Sustained Low-Level Transmission of Zika and Chikungunya Viruses after Emergence in the Fiji Islands

Mike Kama,[1] Maite Aubry,[1] Taina Naivalu, Jessica Vanhomwegen, Teheipuaura Mariteragi-Helle, Anita Teissier, Tuterarii Paoaafaite, Stéphane Hué, Martin L. Hibberd, Jean-Claude Manuguerra, Ketan Christi, Conall H. Watson, Eric J. Nilles, John Aaskov, Colleen L. Lau, Didier Musso, Adam J. Kucharski,[1] Van-Mai Cao-Lormeau[1]

Zika and chikungunya viruses were first detected in Fiji in 2015. Examining surveillance and phylogenetic and serologic data, we found evidence of low-level transmission of Zika and chikungunya viruses during 2013–2017, in contrast to the major outbreaks caused by closely related virus strains in other Pacific Island countries.

Zika virus and chikungunya virus (CHIKV) have caused outbreaks in several tropical regions, including the Pacific (*1*). The first known Zika virus outbreak occurred in Yap Island (Federated States of Micronesia) in 2007 (*2*), followed by an explosive outbreak in French Polynesia in 2013–2014 (*3*), then other Pacific islands (*4*) and Latin America (*5*). CHIKV first appeared in the Pacific in 2011 (*6*), causing multiple outbreaks from 2013 onward (*4*).

Author affiliations: Fiji Centre for Communicable Disease Control, Suva, Fiji (M. Kama); The University of the South Pacific, Suva (M. Kama, T. Naivalu, K. Christi); Institut Louis Malardé, Papeete, Tahiti (M. Aubry, T. Mariteragi-Helle, A. Teissier, T. Paoaafaite, D. Musso, V.-M. Cao-Lormeau); Fiji National University, Suva (T. Naivalu); Institut Pasteur, Paris, France (J. Vanhomwegen, J.-C. Manuguerra); London School of Hygiene and Tropical Medicine, London, UK (S. Hué, M.L. Hibberd, C.H. Watson, A.J. Kucharski); World Health Organization, Suva (E.J. Nilles); Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts, USA (E.J. Nilles); Harvard Humanitarian Initiative, Cambridge, Massachusetts, USA (E.J. Nilles); Queensland University of Technology, Brisbane, Queensland, Australia (J. Aaskov); Australian National University, Canberra, Australian Capital Territory, Australia (C.L. Lau); Aix Marseille University, Marseille, France (D. Musso)

DOI: https://doi.org/10.3201/eid2508.180524

In Fiji, the first confirmed Zika virus infections were detected in July 2015; these were locally acquired. By March 2016, a total of 13 confirmed infections had been reported (*7*). The first recorded CHIKV infection was an imported case detected in March 2015 (*8*); 24 autochthonous infections were identified by June 2016 (*9*). CHIKV and Zika virus were subsequently detected in travelers returning from Fiji (*10*,*11*). Outbreaks of dengue virus (DENV) have been recorded in Fiji (*4*,*12*), and evidence from other settings indicates that DENV and Zika virus can exhibit similar transmission characteristics in the same location (*13*). Despite enhanced surveillance, no large outbreaks of Zika or chikungunya were identified in Fiji, unlike in other settings (*3*,*4*). We describe the introduction, epidemiology, and transmission of Zika virus and CHIKV in Fiji during 2013–2017, in a context of concurrent circulation of DENV (*4*,*12*).

The Study

We retrieved surveillance data for patients with prolonged fever (PF), defined as any fever lasting $\geq$3 days, and acute fever and rash (AFR) in Fiji (Figure), as well as data on suspected and confirmed Zika virus, CHIKV, and DENV infections (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/25/8/18-0524-App1.pdf). We reconstructed phylogenetic trees of Zika virus and CHIKV sequences by using Bayesian inference (Appendix Tables 2, 3). We recruited 778 participants in Fiji during September–November 2013 as part of a community-based serologic survey (Appendix Figure 1). We collected follow-up samples from the same participants in the Central Division (N = 333) during October–November 2015. We tested serum samples by using a recombinant antigen-based microsphere immunoassay to detect Zika virus, CHIKV, and DENV-1–4 IgG (Appendix). Analysis of neutralizing antibodies against Zika virus and DENV in a subset of 69 paired serum samples showed good concordance with the microsphere immunoassay for Zika virus (κ = 0.71) and DENV (κ = 0.80) (Appendix Table 4).

Surveillance data recorded during 2013–2017 indicated cyclical increases in AFR and PF each year, concurrent

[1]These authors contributed equally to this article.

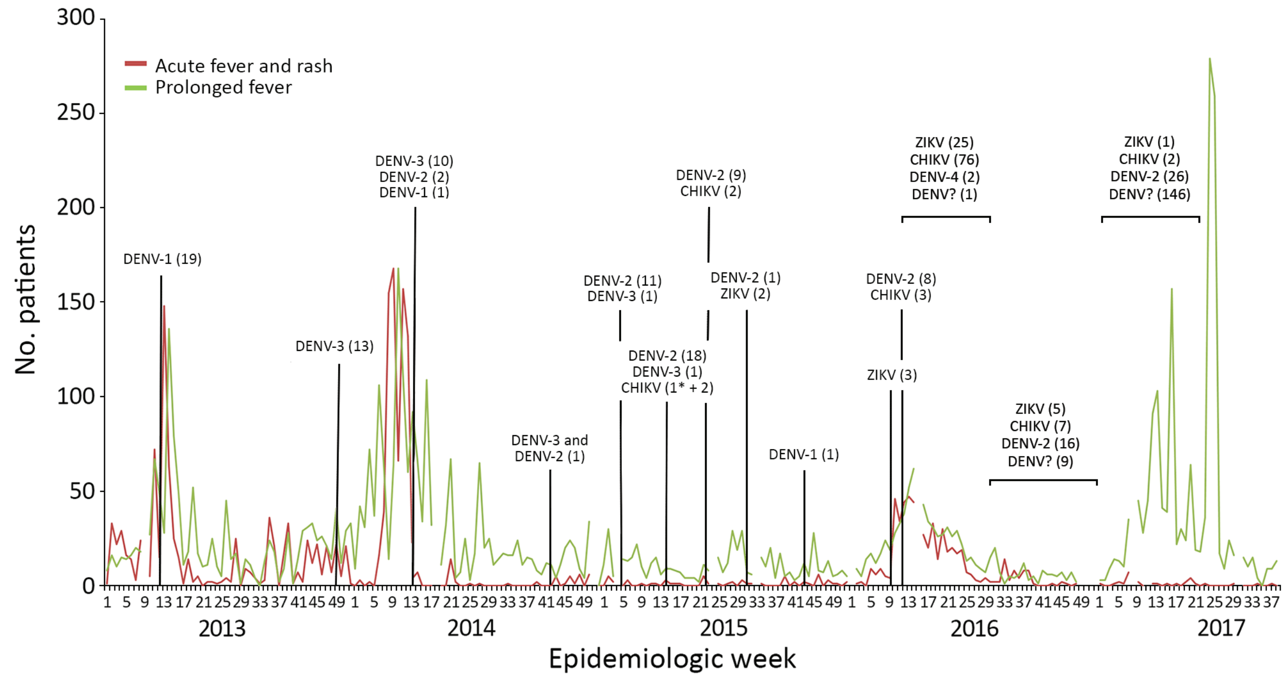

Figure. Reports of patients with acute fever and rash, prolonged fever, and infections with dengue, Zika, or chikungunya viruses confirmed by reverse transcription PCR in Fiji, 2013–2017. Number of dengue, Zika, or chikungunya virus infections were confirmed by reverse transcription PCR. Asterisks (*) indicate imported chikungunya virus infections. CHIKV, chikungunya virus; DENV-1, dengue virus serotype 1; DENV-2, dengue virus serotype 2; DENV-3, dengue virus serotype 3; DENV-4, dengue virus serotype 4; DENV?, information on dengue virus serotype not available; ZIKV, Zika virus.

with the hot and rainy season occurring in December–April (Figure). Molecular testing of blood samples from symptomatic patients suggested outbreaks of DENV-1 in 2013, DENV-3 in 2014, and DENV-2 in 2017. In 2015, Zika virus and CHIKV apparently were co-circulating at low levels alongside DENV-1, DENV-2, and DENV-3. In 2016, an increase in proportional positivity for Zika virus and CHIKV was detected among 804 AFR and PF patients, suggesting higher transmission of these viruses but not widespread circulation (Zika virus, 32/804 [4%]; CHIKV, 86/804 [11%]; DENV-2, 10/804 [1%]; DENV-4, 2/804 [<1%]). Additional CHIKV (n = 2) and Zika virus (n = 1) infections were detected during the first half of 2017.

We aligned the envelope (E) gene sequences of Zika virus strains collected in Fiji during 2015–2016 (Appendix Table 2) with sequences from other countries. All Zika virus strains belonged to the Asia lineage and segregated into 2 separate clades (posterior probability >0.99) (Appendix Figure 2, panel A). The Fiji Zika virus strains belonged to the Asia and Oceania clade; 2 strains collected in 2016 grouped with viruses isolated in Japan in 2016 (posterior probability >0.99), including 1 from a traveler returning from Fiji. The estimated time of most recent common ancestor of this cluster was September 2013 (95% higher probability density [HPD] interval September 2011–August 2015). The remaining Fiji strains formed a distinct cluster with strains from Southeast Asia and other Pacific Islands. We dated the origin of this second cluster to November 2013 (95% HPD interval March 2013–July 2015).

We aligned the E1 gene sequences of Fiji CHIKV strains collected during 2015–2016 (Appendix Table 2) with sequences from other countries. All strains belonged to the Asia genotype; Fiji strains formed a monophyletic group with strains from Tonga sampled in 2014 (posterior probability 1.00) (Appendix Figure 2, panel B). This grouping suggested a single introduction of CHIKV into Fiji in February 2014 (95% HPD interval December 2013–August 2014) and subsequent persistence in the population.

Zika virus seroprevalence in 2013 was 7.8% (95% CI 6.1%–10%); we observed no significant differences between age groups, sexes, residential divisions, or areas (Table). In 2015, seroprevalence was 21.9% (95% CI 17.6%–26.8%), and the only significant difference observed was between rural (14.2% [95% CI 8.3%–22%]) and urban (26.6% [95% CI 19.5%–34.6%]) areas (p = 0.0202). Compared with 2013, Zika virus seroprevalence in 2015 was significantly higher overall (p<0.0001). However, no change was observed in the CHIKV seroprevalence between 2013 (0.8% [95% CI 0.3%–1.7%]) and 2015 (0.9% [95% CI 0.2%–2.6%]), and no significant differences were observed in the demographic variables described for Zika virus. The seroprevalence of DENV in 2013 was 73% (95% CI 69.7%–76.1%) and was lower among

Table. Prevalence of Zika, chikungunya, and dengue virus antibodies in a representative subset of the population sampled during September–November 2013 and October–November 2015, Fiji Islands*

	No. seropositive/no. tested (% [95% CI])					
	Zika virus		Chikungunya virus		Dengue viruses†	
Variable	2013	2015	2013	2015	2013	2015
Total	61/778‡ (7.8 [6.1–10])	73/333 (21.9 [17.6–26.8])	6/778 (0.8 [0.3–1.7])	3/333 (0.9 [0.2–2.6])	568/778 (73 [69.7–76.1])	276/333 (82.9 [78.4–86.8])
Age range (median), y	2–85 (28)	4–80 (29)	2–85 (28)	4–80 (29)	2–78 (28)	4–80 (29)
Age group, y						
0–19	29/282 (10.3 [7–14.4])	29/115 (25.2 [17.6–34.2])	4/282 (1.4 [0.4–3.6])	1/115 (0.9 [0–4.7])	141/282 (50 [44–56])	78/115 (67.8 [58.5–76.2])
20–39	15/239 (6.3 [3.6–10.1])	18/103 (17.5 [10.7–26.2])	1/239 (0.4 [0–2.3])	1/103 (1 [0–5.3])	201/239 (84.1 [78.8–88.5])	93/103 (90.3 [82.9–95.2])
40–59	11/179 (6.1 [3.1–10.7])	13/73 (17.8 [9.8–28.5])	1/179 (0.6 [0–3.1])	1/73 (1.4 [0–7.4])	161/179 (89.9 [84.6–93.9])	68/73 (93.2 [84.7–97.7])
≥60	6/77 (7.8 [2.9–16.2])	13/42 (31 [17.6–47.1])	0/77 (0 [0–4.7])	0/42 (0 [0–8.4])	64/77 (83.1 [72.9–90.7])	37/42 (88.1 [74.4–96])
Sex						
F	28/423 (6.6 [4.4–9.4])	41/190 (21.6 [16–28.1])	4/423 (0.9 [0.3–2.4])	2/190 (1.1 [0.1–3.8])	312/423 (73.8 [69.3–77.9])	165/190 (86.8 [81.2–91.3])
M	33/354 (9.3 [6.5–12.8])	32/143 (22.4 [15.8–30.1])	2/354 (0.6 [0.1–2])	1/143 (0.7 [0–3.8])	255/354 (72 [67–76.6])	111/143 (77.6 [69.9–84.2])
Division						
Central	30/451 (6.7 [4.5–9.4])	73/333 (21.9 [17.6–26.8])	5/451 (1.1 [0.4–2.6])	3/333 (0.9 [0.2–2.6])	331/451 (73.4 [69.1–77.4])	276/333 (82.9 [78.4–86.8])
Northern	7/59 (11.9 [4.9–22.9])	ND	0/59 (0 [0–6.1])	ND	51/59 (86.4 [75–94])	ND
Western	24/268 (9 [5.8–13])	ND	1/268 (0.4 [0–2.1])	ND	186/268 (69.4 [63.5–74.9])	ND
Area						
Periurban	10/135 (7.4 [3.6–13.2])	19/77 (24.7 [15.6–35.8])	2/135 (1.5 [0.2–5.2])	0/77 (0 [0–4.7])	104/135 (77 [69–83.8])	66/77 (85.7 [75.9–92.6])
Rural	24/344 (7 [4.5–10.2])	16/113 (14.2 [8.3–22])	2/344 (0.6 [0.1–2.1])	0/113 (0 [0–3.2])	234/344 (68 [62.8–72.9])	84/113 (74.3 [65.3–82.1])
Urban	27/298 (9.1 [6.1–12.9])	38/143 (26.6 [19.5–34.6])	2/298 (0.7 [0.1–2.4])	3/143 (2.1 [0.4–6])	229/298 (76.8 [71.6–81.5])	126/143 (88.1 [81.6–92.9])

*No participants were recruited in the Northern and Western divisions in 2015. ND, no data.
†Seropositivity for ≥1 serotypes of dengue virus.
‡For 1 participant, demographic data were not available except for the administrative division of residence.

persons in the 0–19 years age group compared with other age groups (p<0.0001) (Table). We observed no significant difference by sex. DENV seropositivity was higher in the Northern than in the Central and Western divisions (p≤0.0368) and higher in urban than in rural areas (p = 0.0136). During 2013–2015, we observed a significant increase in DENV seroprevalence (82.9% [95% CI 78.4%–86.8%]; p = 0.0004) among persons 0–19 years of age (p = 0.0013), women and girls (p = 0.0002), and participants living in the Central Division (p = 0.0018) and urban areas (p = 0.0048). Seroprevalence in 2015 remained lower in persons 0–19 years of age than in other age groups (p≤0.0137) but was significantly higher in women and girls compared with men and boys (p = 0.039) and in urban compared with rural areas (p = 0.0053).

Analysis of paired samples collected in 2013 and 2015 from the same participants supported previous serologic findings on all samples collected (Appendix Table 5). Among these participants, 55/311 (17.7% [95% CI 13.6%–22.4%]) seroconverted to Zika virus, 40/311 (12.9% [95% CI 9.3%–17.1%]) seroconverted to DENV, and 1/311 (0.3% [95% CI 0.008%–1.8%]) seroconverted to CHIKV (Appendix Table 6).

Conclusions

We found evidence of low-level transmission of Zika virus and CHIKV in Fiji for multiple years after their initial introduction into a population that probably was immunologically naive, despite an ecologic environment subject to large and recurrent DENV outbreaks. Similar evidence of low-level Zika virus circulation has been observed in other settings (*14*). Our findings indicate that Zika virus circulated before the first confirmed cases in 2015 and that multiple introductions from other Pacific islands might have occurred, which suggests the possible role of Zika virus in a cluster of Guillain-Barré syndrome cases of unknown etiology in Fiji during February–May 2014 (*15*). However, there was no epidemiologic or serologic evidence that CHIKV circulated in Fiji before it was first reported in 2015. High DENV seroprevalence in 2013 and 2015 suggests that DENV is endemic in Fiji, with seroprevalence increasing with age. Our data also suggest that DENV and Zika virus transmission occurs mostly in urban areas where peridomestic mosquitoes, notably *Aedes aegypti* and *Ae. albopictus*, are abundant.

Our study highlights the difficulties in detecting and anticipating outbreaks of Zika virus and CHIKV and the value of having multiple data sources available. Stronger clinical and laboratory surveillance capacities are needed to ensure the early detection of these and future infectious disease threats.

This work was part of ISID-Pacific and R-ZERO Pacific programs funded by the French Ministry for Europe and Foreign Affairs (Pacific Fund nos. 06314-09/04/14, 12115-02/09/15, 03016-20/05/16, and 04917-19/07/17). The study also received support from the Embassy of France in the Republic of the Fiji Islands. The study was supported by the French Government's "Investissement d'Avenir" Program (Labex IBEID no. ANR-10-LABX-62-IBEID). C.L.L. was supported by an Australia National Health and Medical Research Council Fellowship (grant no. 1109035). A.J.K. was supported by a Wellcome Trust/Royal Society Sir Henry Dale Fellowship (grant no. 206250/Z/17/Z). The seroprevalence study was part of MSc research work by M.K. performed at the University of the South Pacific.

About the Author

Dr. Kama is a medical doctor with the Fiji Ministry of Health. Since 2008, he has worked in communicable disease control in Fiji. His area of interest is public health surveillance and response to communicable disease emergencies.

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Address for correspondence: Van-Mai Cao-Lormeau, Institut Louis Malardé, PO Box 30, 98713 Papeete, Tahiti; email: mlormeau@ilm.pf

Article DOI: https://doi.org/10.3201/eid2508.180524

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Appendix

Materials and Methods

Study Location and Population

The Republic of the Fiji islands is located in the South Pacific and comprises 322 islands divided into 4 administrative Divisions: Central, Western, Northern and Eastern (Appendix Figure 1). The population was 837,271 in 2007 and most of the inhabitants lived in the Central (41%), Western (38%) and Northern (16%) Divisions. The proportion of people living in urban/peri-urban and rural areas was similar (51% versus 49%). The median age of the population was 21 years with a 1:1 ratio of males to females.

Syndromic and Laboratory Surveillance

Surveillance data for patients with prolonged fever (PF, ie any fever lasting three or more days) and acute fever and rash (AFR) in Fiji (week 1 of 2013 to week 37 of 2017) were retrieved from the Pacific Syndromic Surveillance System weekly reports (*1*); the case definitions have been previously reported (*2*).

Data on suspected and confirmed DENV, CHIKV and ZIKV cases including type and location of testing were retrieved from the laboratory surveillance database of the Fiji Centre for Communicable Disease Control (FCCDC, Suva, Fiji) (Appendix Table 1). Molecular assays for DENV, CHIKV and ZIKV were primarily performed at the Institut Louis Malardé (ILM, Tahiti, French Polynesia). From January 2013, serum samples blotted on filter paper cards and saliva samples collected on dry oral swabs from patients with PF or AFR were referred to ILM for testing by real-time RT-PCR for the presence of RNA from the four DENV serotypes, CHIKV and ZIKV following the protocols previously described (*3*–*6*). From 2016, molecular testing for DENV, ZIKV and CHIKV was implemented at the FCCDC Laboratory (Mataika House) using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) and Trioplex Real-time RT-PCR assay provided by the Centers for Disease Control and Prevention (CDC, USA) (*7*). A smaller number of samples were tested using RT-PCR for the same pathogens at the Environmental Science and Research Limited (ESR, New Zealand) and the Environmental Health Institute (EHI, Singapore).

Sequencing of Viral Genes and Phylogenetic Analyses

The envelope (E) gene of ZIKV strains recovered from two saliva samples collected in 2015 and three serum samples collected in 2016, and the E1 gene of CHIKV strains isolated from two serum samples collected in 2015 and three additional serum samples collected in 2016 were sequenced (Appendix Table 2). Briefly, ZIKV and CHIKV RNA were extracted using the Easymag extraction system (bioMérieux, France) as previously detailed (4) and amplified using the One-Step RT-PCR Kit and HotstarTaq DNA Polymerase Kit or HotstarTaq Plus DNA Polymerase Kit (Qiagen, Germany) with primers described in Appendix Table 3. The PCR products were purified using the QIAquick PCR Purification Kit or the QIAquick Gel Extraction Kit (Qiagen, Germany). The subsequent sequencing reactions were performed with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and the products were purified with the DyeEx 96 Kit (Qiagen, Germany). Sequencing of purified cDNA was performed on the 3500 Series Genetic Analyzer (Applied Biosystems, USA). Fragments of nucleotide sequences obtained for each ZIKV and CHIKV strain were cleaned and assembled using the Sequencher 4.10.1 software (Gene Codes Corporation, USA). Complete sequences of ZIKV E gene and CHIKV E1 gene were aligned with sequences retrieved from GenBank using the Clustal W algorithm integrated in the MEGA 7 phylogenetic package (*8*). The sequences retrieved from GenBank were selected using nucleotide BLAST searches (*9*), where all sequences with a reported date of sampling and country of origin sharing more than 99% genetic identity to the Fiji sequences were retained. After removal of duplicates, the ZIKV and CHIKV alignments contained 66 and 62 sequences, respectively. For each alignment, phylogenies in nucleotide substitutions per sites and in unit of time (‘dated’ phylogenies) were reconstructed by Bayesian MCMC inference, using the package BEAST (*10*).

Serosurveys

Sampling Design and Data Collection

Participants (N = 778) were recruited between September and November 2013 in the Northern, Western and Central divisions of Fiji (Appendix Figure 1), as part of a population-representative community-based typhoid/leptospirosis seroprevalence study as previously described (*11*,*12*). Briefly, local nursing zones were initially selected using population-proportionate sampling. Next, one community was randomly selected from each of these zones, then 25 households were selected from each community and finally one individual was selected from each of the households: household members (defined as someone who stayed at the house the previous night) were enumerated, with one selected at random. A questionnaire was used to collect demographic data (age, gender, Division, residential area), and a 5 ml venous blood sample was collected. A second phase to obtain paired samples was conducted in the Central Division between October and November 2015 (Appendix Figure 1). Field officers followed-up participants who had participated in the 2013 study, and who had consented to being contacted again for health research. Similar to 2013, a 5 ml venous blood sample was collected from the participants (N = 333) along with questionnaire data. This resulted in a set of paired samples (N = 311). Insufficient volumes of serum from some of the 2013 samples meant that a subset of participants (N = 22) only had 2015 samples available.

Informed Consent and Ethics Approvals

Ethics approvals were granted by the Fiji National Health Research Ethics Review Committee (FNRERC 2013–03, N°2015.114.NW and N°2015.45.MC), the University of the South Pacific (FSTER/2015/10/Research Proposal Approval/Mike Kama/S90058620 and FSTER/2015/11/Research Proposal Approval/Taina Naivalu/S11127279) and the London School of Hygiene & Tropical Medicine Observational Research Ethics Committee (6344 and 10207).

Serologic Analysis

Serologic assays to detect immunoglobulin class G (IgG) antibodies against ZIKV, CHIKV and the four serotypes of DENV were performed on serum samples collected in 2013 (N = 778) and 2015 (N = 333), including paired samples (N = 311), using a recombinant antigen-based microsphere immunoassay (MIA) as previously described (*13*–*15*), with recombinant antigens of ZIKV, CHIKV, DENV-1, –2, –3, or –4 (GenBank accession no. KJ776791,

AM258994, AF226686.1, FM986654, FJ44740.1, and FM986672.1 respectively). The presence of neutralizing antibodies against ZIKV and each of the four DENV serotypes was assessed in the paired serum samples of the 69 participants for whom at least one of the samples had anti-ZIKV antibodies detected by MIA, using a neutralization assay as previously detailed (*13*,*15*), with ZIKV, DENV-1, –2, –3 or –4 strains (GenBank accession no. KX369547, MG181997, JQ650020, AY744680, and KY933793, respectively). Among the samples reactive by MIA, 66/83 (79.5%) exhibited neutralizing activity for ZIKV ($\kappa = 0.71$) and 109/112 (97.3%) for DENV ($\kappa = 0.80$) (Appendix Table 4); showing good concordance between the MIA and neutralizing antibody assays for anti-ZIKV and anti-DENV IgG respectively.

Statistical Analysis

Statistical analysis of seroprevalence data was conducted with the GraphPad Prism 6 version 6.03 software using the Fisher's test. P values less than 0.05 were considered as statistically significant.

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Appendix Table 1. Diagnosis of dengue, chikungunya and Zika virus infections in Fiji by RT-PCR between 2013–2017*

			Number positive		
Period	Laboratory	Number tested	DENV	ZIKV	CHIKV
2013					
January–June	ILM	29	19	NT	NT
July–December	ILM	35	13	NT	NT
2014					
January–June	ILM	36	13	0	0
July–December	ILM	3	1	0	0
2015					
January–June	ILM	344	40	0	5
July–December	ILM	69	2	2	0
2016					
January–June	ILM	187	12	3	3
January–June	Mataika House	325	2	6	67
January–June	ESR	96	3	16	16
January–June	EHI	196	NT	7	NT
July–December	ILM	15	11	0	0
July–December	Mataika House	233	8	1	0
2017					
January–June	ILM	27	26	NT	0
January–June	Mataika House	572	146	1	2

*ZIKV, Zika virus; CHIKV, chikungunya virus; DENV, dengue viruses; ILM, Institut Louis Malardé (French Polynesia); ESR, Environmental Science and Research Limited (New Zealand); EHI, Environmental Health Institute (Singapore); NT, not tested.

Appendix Table 2. Characteristics of Zika and chikungunya virus strains sequenced in this study

Virus	Strain ID	Geographic origin	Collection date	Sample origin	GenBank accession number
Zika	Fiji2015–110715–17A	Suva (Central division)	11 July 2015	Saliva	MG216928
Zika	Fiji2015–110715–18A	Suva (Central division)	11 July 2015	Saliva	MG216929
Zika	Fiji2016–260516–7284	Lautoka (Western division)	26 May 2016	Serum	MG216930
Zika	Fiji2016–220716–1568	Suva (Central division)	22 July 2016	Serum	MG216931
Zika	Fiji2016–030816–1580	Lautoka (Western division)	03 August 2016	Serum	MG216932
Chikungunya	Fiji2015–070315–0515	Lautoka (Western division)	07 March 2015	Serum	MG271970
Chikungunya	Fiji2015–250315–0815	Nadi (Western division)	25 March 2015	Serum	MG271971
Chikungunya	Fiji2016–060516–5530	Suva (Central division)	06 May 2016	Serum	MG271972
Chikungunya	Fiji2016–070516–5541	Suva (Central division)	07 May 2016	Serum	MG271973
Chikungunya	Fiji2016–060517–5561	Suva (Central division)	06 May 2016	Serum	MG271974

Appendix Table 3. Primers used for sequencing of the envelope gene of Zika virus and envelope gene (E1) of chikungunya virus

Virus	Primer	Genome position	Sequence (5′→3′)	Reference
Zika	ZIKVF2	782–801	CGCAAACCTGGTTGGAATCA	(*16*)
Zika	ZIKV 835	882–904	TTGGTCATGATACTGCTGATTGC	(*17*)
Zika	ZIKV 911c	958–937	CCTTCCACAAAGTCCCTATTGC	(*17*)
Zika	ZIKV 1086	1133–1149	CCGCTGCCCAACACAAG	(*17*)
Zika	ZIKV 1162c	1209–1186	CCACTAACGTTCTTTTGCAGACAT	(*17*)
Zika	ZIKVF3	1510–1530	GGAAGCCTAGGACTTGATTGT	(*16*)
Zika	ZIKVR2	1729–1709	CCACGACAGTTTGCCTTTTGG	(*16*)
Zika	ZIKVF4	2172–2193	CAGCACCATTGGAAAAGCATTT	(*16*)
Zika	ZIKVR3	2487–2466	CGAGCACCCCACATCAGCAGAG	(*16*)
Zika	ZIKVR4	2952–2928	GAACCCATGATCCTCCACAAGAAAG	(*16*)
Chikungunya	CHIK17F	8976–8997	GGAACTACCTTGCAGCACGTAC	(*18*)
Chikungunya	CHIKE1S1	10118–10139	ACATCACGTGCGAGTACA	(*19*)
Chikungunya	CHIK19F	10172–10191	GTACAGCAGAGTGTAAGGACCA	(*18*)
Chikungunya	F-CHIK	10366–10387	AAGCTYCGCGTCCTTTACCAAG	(*20*)
Chikungunya	R-CHIK	10574–10554	CCAAATTGTCCYGGTCTTCCT	(*20*)
Chikungunya	CHIK 18R	10989–10969	GGCGTTAGTCATCGAGTGCAC	(*18*)
Chikungunya	CHIKE1R1	11491–11470	TCTCTTAAGGGRCACATATACC	(*19*)
Chikungunya	CHIK 21F	10803–10824	GAACATGCCTATCTCCATCGAC	(*18*)

Appendix Table 4. Comparison of the detection of anti-Zika or anti-dengue virus IgG by microsphere immunoassay and seroneutralization assay in 69 paired samples from participants collected during September-November 2013 and October-November 2015 in the Central division in Fiji*

Year (no. samples tested)	MIA / NTA							
	ZIKV + / +	ZIKV - / -	ZIKV + / -	ZIKV - / +	DENV + / +†	DENV - / -	DENV + / -	DENV - / +†
2013 (N = 69)	6	51	9	3	46	17	3	3
2015 (N = 69)	60	1	8	0	63	4	0	2
Total (N = 138)	66	52	17	3	109	21	3	5

*MIA, microsphere immunoassay; NTA, Neutralization assay; ZIKV, Zika virus; DENV, dengue viruses; +, positive serologic result; -, negative serologic result.
†Neutralization of one or more serotypes of dengue virus.

Appendix Table 5. Prevalence of anti-Zika, chikungunya or dengue virus antibodies in the paired samples (N = 311) collected during September-November 2013 and October-November 2015 in the Central division in Fiji*

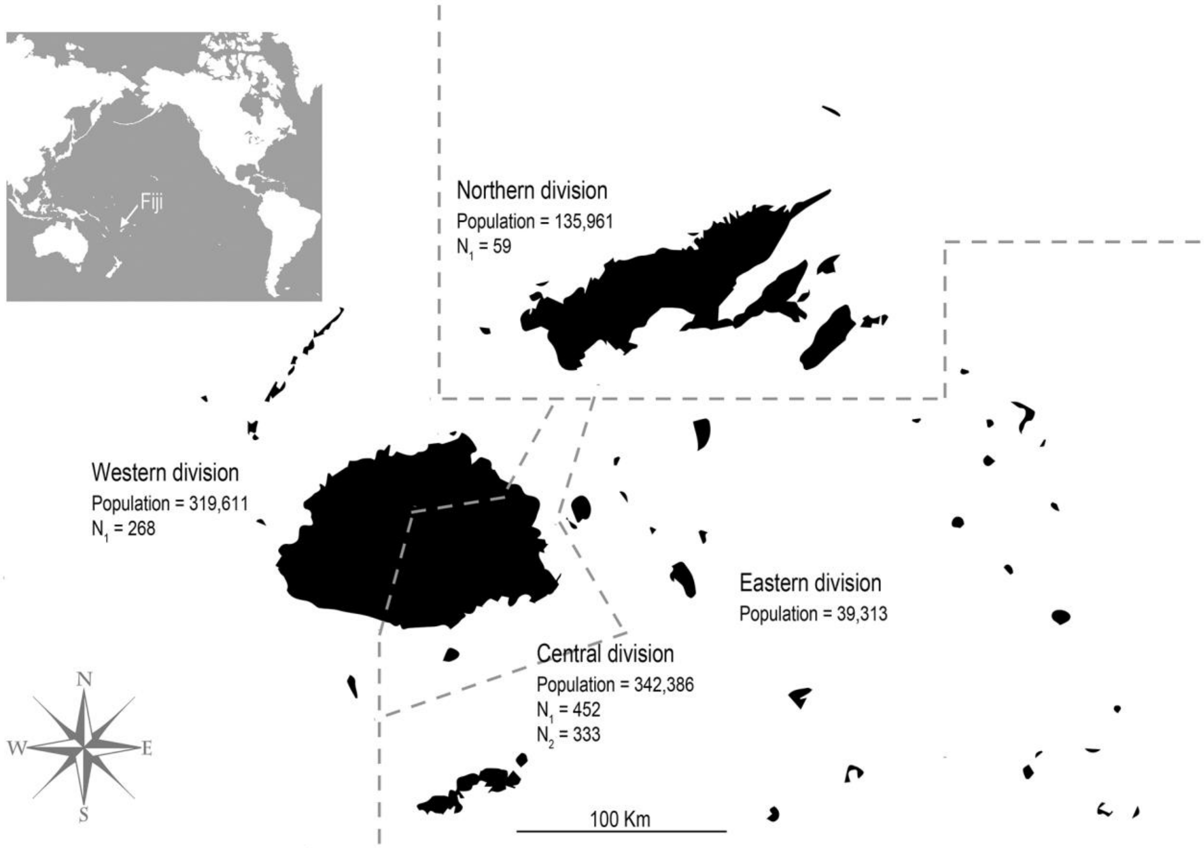
	No. seropositive / No. tested (% [95% CI])					
	ZIKV		CHIKV		DENV†	
Variable	2013	2015	2013	2015	2013	2015
Total	16/311 (5.1 [3–8.2])	69/311 (22.2 [17.7–27.2])	3/311 (1 [0.2–2.8])	3/311 (1 [0.2–2.8])	228/311 (73.3 [68–78.1])	256/311 (82.3 [77.6–86.4])
Age range (median)	2–78 (28)	4–80 (29)	2–78 (28)	4–80 (29)	2–78 (28)	4–80 (29)
Age groups						
0–19	10/121 (8.3 [4–14.7])	25/105 (23.8 [16–33.1])	2/121 (1.7 [0.2–5.8])	1/105 (1 [0–5.2])	63/121 (52.1 [42.8–61.2])	69/105 (65.7 [55.8–74.7])
20–39	3/91 (3.3 [0.7–9.3])	18/97 (18.6 [11.4–27.7])	0/91 (0 [0–4])	1/97 (1 [0–5.6])	73/91 (80.2 [70.6–87.8])	85/97 (87.6 [79.4–93.4])
40–59	1/65 (1.5 [0–8.3])	14/72 (19.4 [11.1–30.5])	1/65 (1.5 [0–8.3])	1/72 (1.4 [0–7.5])	60/65 (92.3 [83–97.5])	67/72 (93.1 [84.5–97.7])
60+	2/34 (5.9 [0.7–19.7])	12/37 (32.4 [18–49.8])	0/34 (0 [0–10.3])	0/37 (0 [0–9.5])	32/34 (94.1 [80.3–99.3])	35/37 (94.6 [81.8–99.3])
Gender						
Female	7/177 (4 [1.6–8])	39/177 (22 [16.2–28.9])	12/177 (6.8 [3.6–11.5])	2/177 (1.1 [0.1–4])	133/177 (75.1 [68.1–81.3])	152/177 (85.9 [79.9–90.6])
Male	9/134 (6.7 [3.1–12.4])	30/134 (22.4 [15.6–30.4])	1/134 (0.7 [0–4.1])	1/134 (0.7 [0–4.1])	95/134 (70.9 [62.4–78.4])	104/134 (77.6 [69.6–84.4])
Zone						
Periurban	4/65 (6.2 [1.7–15])	18/65 (27.7 [17.3–40.2])	0/65 (0 [0–5.5])	0/65 (0 [0–5.5])	53/65 (81.5 [70–90.1])	54/65 (83.1 [71.7–91.2])
Rural	4/110 (3.6 [1–9])	16/110 (14.5 [8.5–22.5])	1/110 (0.9 [0–5])	0/110 (0 [0–3.3])	70/110 (63.6 [53.9–72.6])	83/110 (75.5 [66.3–83.2])
Urban	8/136 (5.9 [2.6–11.3])	35/136 (25.7 [18.6–33.9])	2/136 (1.5 [0.2–5.2])	3/136 (2.2 [0.5–6.3])	105/136 (77.2 [69.2–84])	119/136 (87.5 [80.7–92.5])

*ZIKV, Zika virus; CHIKV, chikungunya virus; DENV, dengue viruses.
†Seropositivity for one or more serotypes of dengue virus.

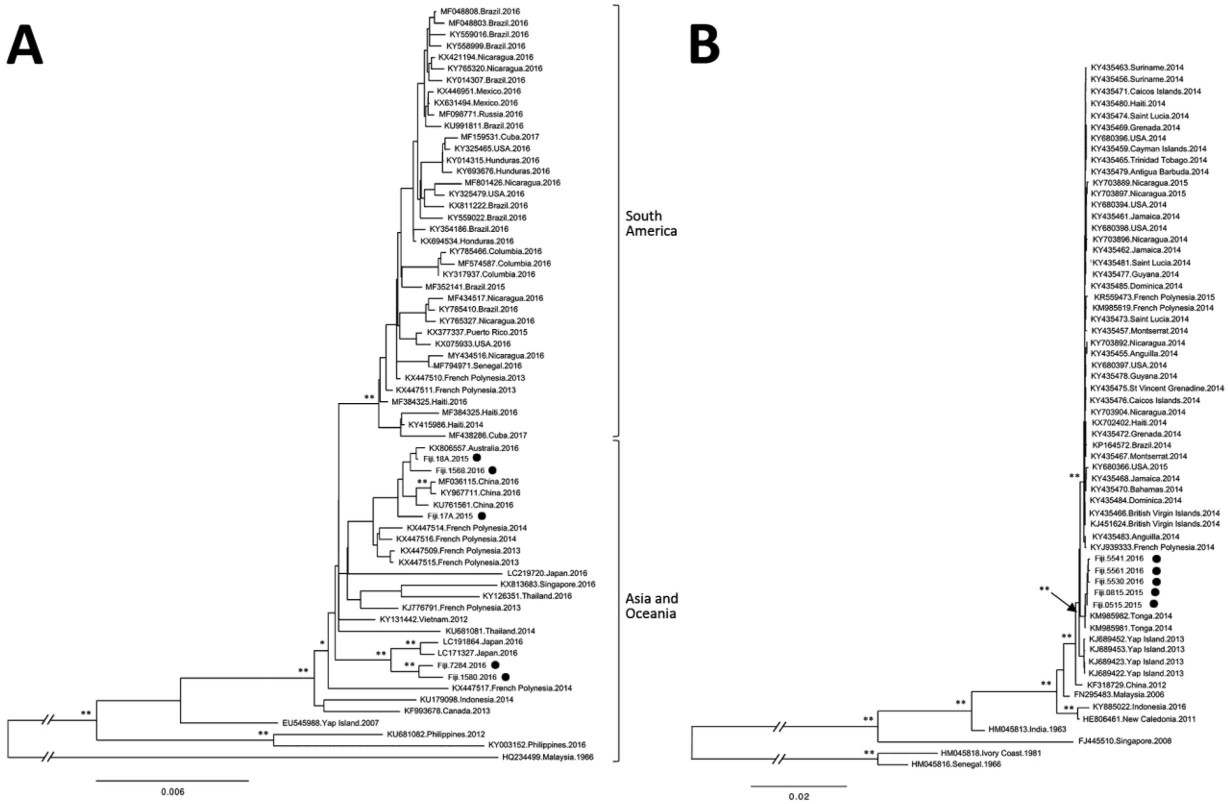
Appendix Table 6. Detection of IgG against Zika, chikungunya and dengue viruses detected by microsphere immunoassay in the paired samples from participants (N = 311) collected during September-November 2013 and October-November 2015 in the Central division in Fiji*.

	2015					
2013	ZIKV+	ZIKV-	CHIKV+	CHIKV-	DENV+†	DENV-
ZIKV+	14	2	–	–	–	–
ZIKV-	55	240	–	–	–	–
DENV+†	–	–	–	–	216	12
DENV-	–	–	–	–	40	43
CHIKV+	–	–	2	1	–	–
CHIKV-	–	–	1	307	–	–

*ZIKV, Zika virus; CHIKV, Chikungunya virus; DENV, dengue viruses.
†Seropositivity for one or more serotypes of dengue virus.



Appendix Figure 1. Geographic distribution of participants recruited among the general population of Fiji between September-November 2013 and October-November 2015. N1 and N2 indicate areas of recruitment in 2013 and 2015, respectively. The total population for each area is given. Gray dashed lines delineate the 4 divisions that comprise Fiji (Northern division, Central division, Western division, Eastern division). Inset Map at upper left shows location of Fiji in the Pacific Ocean (white arrow). Source: map downloaded from: https://www.shutterstock.com/fr/image-vector/vector-map-fiji-isolated-illustration-black-379408168



Appendix Figure 2. Phylogenetic relationship between viruses recovered in Fiji and those from other locations. (A) Zika virus E gene and (B) chikungunya virus E1 gene. Analyses were performed using the GTR model of nucleotide substitution with gamma-distributed rate heterogeneity, a lognormal relaxed molecular clock model, and the Bayesian Skyline coalescent tree prior. A mean substitution rate prior of 4x10–4 substitutions per site per year was used for both datasets. The MCMC chains were run for 100 million generations, with 2–4 runs performed for each file. Convergence of the estimates was considered satisfactory when the effective sample size (ESS) calculated in Tracer v1.6.0 (available at: http://tree.bio.ed.ac.uk/software/tracer/) was >200. BEAST log and tree files obtained for a given dataset were combined with LogCombiner v1.8.2, using a burn-in of 10%. Maximum clade credibility trees (MCCT) were summarized using TreeAnnotator v1.8.2, from the BEAST package, keeping the median height over the posterior distribution of trees. MCCT were visualized and edited with FigTree v1.4.2. (available at: http://tree.bio.ed.ac.uk/software/figtree/). Strains collected in Fiji in 2015 and 2016 are tagged with a black circle. Branches with a posterior probability >0.70 or >0.99 are labeled with one (*) or two (**) asterisks, respectively. Each strain is labeled by GenBank accession number/Country/Collection date. Bars indicate the number of nucleotide substitution per site.