immune protection falls below a threshold that could allow widespread transmission is critical for public health forecasting and response, both in the Dominican Republic and in settings with similar immunologic and epidemiologic profiles.

Our analyses suggest that population immune protection derived from the 2014 outbreak is likely to minimize the risk for another large outbreak through at least 2035 (Figure 2, panel A) if other factors remain unchanged. However, changes in population behavior or environmental conditions, or emergence of new strains, could lead to increased transmission, as documented in the 2022–2023 outbreak in Paraguay, when transmission expanded to multiple previously unaffected regions (12).

Limitations included lack of generalizability of the data sources. We largely addressed this limitation

by using multiple data sources to confirm key findings, with the exception of population seroprevalence estimates, for which we relied on a single source. The number of study participants was limited, and data were restricted to 2 provinces; therefore, our point estimates might not be representative of national seroprevalence. In addition, the specificity of CHIKV immunoassays may be affected by other circulating alphaviruses (13), although substantially lower seroprevalence among those born after 2014 suggests that effect was unlikely.

In conclusion, chikungunya immune protection generated during the 2014 outbreak in the Dominican Republic will likely minimize the risk for widespread and intense transmission in the next decade, similar to findings from Jamaica and Puerto Rico. However, changes in behaviors, environmental conditions, or emergence of new strains could affect those predictions. Therefore, public health authorities should closely monitor chikungunya activity and develop preparedness plans to mitigate effects of future outbreaks.

#### About the Author

Dr. Loevinsohn is chief resident in the Department of Emergency Medicine at Massachusetts General Hospital and Brigham and Women's Hospital in Boston, Massachusetts, USA. His clinical and research interests focus on emergency care in resource-limited settings, epidemiology of acute illnesses, and multifaceted determinants of health.

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# Chikungunya Outbreak Risks after the 2014 Outbreak, Dominican Republic

## Appendix

### Methods

#### Molecular detection of CHIKV

RNA was extracted from sera using the QIAamp Viral RNA Mini kit (QIAGEN). RNA extracts were then tested using real-time RT-PCR technique and a ZCD multiplex Zika, chikungunya, and dengue virus (DENV1-4; ZCD) assay on an ABI 7500 Fast (Applied Biosystems) using commercially available primers and probes. Real-time RT-PCR was performed according to the manufacturer's instructions.

#### IgG Enzyme-Linked Immunosorbent Assay (ELISA)

The IgG ELISA was performed as previously described and has been demonstrated to correlate closely with plaque-reduction neutralization assays (1). Briefly, the inner 60 wells of a 96 well flat-bottom plate (Immulon II HB, ThermoFisher) were coated with the alphavirus cross-reactive monoclonal antibody 1A4B-6 (Arboviral Diseases Branch, Arboviral Reference Collection) diluted in carbonate coating buffer and incubated overnight at 4°C. The coating buffer was removed, and plates blocked with blocking buffer (PBS, 0.1% Tween20, 3% goat serum [Colorado Serum Co.]) for 1 hour at room temperature. Plates were washed 5 times with PBS, 0.05% Tween20 wash buffer. CHIKV suckling mouse brain antigen (Arboviral Diseases Branch, Arbovirus Reference Collection) was diluted in wash buffer and 50 µl added to 3 wells per test sample, normal mouse brain antigen was also added to 3 wells per test sample, and incubated overnight at 4°C. Antigens were washed 5 times with wash buffer, and test serum was diluted 1:400 in wash buffer, 50 µl added to 6 wells (3 wells of CHIKV antigen and 3 wells of negative antigen), and incubated for 1 hour at 37°C in a humid chamber. Known positive control

serum and normal human serum were included as controls. The plates were washed 5 times, and 50  $\mu$ l alkaline-phosphate conjugated goat anti-human IgG (Jackson ImmunoResearch) was added to the wells and incubated for 1 hour at 37°C in a humid chamber. The plates were washed 10 times and developed with 3 mg/ml p-nitrophenyl phosphate disodium (Sigma) in 1M Tris base pH 8.0. for 30 minutes at room temperature. 3M sodium hydroxide was added to stop the development and plates were read in a plate reader (BioTek) at 405nm. Positive samples were determined by the mean OD of the sample serum on viral antigen/the mean OD of the negative human serum on viral antigen (P/N). A P/N greater than 2.0 is considered positive.

#### **Plaque reduction neutralization test (PRNT<sub>90</sub>)**

Serum samples were screened for neutralizing antibodies against CHIKV East, Central and South African- Indian Ocean (ECSA-IOL) strain 91064 (GenBank accession EF451144) in Vero cells (ATCC CCL-81). Briefly, samples were diluted 1:10 in BA-1 (M199-H, Sigma-Aldrich; 0.05 M Tris-HCl, Invitrogen; 1% bovine serum albumin, Millipore) and heat inactivated at 56°C for 30 minutes. 60  $\mu$ l of diluted serum was incubated with an equal volume of CHIKV at 200 plaque forming units (pfu)/0.1 mL supplemented with 8% Labile Serum Factor (LSF) (Millipore) for one hour at 37°C. Back titrations of 10-fold dilutions, representing 100 plaques, 10 plaques, and 1 plaque were also incubated for one hour at 37°C. 100  $\mu$ l of sample/virus and back titration were added to Vero cells seeded in 6-well cell culture plates. Plates were incubated for one hour at 37°C then overlaid with 3 mL of yeast extract-lactalbumin (Ye-Lah) media containing 0.5% (w/v) Agarose (Lonza), 3% (v/v) sodium bicarbonate (Invitrogen), 2% (v/v) FBS (Hyclone), 0.1% (v/v) Gentamicin (MP Biomedicals), 0.4% (v/v) Fungizone (Hyclone), and 1% (v/v) Pen/Strep (Invitrogen). Two days post incubation, a second Ye-Lah overlay with 3% (v/v) neutral red (Sigma-Aldrich) was applied. Plaques were counted the next day. Samples were compared to the back titration and considered positive if they had  $\geq$ 90% neutralizing at 1:20.

#### **Calculation of population immunity**

We conducted a time-series analysis from January 1, 2012, to December 31, 2045, to examine the dynamics of immunity and the effective reproductive number in a population (Fig 1). An initial population of 1,000 persons were considered, with varying birth and death rates from 2012 through 2045. We assumed interval birth and death rates were linear between these timepoints. We also assumed that prior to 2014 the population was fully susceptible. A sigmoid function was applied in 2014 to simulate a rapid increase in immunity. Post-2014, adjustments

were made to the immune population by adding new births to the susceptible pool and removing persons that died from the immune pool. Data source for country-specific births and deaths was the United Nations World Population Prospects, accessed from <http://www.macrotrends.net/countries>.

### **Calculation of $R_0$ and $R_{\text{eff}}$**

We estimated the basic reproduction number for different infection scenarios based upon the estimated seroprevalence of the simulated population following the outbreak, where  $\chi$  = the final susceptible proportion of the population post-outbreak.

$$R_0 = -\ln(\chi)/(1 - \chi)$$

$R_0$  values were used to estimate the  $R_{\text{eff}}$ , which was calculated iteratively for each day of the dataset from January 1, 2012 to Dec 31, 2045, as follows, where  $A$  = number of immune persons and  $B$  = total simulated population.

$$R_{\text{eff}} = R_0 \times \left(1 - \frac{A}{B}\right)$$

### **Times series of national reported cases**

Using data on reported CHIKV cases provided by the Dominican Republic Ministry of Public Health and Social Welfare, we aggregated the case counts by week and year. Reported cases included both laboratory confirmed and suspected cases.

### **Data sources**

National chikungunya surveillance data that includes reported cases from 2013 through 2021 were provided by the Dominican Republic Ministry of Public Health and Social Welfare. Data on birth and death rates are from the United National Department of Economic and Social Affairs, Population Division, and are available from <https://www.macrotrends.net/countries/DOM/dominican-republic>. Data on seroprevalence for Jamaica and Puerto Rico were obtained from the cited studies. All other data were enumerated through the current study.

### **Programming language**

Analyses and data visualization were performed using the R statistical programming language (R version 4.2.3, 2023-03-01), with ggplot2 for data visualization.

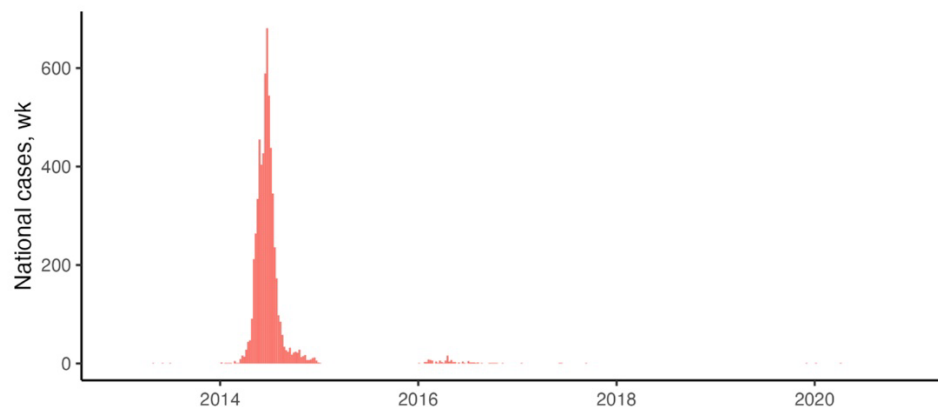


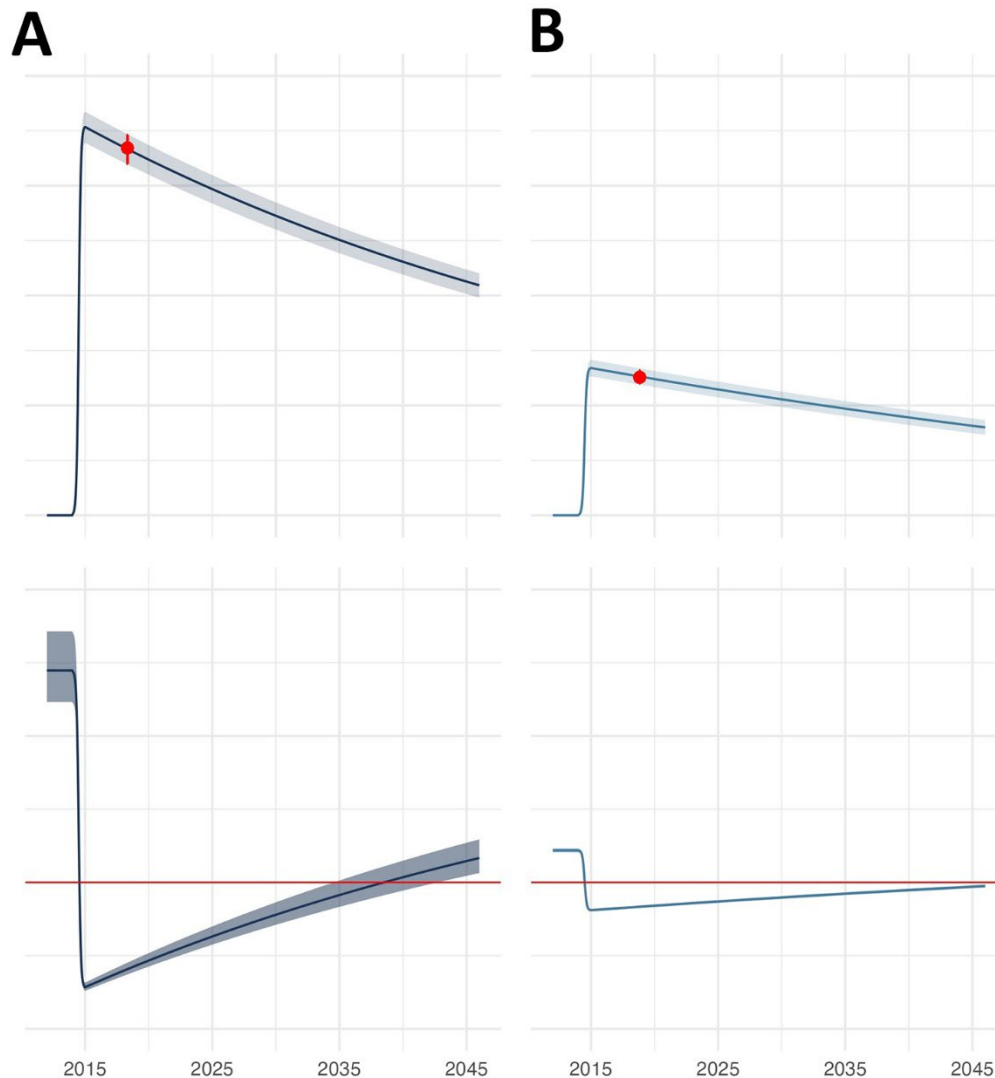
**Appendix Table 1.** Acute Febrile Infection Surveillance Study Population (N = 2792)

Characteristic	Study participants, n (%)
Home province	
San Pedro de Macoris	1136 (41)
Espaillat	1487 (53)
Other	169 (6)
Median age, y (IQR)	27 (12–41)
Age range, y	
1–5	408 (15)
6–10	206 (7)
11–20	456 (16)
21–40	1006 (36)
41–60	534 (19)
≥61	182 (7)
Gender	
F	1615 (58)
M	1169 (42)
Other	1 (0)
Country of birth	
Dominican Republic	2701 (97)
Other	88 (3)
Unknown	2 (0)
Symptoms	
Chills	2202 (79)
Malaise	224 (8)
Headache	200 (7)
Cough	2121 (76)
Vomiting	62 (2)
Diarrhea	63 (2)
Myalgia	1835 (66)
Arthralgia	69 (2)
Erythematous rash	94 (3)

**Appendix Table 2.** Estimated CHIKV seroprevalence and basic reproductive numbers in the Dominican Republic, Jamaica, and Puerto Rico

Category	Dominican Republic	Jamaica	Puerto Rico
Year of survey	2021	2017–2019	2018–2019
Sample size	397	584	4,035
Estimated seroprevalence at the time of survey, % (95% CI)	69.6 (64.5–74.8)	83.6% (80.0–86.5)	31.4 (30.0–32.9)
Estimated seroprevalence at the end of 2014, % (95% CI)	80.6 (74.6–86.7)	88.6 (85.0–92.1)	33.6 (32.0–35.1)
Basic reproductive number (95% CI)	2.0 (1.84–2.33)	2.45 (2.23–2.71)	1.22 (1.21–1.23)

**Appendix Figure 1.** National reported CHIKV cases by week, Dominican Republic. X-axis tick marks indicate January 1 of the respective year. Data obtained from the Dominican Republic Ministry of Public Health and Social Welfare.



**Appendix Figure 2.** Projected chikungunya population immunity and effective reproduction number ( $R_{eff}$ ) in other Caribbean settings. Top row represents estimated population immunity during 2012–2045 using a simulated population parameterized to the current population seroprevalence (red dots) with 95% CIs (grey bands), with estimates from previously reported seroprevalence studies from A) Jamaica (among women attending antenatal care) and B) Puerto Rico (among a cohort of persons  $\leq 50$  years of age). Dark lines represent the point estimates with grey bands representing 95% CIs. Changes in population immunity over time reflect the introduction of additional susceptible persons through births and decrease in immune persons through deaths. Bottom row presents projected changes in  $R_{eff}$  over time calculated from  $R_0$  and population immunity. Dark lines represent changes in  $R_{eff}$  based on the simulated proportion of the immune population shown in the top row.