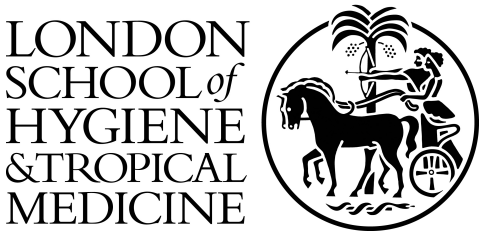

Mathematical Modelling of Arbovirus Outbreak Dynamics in Fiji and the Wider Pacific

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Declaration

Statement of Own Work

I, Alasdair Henderson, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, this has been indicated in the thesis. I have read and understood the School's definition of plagiarism and cheating given in the Research Degrees Handbook.

Alasdair Henderson,

November 2020

Abstract

Diseases spread by the *Aedes* genus of mosquitoes are some of the fastest growing and fastest spreading viral pathogens in the world. Large dengue virus (DENV) epidemics have emerged in Fiji since the Second World War and there was widespread transmission of Zika virus (ZIKV) throughout the Pacific between 2013 and 2017. However, very few ZIKV cases were confirmed in Fiji. I conducted a serological survey in Fiji in 2017 and used these serological data, combined with mathematical modelling, to analyse transmission dynamics of arboviruses in Fiji.

I found evidence for ZIKV circulation in Fiji between 2013 and 2015 followed by low ZIKV seroprevalence in 2017. I used paired serum samples to analyse the immunological response to ZIKV following outbreaks in Fiji and French Polynesia and found that neutralising antibodies declined in adults within two years of the outbreak in each country. I combined serological data with surveillance and molecular data to model unobserved ZIKV transmission and compare ZIKV and DENV transmission dynamics in Fiji. I found that the introduction time of a virus in Fiji could explain different transmission dynamics and concluded that ZIKV was likely introduced to Fiji in late 2014.

I found high seroprevalence for all four DENV serotypes. I used a mathematical model of DENV transmission to analyse a DENV outbreak in 2017 to predict the duration and peak of the outbreak in real-time. I found that jointly fitting the model to a historic outbreak as well as the emerging outbreak improved the accuracy of the predictions from the model.

Overall, I combined multiple data sources with mathematical modelling to reveal a diverse range of outbreak dynamics and serological responses to outbreaks of closely related viruses in the same location. I found that ZIKV and DENV do not necessarily generate a similar immune response in the same population and that both can cause low level multi-year outbreaks as well as large single season epidemics. Despite these challenges, mathematical modelling can improve our understanding of arbovirus outbreak dynamics such that it is possible to accurately forecast outbreak dynamics in real-time.

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Thank you Dad.

Acronyms

ADE Anitbody dependent enhancement

AFR Acute fever and rash

BEAST Bayesian evolutionary analysis sampling trees

BEAUti Bayesian evolutionary analysis utility

CHIKV Chikungunya virus

CI Confidence interval

CrI Credible interval

DENV Dengue virus

DHF Dengue hemorrhagic fever

DIC Deviance information criterion

DLI Dengue-like illness

EIP Extrinsic incubation period

ELISA Enzyme linked immunosorbent assay

ESS Effective sample size

FNHRERC Fiji National Health Research and Ethics Committee

GAM Generalised additive model

GBS Guillain-Barré syndrome

IgG Immunoglobulin class G

IgM Immunoglobulin class M

IIP Intrinsic incubation period

ILM Institut Louis Malardé

IVM Integrated vector management

LSHTM London School of Hygiene & Tropical Medicine

MCMC Markov chain Monte Carlo

MIA Microsphere immunoassay

MOH Ministry of Health (Fiji)

NABs Neutralising antibodies

NB Negative binomial

NS1 Non-structural protein 1

NTCOPD National Taskforce for the Control of Outbreak Prone Diseases

PICTs Pacific island countries & territories

PPHSN Pacific Public Health Surveillance Network

PRNT Plaque reduction neutralisation test

PSSS The Pacific Syndromic Surveillance System

R_0 Basic reproduction number

RRV Ross River virus

R_t Effective reproduction number

RT-PCR Reverse transcription polymerase chain reaction

SEIR Susceptible, pre-infectious, infectious, recovered (model)

SIR Susceptible, infectious, recovered (model)

tMRCA Time to most common recent ancestor

WHO World Health Organisation

ZIKV Zika virus

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Chapter 1

Introduction

1.1 Background

Diseases spread by the *Aedes* genus of mosquitoes are responsible for millions of viral infections globally (*James et al.*, 2018). This doctoral thesis focuses on dengue virus (DENV) and Zika virus (ZIKV) which are two viruses that share the same primary vector of transmission, the *Aedes aegypti* mosquito. The public health burden of DENV and ZIKV is increasing annually as temperatures increase and the viable range of *Aedes* mosquitoes expands (*Kraemer et al.*, 2019). The devastating impact of these arboviruses means that more needs to be understood about how these viruses transmit.

This PhD thesis investigates the transmission dynamics of DENV, ZIKV and other related arboviruses in Fiji and the wider Pacific. This thesis describes a population-representative longitudinal serological data collection study I led in Fiji in 2017. These data have been used to understand serological dynamics following arbovirus outbreaks and, combined with mathematical modelling, I have developed transmission dynamic models for ZIKV and DENV in Fiji. The overall aims of this research are (i) to improve understanding of population immune dynamics following arbovirus outbreaks and (ii) identify the determinants of arbovirus transmission dynamics in island outbreaks. This introduction will introduce the three main topics in the thesis title in turn: 'arbovirus outbreaks', 'Fiji and the wider Pacific' and 'mathematical modelling'.

1.1.1 Dengue virus and Zika virus outbreaks

DENV and ZIKV are two closely related members of the virus family *Flaviviridae*, genus flavivirus. Both are arboviruses, a contraction of arthropod-borne viruses, and therefore exist in nature because of transmission between a susceptible vertebrate host – in this case, humans – and a hematophagous arthropod – in this case, mosquitoes (*Gubler*, 1998; *Musso and Gubler*, 2016).

ZIKV is a positive-sense, single-stranded RNA virus that can cause mild, acute febrile illness in humans (*Musso and Gubler*, 2016). The flavivirus ZIKV was first isolated in a sentinel *Rhesus* Macaque in the Zika forest, near the East African Virus Research

Institute in Entebbe, Uganda in 1947 (*Dick*, 1952). The first phylogenetic study of ZIKV based on the full genome found two distinct lineages, African and Asian. *Haddow et al.* (2012), found evidence that the ZIKV that caused outbreaks in the Pacific was the Asian lineage.

DENV is another flavivirus and has four antigenically distinct serotypes. DENV-1 was first isolated in 1943 in Japan (*Hotta*, 1952) and 1945 in Calcutta (*Sabin and Walter Schlesinger*, 1945), and the first full sequence was published in 1987 (*Mason et al.*, 1987). DENV-2 was first isolated in Trinidad in 1954 (*Anderson et al.*, 1956) and the full sequence published in 1988 (*Hahn et al.*, 1988). DENV-3 was isolated and sequenced in 1963 and 1988 respectively (*Osatomi et al.*, 1988; *Russell et al.*, 1966). Finally, DENV-4 was isolated in 1981 and sequenced in 1987 (*Lanciotti et al.*, 1997; *Mackow et al.*, 1987). There is considerable genetic variation between these serotypes. The genomes of the four DENV serotypes share approximately 60% sequence identity (*Blok*, 1985; *Holmes and Burch*, 2000; *Yap et al.*, 2007). Even within serotypes, distinct genotypes have been identified (*Rico-Hesse*, 1990) and the serotypes are not antigenically homogeneous (*Katzelnick et al.*, 2015). Despite this genetic variation the disease and clinical symptoms caused by infection with any serotype are consistent.

Virus infection and vectors

The primary route of transmission of ZIKV and DENV is through the bite of an infected female *Aedes* mosquitoes but other transmission routes are possible (*Epelboin et al.*, 2017). Other mosquito species, including from genera *Anopheles* and *Mansonia*, carried detectable ZIKV in Senegal in 2011 (*Diallo et al.*, 2014). Similarly, DENV is transmitted to humans by the *Aedes* genus of mosquitoes (*Halstead*, 2008; *Simmons et al.*, 2012).

The main mosquito species for ZIKV and DENV transmission in Fiji and the wider Pacific is *Aedes aegypti* because it is an urban-adapted mosquito and thrives in close proximity to humans (*Gubler*, 1998). *Aedes aegypti* are day-biting mosquitoes and propagate when the female mosquito lays eggs in standing water, often near human-inhabited dwellings (*Gubler*, 1987; *Kraemer et al.*, 2015). Transmission of ZIKV and

DENV is almost always seasonal and is affected by temperature and rainfall. Transmission increases in warmer and wetter seasons (*Halstead*, 2008). Notable effects of increased temperature are the shortening of the extrinsic incubation period and increased biting rate (*Mordecai et al.*, 2017). *Aedes aegypti* mosquitoes are the most efficient arbovirus vector in Fiji. However, there are 26 known species of mosquitoes in Fiji of which several are recognised as vectors of DENV including *Aedes albopictus*, *Aedes polynesiensis* and *Aedes pseudoscutellaris* (*Prakash et al.*, 2001).

ZIKV virus can also persist in semen and there are reports of sexual transmission of ZIKV (*Foy et al.*, 2011; *Musso et al.*, 2015a). There is also evidence of perinatal transmission of ZIKV from mother to child and through transfusion of infected blood (*Musso et al.*, 2014). The evidence on transmission through breastfeeding is not conclusive (*Colt et al.*, 2016). Mosquito-borne transmission remains the primary route of transmission (*Althaus and Low*, 2016; *Yakob et al.*, 2016).

Disease burden and epidemiology

Between 1947 and 2007 there were only 14 documented cases of ZIKV infection in humans. The first ZIKV epidemic was recorded in the Yap islands of the Federated States of Micronesia in 2007 (*Duffy et al.*, 2009) and outbreaks followed across the Pacific from 2013 (*Baud et al.*, 2017; *Musso and Gubler*, 2016) then the Americas where over 750,000 cases were confirmed or suspected (*Pan American Health Organization*, 2018b).

DENV virus outbreaks have a longer recorded history and are ubiquitous in tropical regions where *Aedes* species of mosquitoes thrive. An estimated 2.5 billion people live in DENV endemic countries (*World Health Organisation*, 2009). DENV-like epidemics have been recorded for centuries (*Henchal and Putnak*, 1990) and DENV incidence has increased 30-fold since the middle of the 20th century (*World Health Organisation*, 2009). This rapid increase in transmission is attributed to a combination of increasing urbanisation within tropical countries, increased travel between countries and ineffective control strategies (*Simmons et al.*, 2012). Another key factor in the increase of DENV

incidence is the expanding geographical range of *Aedes* mosquitoes because of increasing global temperatures, attributed to climate change (*Messina et al.*, 2014).

In 2009, the World Health Organisation (WHO) reported an estimated annual incidence of DENV of 50-100 million (*Rigau-Pérez et al.*, 1998; *World Health Organisation*, 2009). Over the past decade, methods to estimate and map the annual incidence of DENV have improved. *Bhatt et al.* (2013), estimated that there were 390 million DENV infections per year in 2013. This estimate was based on risk maps which have since been updated with global climate projections (*Messina et al.*, 2019). The authors estimate that, in 2015, approximately half the world's population lived in areas that are environmentally suitable for DENV transmission and by 2080 this will increase to 62% of the world's population.

Clinical presentation and complications

The first clinical description of ZIKV is from a human challenge study conducted in 1956 and the patient reported a fever and slight headache (*McFadzean and Tsang*, 1956). During the first ZIKV epidemics in Yap and French Polynesia the most common clinical symptoms reported were fever, rash, arthritis and/or arthralgia and/or myalgia, conjunctivitis and fatigue (*Musso and Gubler*, 2016).

Analysis of blood samples after the French Polynesia outbreak estimated that 50-66% of the population experienced a ZIKV infection (*Aubry et al.*, 2015). During the outbreak an estimated 11.5% of the population reported with symptoms (*Musso et al.*, 2014), which suggests that approximately 10-20% of ZIKV infections were symptomatic during this outbreak. The proportion of asymptomatic infections is likely to be highly dependent on setting due to the variety of laboratory tests used for case definition and cross-reactivity in serum samples from variable levels of other circulating flaviviruses. A systematic review of the prevalence of asymptomatic ZIKV infections found that a pooled estimate would not be robust due to the large heterogeneity in estimates, which ranged from 29% to 82% (*Haby et al.*, 2018). Causes for this heterogeneity could include the representativeness of sampled individuals, different case definitions and different age

structures in studied populations (*Burger-Calderon et al.*, 2020; *Haby et al.*, 2018).

ZIKV can also cause severe neurological and neonatal complications. Microcephaly is a severe congenital malformation in neonates where the head circumference is smaller than normal because the brain has not developed properly. In Brazil there was evidence that an increase in microcephaly cases was causally associated with ZIKV (*Schuler-Faccini et al.*, 2016) and a study of babies born during the ZIKV epidemic found that 14% had severe developmental problems (*Elisabeth Lopes Moreira et al.*, 2018). In French Polynesia, ZIKV infection in the first trimester dramatically increased the rate of microcephaly cases (*Cauchemez et al.*, 2016). Another neurological complication potentially associated with ZIKV is Guillain-Barré syndrome (GBS), an autoimmune disease causing acute, or subacute flaccid paralysis. No clear etiologies were found for a cluster of nine GBS cases in Fiji in 2014 (*Pastula et al.*, 2016). However, a larger cluster of 42 GBS cases during the French Polynesia outbreak was associated with the circulating ZIKV (*Cao-Lormeau et al.*, 2016). A systematic review of evidence was conducted by *Krauer et al.* (2017), and concluded that ZIKV is a cause of congenital brain abnormalities including microcephaly and that ZIKV is a trigger for, but insufficient to cause, GBS.

DENV infection leads to a wide range of clinical presentations. The majority of DENV infections are subclinical (*Gubler*, 1998) and 300 million of the estimated 390 million annual infections are subclinical or mildly symptomatic (*Bhatt et al.*, 2013). There is strong evidence that human hosts with asymptomatic infection can still transmit DENV to mosquitoes (*Duong et al.*, 2015).

In those that become symptomatic, these symptoms follow an incubation period of 3-7 days. Symptom onset after the incubation period is acute, typically a fever accompanied by headache (*Henchal and Putnak*, 1990; *Rigau-Pérez et al.*, 1998). A generalised muscular rash may be seen during the first 1-2 days of fever, followed by anorexia, nausea and vomiting and the fever typically persists for 4-6 days (*Henchal and Putnak*, 1990). A small proportion of patients will progress to a critical phase that results in the severe form of the disease, dengue hemorrhagic fever (DHF) (*Gubler*, 1998; *Rigau-Pérez et al.*, 1998; *Stephenson*, 2005).

Non-pharmaceutical interventions

The most common method to attempt to reduce the burden of these arboviruses is through management of the main vector, *Aedes* mosquitoes. The increase of DENV transmission and the emergence of a ZIKV pandemic is evidence that current control strategies are insufficient to slow the spread of these arboviruses. However, they remain the most effective means of control until a vaccine becomes available. Integrated vector management (IVM) is "a rational decision-making process for the optimal use of resources for vector control" and recommended by WHO (*Pan American Health Organization*, 2018a; *World Health Organisation*, 2009). Strategies for controlling the *Aedes aegypti* population can be broadly split into three categories: strategies to stop mosquito propagation, strategies to kill adult mosquitoes and strategies to change individual human behaviour and risk of exposure.

Environmental management is a principal strategy to stop mosquito propagation through the elimination of non-essential containers that provide larval habitats for *Aedes* mosquitoes (*Buhler et al.*, 2019). Long-term strategies may include improved sanitation and water access, which would remove the need for water tanks at dwellings. In the short-term it is often the physical removal of breeding sites and collection of waste (*Vanlerberghe et al.*, 2009). Another strategy to stop mosquito propagation is larviciding, chemical treatment of potential breeding sites to kill mosquito larvae (*Kroeger et al.*, 2006). The WHO instructions state that this is to be considered complimentary to environmental management, not a primary means of reducing mosquito propagation.

A second technique for reducing mosquito density is adulticides, which are insecticides that are targeted to kill adult mosquitoes. These can be applied as either residual surface treatments or as space treatments, fogging areas with adulticide to reduce adult mosquito density. Residual surface application is challenging with *Aedes aegypti* since these female mosquitoes lay a small number of eggs at multiple cites (*Reiter*, 2007, 2016). Space spraying is used in emergency situations and is designed to rapidly and significantly reduce the adult mosquito population to stop the spread of a virus (*Esu et al.*, 2010).

Finally, the interactions between humans and mosquitoes can be minimised through guidance and individual behavioural change (*Parks and Lloyd*, 2004). In response to the emergence of ZIKV in the Americas, the Pan-American Health Organisation outlined "personal prevention measures" to be promoted alongside IVM (*Pan American Health Organization*, 2018a). These included sleeping under mosquito nets, wearing long sleeves and application of repellents containing DEET, IR3535 or Icaridin (*Pan American Health Organization*, 2018a).

The evidence for the effectiveness of IVM and personal prevention measures in reducing arbovirus transmission is sparse and weak. *Buhler et al.* (2019), reviewed studies on environmental management and found some evidence of effectiveness in reducing larval and pupal densities of *Aedes* mosquitoes. *Bowman et al.* (2016), conducted a systematic review of studies that evaluated vector control against *Aedes aegypti* or *Aedes albopictus* for a period of at least three months. The authors concluded that their review and analysis "demonstrate the remarkable paucity of reliable evidence for the effectiveness of any dengue vector control method". *Heintze et al.* (2007), similarly concluded that "evidence that community-based dengue control programmes alone and in combination with other control activities can enhance the effectiveness of dengue control programmes is weak". *Reiter* (2016), stated that "there are very few published studies and even fewer studies that formally assess the impact of existing insecticide-based strategies on dengue". Studies have even shown that vector control can create a false sense of security that exacerbates transmission (*Bouzid et al.*, 2016).

Investigating the impact of vector control on epidemics is challenging because outbreaks can end due to a combination of several factors, including population immunity, seasonal factors and human movement. Until the point that future pharmaceutical interventions prove effective, and several are being developed (*Pang et al.*, 2017; *Yakob et al.*, 2016), the effectiveness of vector control in the prevention of DENV, ZIKV and related arbovirus transmission remains a major gap in our understanding.

Finally, a novel non-pharmaceutical intervention against arbovirus transmission is the use of *Wolbachia* in mosquitoes which blocks the transmission of many important human pathogens. There is research that shows introduction of *Wolbachia* in a mosquito

population reduces R_0 by 66-75% (*Ferguson et al.*, 2015). *Ndii et al.* (2015), show that the effect of *Wolbachia* on DENV transmission can be via multiple routes such as a reduction in lifespan and cytoplasmic incompatibility which gives *Wolbachia* carrying females a competitive advantage in mating. Research also shows that this intervention is effective against ZIKV transmission (*Dutra et al.*, 2016). Introduction of *Wolbachia* needs to be combined with a broader integrated vector control programme for their release to be effective (*Yakob et al.*, 2017).

Pharmaceutical interventions

There are no specific anti-viral treatments available for either DENV or ZIKV. There is one licensed vaccine available for DENV, a recombinant, live-attenuated DENV vaccine (*Dengvaxia*) that was approved in several countries in 2016. However, due to the complexity in DENV pathogenicity, this vaccine is only recommended in specific settings (*Ferguson et al.*, 2016b; *Flasche et al.*, 2016) and is not distributed in Fiji or other Pacific countries studied in this doctoral project. There is no vaccine currently available for ZIKV.

1.1.2 Diagnosis, serology and cross-reaction of DENV and ZIKV

Laboratory diagnosis of DENV or ZIKV can be performed either directly or indirectly. Direct methods involve detection of viral components in serum. Indirect methods involve detection of short-term immunoglobulin class M (IgM) or long-term immunoglobulin class G (IgG) antibodies against the virus. The choice of method depends on the duration of the illness in the patient with the suspected infection and affects the sensitivity of the diagnosis (*Peeling et al.*, 2010) (Figure 1.1). In the case of DENV, during the febrile phase of infection viral nucleic acid can be detected by means of reverse transcription polymerase chain reaction (RT-PCR). After the onset of illness, the virus can be detected in serum, plasma, circulating blood cells and other tissues for 4–5 days (*Gubler*, 1998; *World Health Organisation*, 2009). Alternatively, detection of the virus-expressed soluble nonstructural protein 1 (NS1) by means of enzyme-linked

immunosorbent assay (ELISA) or the lateral-flow rapid test is sufficient to confirm a diagnosis of DENV (*Simmons et al.*, 2012). The RT-PCR is very sensitive but NS1 detection by ELISA is less so with approximately 60 to 80% sensitivity in secondary infections, but sensitivity exceeds 90% in primary infections (*Simmons et al.*, 2012).

Diagnosis of ZIKV is also ideally performed with detection of viral nucleic acid by RT-PCR. A rapid NS1 detection diagnostic test is not currently available (*Musso and Gubler*, 2016). ZIKV is also detectable in saliva samples and filter papers spotted with dried blood that can be shipped to reference laboratories if local facilities cannot perform these diagnostic tests, as has been done in the Pacific (*Musso and Gubler*, 2016; *Musso et al.*, 2015b).

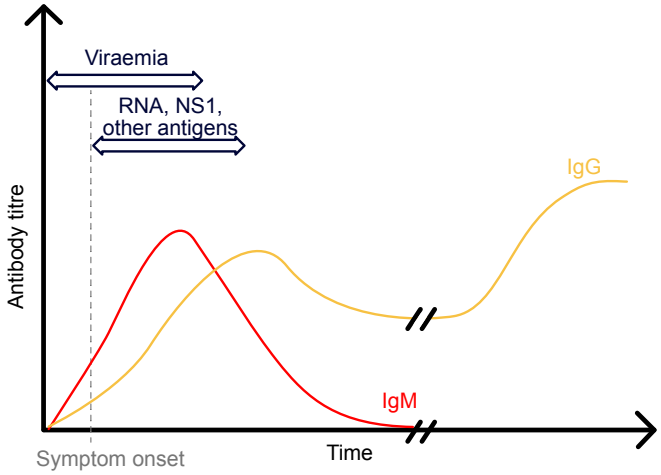

Figure 1.1: *Antibody dynamics following suspected ZIKV or DENV infection (adapted from Peeling et al. (2010)). The earliest opportunity to detect DENV is through reverse transcription polymerase chain reaction to detect viral nucleic acid during the febrile phase of infection. Non-structural protein 1 (NS1) antigen and short-term immunoglobulin class M (IgM) antibodies are detectable with serology shortly after. Long-term antibodies immunoglobulin class G (IgG) antibodies are detectable for the rest of the patient's life*

At the end of the acute phase of infection, serology is the method of choice for diagnosis of DENV (*Gubler*, 1998; *Innis et al.*, 1989; *Peeling et al.*, 2010). Antibody response to infection is not consistent and depends on the immune status of the host including previous flavivirus infections and vaccinations against tick-borne encephalitis virus (TBEV), Japanese encephalitis virus (JEV) and yellow fever virus (YFV) (*Mansfield*

et al., 2011). IgM are the first antibodies to appear following DENV infection and are detectable in 50% of patients by days 3-5 after onset of illness, increasing to 80% by day 5 and 99% by day 10 (*Innis et al.*, 1989; *World Health Organisation*, 2009).

Serological diagnosis can be used for ZIKV as well, as was done during the 2007 Yap outbreak with IgG and IgM ZIKV ELISA. A post-outbreak review of diagnostic testing for ZIKV found that IgM antibodies to ZIKV were detectable approximately 1 week following infection. In over 80% of individuals however, IgM antibodies were still detectable more than 2 months following infection. Using detection of IgM antibodies as evidence of recent ZIKV infection could therefore be misleading since the individual could have been infected several months prior to sample collection (*Theel and Jane Hata*, 2018).

In Fiji, DENV outbreaks are confirmed through non-structural protein 1 (NS1) antigen detection or IgM ELISA. This laboratory reporting switches to clinical-based reporting during outbreaks when laboratory capacity is exceeded (*Kucharski et al.*, 2018). Clinical-based reporting relies on a standard case definition for DENV. Clinical cases are defined as suspected cases if they presented to health practitioners with rash and/or mild fever and at least two of the following signs: conjunctivitis, arthralgia, or oedema (*Kucharski et al.*, 2018). ZIKV diagnosis of suspected cases in Fiji are performed at Institut Louis Malardé in French Polynesia. Serum samples blotted on filter paper cards and saliva samples collected on dry oral swabs from suspected cases are tested by real-time RT-PCR for the presence of RNA from the four DENV serotypes, ZIKV and chikungunya virus (CHIKV) (*Aubry et al.*, 2012; *Kama et al.*, 2019).

The consequences of cross-reaction between DENV and ZIKV

Cross-reactivity is the reaction between an antibody raised against one virus recognising another virus. Cross-reactivity between flaviviruses has been well documented (*Calisher et al.*, 1989; *Mansfield et al.*, 2011; *Scott et al.*, 1983). The window to diagnose flavivirus infection through detection of viraemia is short and is often missed so serological diagnosis is necessary. However, differential classification in serological diagnosis is more

challenging because of cross-reactivity (*Kerkhof et al.*, 2020). The main problem when using serological detection – for either recent or historical flavivirus infection – is that it increases the probability of a false positive result (*Hirota et al.*, 2010). For example, vaccination for another flavivirus, yellow fever virus, can result in false DENV positive results by enzyme ELISA (*Houghton-Triviño et al.*, 2008). It was apparent following the ZIKV outbreak in Yap island in 2007 that cross-reaction could complicate ZIKV diagnostics (*Duffy et al.*, 2009; *Lanciotti et al.*, 2008). Antibodies generated following DENV and ZIKV infections are highly cross-reactive (*Balmaseda et al.*, 2017; *Musso and Gubler*, 2016; *Priyamvada et al.*, 2016; *Speer and Pierson*, 2016; *Van Meer et al.*, 2017), which can affect results from commercially available test kits (*Felix et al.*, 2017; *Kikuti et al.*, 2018). In the absence of a perfect diagnostic test, serological studies in settings where flaviviruses co-circulate must be accompanied with sensitivity analyses to validate findings from any one assay because of this cross-reactivity.

Cross-reactivity can complicate serological testing, but it can also lead to complex immune responses to repeat infections. There is evidence that previous infection with a DENV serotype or ZIKV can both suppress and enhance subsequent infection with a heterologous DENV serotype or ZIKV. I will introduce the evidence of the impact of cross-reactivity as follows: the effect of prior DENV infection on subsequent heterotypic DENV infection, prior DENV infection on subsequent ZIKV infection, finally prior ZIKV infection on subsequent DENV infection.

Human infection with one DENV serotype confers long-term immunity to that serotype (*Halstead*, 1974; *Simmons et al.*, 2012). It is well established that infection with a single DENV serotype confers a strong cross-protective immunity against heterologous serotypes but that this protection is temporary (months) (*Montoya et al.*, 2013; *Reich et al.*, 2013; *Sabin*, 1952; *Snow et al.*, 2014). *Clapham et al.* (2016), found that antibody titres increased from convalescence to 6 months following DENV infection. After this period of cross-protection prior DENV infection may be a significant risk factor for more severe disease from a secondary heterotypic DENV infection (*Burke et al.*, 1988; *Cummings et al.*, 2005; *Dejnirattisai et al.*, 2010; *Halstead et al.*, 1970; *Kliks et al.*, 1988; *Simmons et al.*, 2012). This phenomenon – antibody-dependent enhancement

(ADE) – is one in which pre-existing DENV antibodies bind to the virus particles but are non-neutralising and subsequently enhance the growth of the virus. *Salje et al.* (2018), characterised antibody dynamics in a cohort in Thailand and found that one year after infection individuals with moderate antibody titres ($\leq$ 1:40) were at much greater risk of the more severe dengue hemorrhagic fever than those with high titres (>1:40) or DENV naïve individuals. Post-secondary infections are rarely reported and appear to reduce, but not eliminate, the risk of disease (*Gibbons et al.*, 2007; *Olkowski et al.*, 2013; *Wikramaratna et al.*, 2010).

Secondly, prior DENV infection could affect future ZIKV infection. ZIKV outbreaks spread rapidly from 2013 in the Pacific and in the Americas which are both areas with a high burden of DENV infection. Urgent research was therefore necessary to assess whether prior DENV immunity would enhance the severity of ZIKV disease as it could for heterotypic DENV infections. Experimental evidence demonstrated enhanced ZIKV infection in the presence of DENV antibodies (*Bardina et al.*, 2017; *Dejnirattisai et al.*, 2016; *Paul et al.*, 2016; *Priyamvada et al.*, 2016). However, other *in vivo* experiments have shown no evidence that prior flavivirus immunity had a detrimental effect on ZIKV infection (*Castanha et al.*, 2016; *McCracken et al.*, 2017; *Pantoja et al.*, 2017). Conversely, experimental evidence that a previous DENV infection can cross-neutralise and protect from ZIKV infection has been supported in studies of longitudinal seroepidemiological cohorts. In Salvador, northeast Brazil, a cohort of 1436 urban residents were followed and pre-existing high antibody titres to DENV were associated with reduced risk of ZIKV infection and symptoms (*Rodriguez-Barraquer et al.*, 2019). Within a large paediatric cohort in Nicaragua, prior DENV infection was protective against ZIKV (*Gordon et al.*, 2019). *Montoya et al.* (2018), analysed antibody dynamics in this cohort with others from Latin America and Asia. The authors found that neutralising antibody titres can distinguish ZIKV from the DENV serocomplex and did not enhance ZIKV infection. Evidence from Brazil shows that multitypic DENV infection may protect from development of more severe ZIKV disease (*Pedroso et al.*, 2019). These results support a large body of experimental evidence (*Barba-Spaeth et al.*, 2016; *Swanstrom et al.*, 2016; *Wen et al.*, 2017). The duration of cross-neutralising antibodies from DENV infection is less certain. It has also been shown *in vitro* that cross-neutralising

antibodies are not present in the late DENV convalescent stage of infection ($\geq$ 6 months after infection) (*Collins et al.*, 2017).

There is less evidence about the effect of ZIKV on subsequent DENV infection because the ZIKV epidemic was so recent. Models in nonhuman primates (NHP) have demonstrated that cross-reactive antibodies generated from ZIKV infection enhanced a subsequent DENV infection (*George et al.*, 2017; *Valiant et al.*, 2018). However, another NHP model showed no evidence of enhanced DENV infection following ZIKV (*Pérez-Guzmán et al.*, 2019). It has been hypothesised that the DENV season following the ZIKV epidemic in many locations across the Americas was less severe because of protection from ZIKV (*Borchering et al.*, 2019; *Perez et al.*, 2019; *Ribeiro et al.*, 2018).

Cross-reactivity between DENV and ZIKV is unquestionably a limitation in serological studies. However, the impact it has on outbreak dynamics when both viruses can circulate in the same location at the same time is less clear and needs further study (*Culshaw et al.*, 2017; *Langerak et al.*, 2019). Fiji and the wider Pacific are locations where such studies are appropriate because of the presence of *Aedes aegypti* mosquitoes on many islands which can transmit both DENV and ZIKV.

1.1.3 Fiji and the wider Pacific

This doctoral project analysed data from two countries from the South Pacific: Fiji and French Polynesia. Fiji is an island country in the South Pacific, situated approximately 2,000 kilometres northeast of New Zealand. The country is made up of over 330 islands, approximately one third of which are permanently inhabited. It is an economically developing country and is classified as "upper middle income" by the World Bank (*World Health Organisation Western Pacific Region*, 2011).

There are two major islands in Fiji in terms of population and economic activity. Vanua Levu, in the Northern Division, and Viti Levu which is split between the Central and Western Divisions (Figure 1.2). Approximately 80% of the Fijian population of 884,887 people live in Central and Western Division. The capital city, Suva, is in the southeast

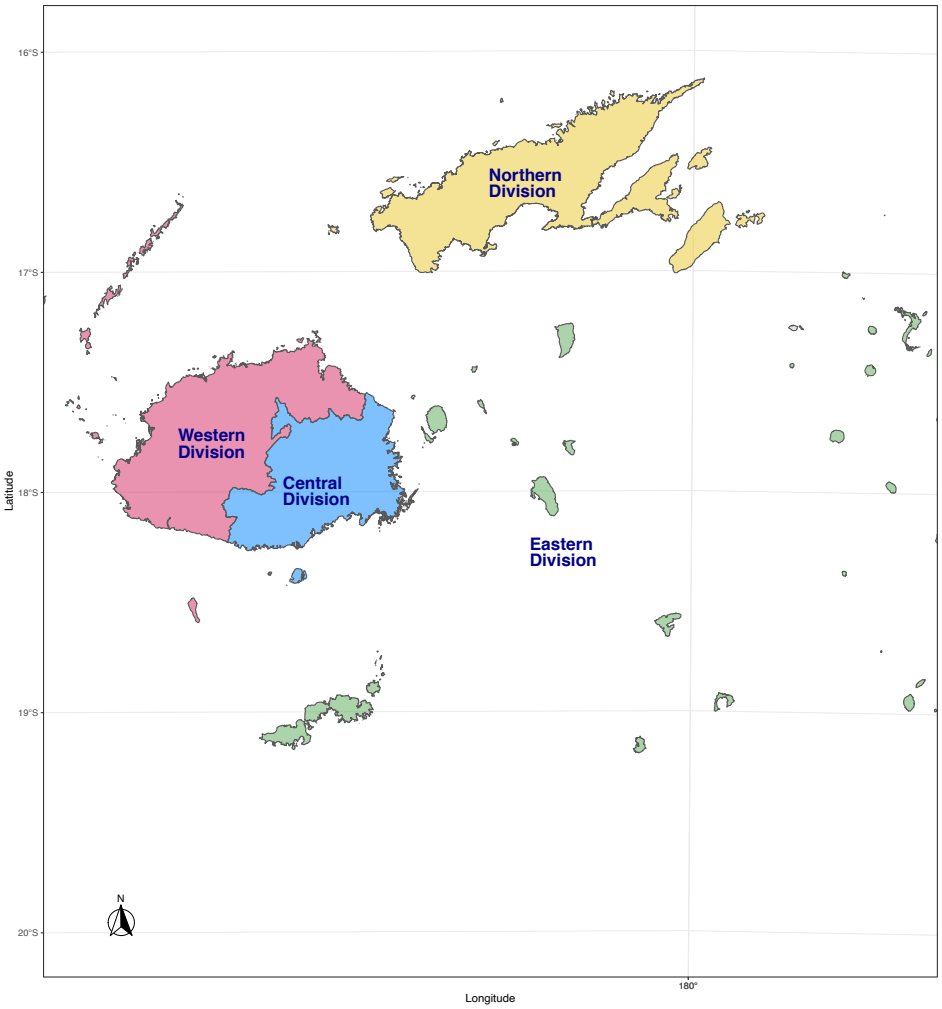

Figure 1.2: *Map of Divisions within Fiji*

corner of Viti Levu and is home to 94,088 Fijians (*Fiji Bureau of Statistics*, 2018a).

French Polynesia is the other country with data presented in this doctoral project. It is of particular interest because, like Fiji, it has a long history of DENV epidemics (*Teissier et al.*, 2020) and a recent ZIKV epidemic (*Cao-Lormeau et al.*, 2014b). French Polynesia is to the east of Fiji and the other side of the international date line. French Polynesia is a collection of 118 dispersed islands split into five archipelagos. The largest of these are the Society Islands, including French Polynesia's largest island Tahiti and capital city Pape'ete. The population of French Polynesia recorded in 2017 is smaller than Fiji at 281,764 (*United Nations*, 2019).

Age has been shown to be an important risk factor for the severity of DENV clinical presentation since older people are more likely to have experienced DENV infection even in endemic settings (*Flasche et al.*, 2016; *Simmons et al.*, 2012). The mean age of a confirmed or suspected DENV-3 case during the 2013-14 epidemic was 27.7 years old (IQR: 16-38) (*Kucharski et al.*, 2018). Consideration of the age distribution of a population is therefore important when studying arbovirus transmission. The median age in the Fijian population as recorded in the 2017 census was 27.5 years and 20% of the population are children younger than 10 (*Fiji Bureau of Statistics*, 2018b) (Figure 1.3). The population in French Polynesia has fewer young children (15.7% of the population) but similar levels of people aged over 50 (20.5% in French Polynesia and 19/3% in Fiji) according to a census from 2012 (*Institut de la statistique de la Polynésie française*, 2020) (Figure 1.4).

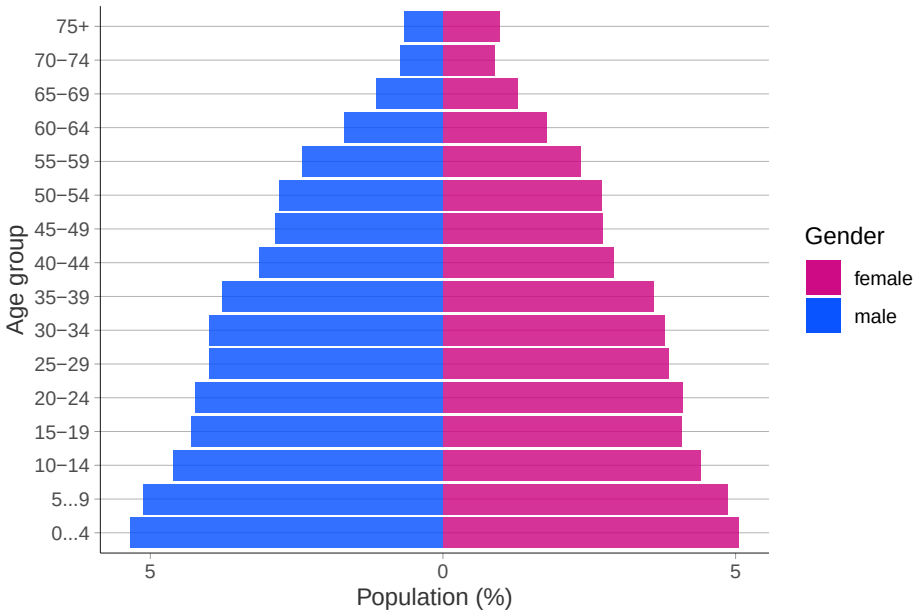

Figure 1.3: *Age pyramid of Fiji from census data collected in 2017 (Fiji Bureau of Statistics, 2018b)*

Urbanisation is another important consideration in the study of DENV and ZIKV. The primary vector of these flaviviruses, and therefore the burden of disease, are typically concentrated in urban areas with dense populations. Fiji has seen increased urbanisation between censuses conducted in 2007 and 2017. The proportion of the population living in urban areas increased from 50% in 2007 to 56% in 2017. The population

Figure 1.4: *Age pyramid of French Polynesia from census data collected in 2012 (Institut de la statistique de la Polynésie française, 2020)*

of Rewa province – which includes Suva – increased by 7% from 100,995 to 108,016 between 2007 and 2017 (*Fiji Bureau of Statistics*, 2018b). By contrast, the population in French Polynesia is dispersed over five separate archipelagos with fewer urban centres. However, the capital city Pape'ete is comparable to Suva, as each houses approximately 10% of that country's total population (25,763 people in Pape'ete) (*Institut de la statistique de la Polynésie française*, 2020).

Temperature is another critical factor in arbovirus transmission. Fiji and French Polynesia are both tropical countries with warm climates that suit virus transmission by *Aedes* mosquitoes (*Mordecai et al.*, 2017; *Nishiura et al.*, 2016a; *Richard et al.*, 2016; *Roth et al.*, 2014). Average temperatures are very similar in both countries, at 26.4°C in Fiji and 27°C in French Polynesia (*Fiji Meteorological Service*, 2017; *Institut de la statistique de la Polynésie française*, 2020). However, French Polynesia is closer to the equator and, as a result, temperature is less variable over a year compared to Fiji where fluctuations are greater (Figure 1.5).

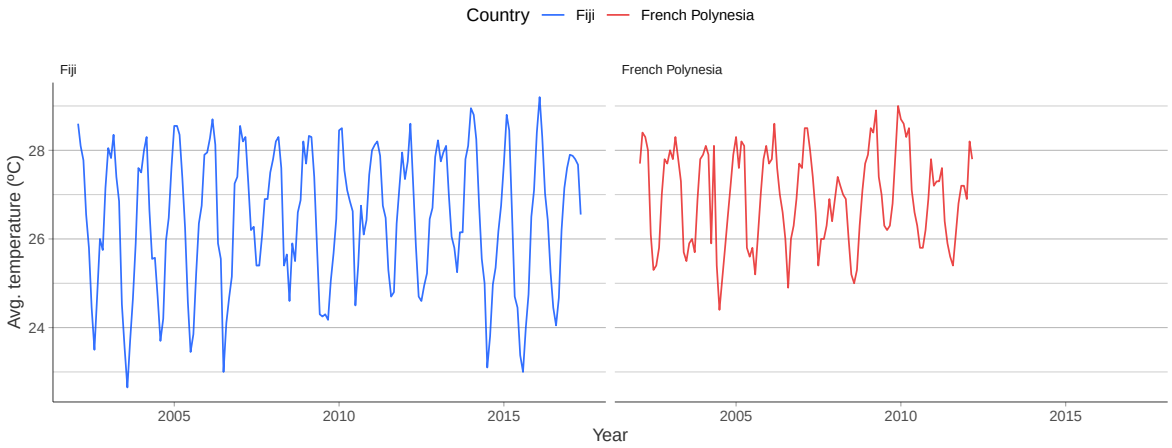

Figure 1.5: *Average monthly temperature (°C) in Fiji and French Polynesia (Fiji Meteorological Service, 2017) (Institut de la statistique de la Polynésie française, 2020)*

Arboviruses in the Pacific

Island populations are isolated and typically have smaller populations so are therefore less likely to sustain endemic transmission (*Black*, 1966; *Keeling and Grenfell*, 1997). This is especially true of diseases that have a seasonal pattern of transmission – such as DENV and ZIKV – with a low-transmission period through which an epidemic cannot easily persist. This generates a pattern of self-contained single serotype epidemics in the South Pacific with reintroduction from outside sources after an interval period of several years (*Cao-Lormeau et al.*, 2014a). DENV circulation in Pacific island countries and territories (PICTs) in the 20$^{\text{th}}$ century began with the first recorded major outbreak of DENV-1 towards the end of the Second World War (*Imrie et al.*, 2007; *Rosen*, 1958). Decades later, there was a DENV-2 outbreak in 1971 (*Maguire et al.*, 1974) which was closely followed by DENV-1 in Fiji in 1975 (*Reed et al.*, 1977) and DENV-4 in 1979 in French Polynesia (*Chungue et al.*, 1999).

A pattern emerged of a single large outbreak invading a country with no transmission for several years afterwards. This pattern can be clearly seen in data from French Polynesia between 1978 and 2014 that has been collated by *Teissier et al.* (2020) (Figure 1.6). The authors defined eight epidemic periods and six inter-epidemic periods over the 35 year time frame. Multiple serotypes were reported in two of these epidemic

periods but most were monotypic or primarily single-serotype outbreaks. This pattern of intermittent epidemics is observable at the regional level as well. Following DENV-4 in 1979, the next large regional outbreak was in 1989-90 (*Chungue et al.*, 1999; *Fagbami et al.*, 1995; *Taleo et al.*, 2000). This was followed in 1996-1999 with DENV-2 cases in French Polynesia (*Deparis et al.*, 1998) and Fiji (*Prakash et al.*, 2001).

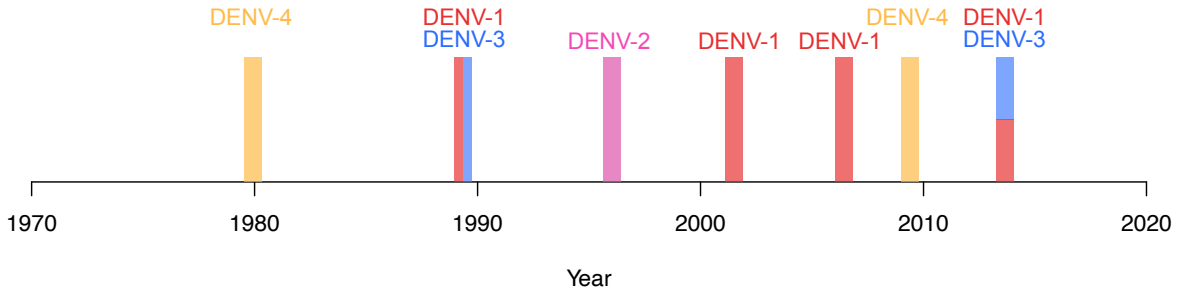

Figure 1.6: *Timing and serotype of DENV outbreaks in French Polynesia between 1978 and 2014, using data from Teissier et al. (2020)*

Since 2000, DENV outbreaks have become more frequent (*Kiedrzynski et al.*, 1996; *Singh et al.*, 2005). Taking Fiji as a case study, a large DENV-1 epidemic in 2001-04 (*Singh et al.*, 2005) was followed by DENV-4 circulation in 2007 (*Li et al.*, 2010; *Warrilow et al.*, 2012). DENV-3 then spread in 2013-2014 (*Cao-Lormeau et al.*, 2014a) and most recently, the Fiji Ministry of Health declared a DENV-2 outbreak in April 2017 (*Fijivillage*, 2017). Increased birth and immigration rates may be creating enough susceptible hosts for DENV to circulate every 3-4 years with a single serotype reappearing every 10-12 years (*Cao-Lormeau et al.*, 2014a). However, the relative contribution of these causes remain poorly understood.

The first recorded outbreak of ZIKV occurred on the Pacific island of Yap, part of the Federated States of Micronesia in 2007 (*Duffy et al.*, 2009; *Hayes*, 2009). This was a large and quick outbreak with an estimated 73% of the residents of Yap state infected with ZIKV between April and July 2007 (*Duffy et al.*, 2009). ZIKV next emerged in French Polynesia in October 2013. The outbreak was short as well, lasting 21 weeks and peaking at the end of February 2014 (*Musso and Gubler*, 2016). By the end of the outbreak an estimated 11.5% of the population had ZIKV fever (*Musso et al.*, 2014). A serological survey conducted in 2015 in the general population estimated an infection

rate of 50 to 66% (*Aubry et al.*, 2015).

Following the French Polynesia outbreak ZIKV spread quickly through the Pacific (*Musso and Gubler*, 2016; *Roth et al.*, 2014). In 2014 ZIKV outbreaks were confirmed in New Caledonia, Cook Islands and Easter Island. In New Caledonia, less than 1% of the population reported as confirmed ZIKV compared to 11.5% in the French Polynesia outbreak (*Musso and Gubler*, 2016; *Musso et al.*, 2018). The cause of this smaller outbreak is still unknown but could be related to mosquito species, different populations, or the lack of a "cold season" in French Polynesia (*Musso and Gubler*, 2016). The outbreak in the Cook Islands was small, with 905 cases reported and there were 50 suspected ZIKV cases reported in Easter Islands (*Musso and Gubler*, 2016). In 2015 ZIKV continued to spread through the Pacific as local transmission of ZIKV was confirmed in Vanuatu, Solomon Islands, Samoa and Fiji. DENV has spread in all of these countries and there were reports of concurrent circulation of DENV, ZIKV and chikungunya virus (CHIKV) throughout the Pacific (*Roth et al.*, 2014).

It is probable that the virus spread from the Pacific Islands to Brazil with some evidence from molecular data and phylogenetics that ZIKV was introduced to Brazil from French Polynesia and Easter Island around the same time (*Delatorre et al.*, 2018). Once ZIKV emerged in northeast Brazil, likely introduced between August 2013 and April 2014 (*Faria et al.*, 2016), it spread through the Americas and was declared a Public Health Emergency of International Concern by WHO (*World Health Organisation*, 2016).

Disease monitoring in the Pacific was enhanced in 2010 with the launch of the Pacific Syndromic Surveillance System (PSSS). This is a sentinel surveillance system with 121 sentinel surveillance sites across twenty-one countries in the Pacific that report weekly on five syndromes: (i) diarrhoea, (ii) influenza-like illness, (iii) prolonged fever, (iv) acute fever and rash, and (v) dengue-like illness (*Craig et al.*, 2016). However, most countries in the PSSS are small and adapting to emerging diseases can be challenging. *Craig et al.* (2016), found that existing reporting on acute flaccid paralysis (AFP) for Polio eradication programmes was insufficient for the identification of ZIKV emergence in the Pacific.

Arboviruses in Fiji

There have been regular outbreaks of DENV in Fiji since the virus emerged after World War II (*Kiedrzynski et al.*, 1996; *Kucharski et al.*, 2018; *Singh et al.*, 2005). Fiji has regular epidemics but has no evidence of sustained endemic DENV transmission. Most of these outbreaks record thousands of cases which is consistent with a large monotypic epidemic. Such outbreaks can deplete the susceptible population and prevent any other DENV serotype from emerging for half a decade, and the same serotype from emerging for 10-12 years (*Cao-Lormeau et al.*, 2014a). Figure 1.7 shows a schematic of reported DENV outbreaks in Fiji since 1970 and demonstrates this pattern of intermittent monotypic epidemics.

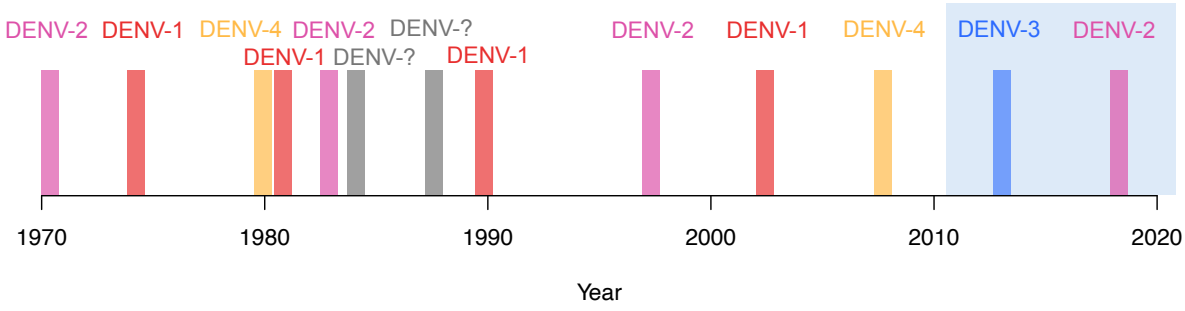

Figure 1.7: *Timing and serotype of DENV outbreaks in Fiji between 1970 and 2017, using data from Kucharski et al. (2018). Blue region highlights the main study period for this doctoral project*

Details on these outbreaks are shown in Table 1.1, adapted from *Kucharski et al.* (2018) and demonstrate the diverse range of dynamics of these individual outbreaks, with some very large outbreaks and some much smaller. There is a limited amount of post-outbreak serological data to validate the size of these outbreaks in the population.

As reported elsewhere in the Pacific (*Roth et al.*, 2014), there were concurrent outbreaks of DENV, ZIKV and CHIKV between 2013 and 2018. This six-year window is the focus of this doctoral project and includes four notable outbreaks of arboviruses in Fiji (Figure 1.8). Firstly a large DENV-3 outbreak in 2013-14 (*Cao-Lormeau et al.*, 2014a). There were a small number of confirmed locally-acquired ZIKV and CHIKV cases between 2015 and 2016 (*Kama et al.*, 2019). Finally, a DENV-2 epidemic emerged in 2017

Table 1.1: *Reported arbovirus outbreaks in Fiji between 1930-2017. Adapted from Kucharski et al. (2018)*

Year	Main virus	Reported cases	Seroprevalence	Source
1930	?	Thousands		(*Maguire et al.*, 1971)
1944-5	DENV-1	Thousands		(*Reed et al.*, 1977)
1971-3	DENV-2	3,413	25%*	(*Maguire et al.*, 1974)
1974-5	DENV-1	16,203		(*Reed et al.*, 1977)
1980	DENV-4	127		(*Fagbami et al.*, 1995)
1981	DENV-1	18		(*Kiedrzynski et al.*, 1996)
1982	DENV-2	676		(*Kiedrzynski et al.*, 1996)
1984-6	DENV-?	490		(*Fagbami et al.*, 1995)
1988	DENV-?	22		(*Fagbami et al.*, 1995)
1989-90	DENV-1°	3,686	54%*	(*Fagbami et al.*, 1995; *Waterman et al.*, 1993)
1997-8	DENV-2	24,780		(*World Health Organisation*, 2015)
2001-3	DENV-1	?		(*Halstead*, 2008)
2008	DENV-4	1,306		(*Pacnet Report*, 2008; *ProMED-mail*, 2008)
2013-14	DENV-3	25,496	53.2%**	(*Kucharski et al.*, 2018)
2015-17	ZIKV	16**	21.9%**	(*Kama et al.*, 2019)
2015-17	CHIKV	93**		(*Aubry et al.*, 2019; *Kama et al.*, 2019)
2017	DENV-2	755*		Fiji MOH data

* Suva

** Central Division

°There is also evidence of DENV-3 circulation during this period (*Singh et al.*, 2005) .

(*Fijivillage*, 2017).

Previous serological surveys in Fiji

This thesis uses data from two previous serological surveys conducted in Fiji. In 2013, *Watson et al.* (2017), collected 1,781 samples from across mainland Fiji; 695 of them in Central Division. *Watson et al.* (2017), characterised the epidemiology of typhoid fever and *Lau et al.* (2016), used the samples to analyse risk factors for leptospirosis transmission in Fiji. The authors conducted a representative, clustered, cross-sectional seroepidemiological survey of the two main Fijian islands. A follow-up study was conducted in November 2015 in Central Division after the DENV-3 epidemic. 333 of the same participants were resampled in 2015 to collect a data set of pre- and post-outbreak serology (*Kama et al.*, 2019; *Kucharski et al.*, 2018).

Data from these serological surveys showed evidence of increased DENV-3 infections, concentrated in DENV naïve children, as expected given the large reported outbreak. Analysis of serology also showed that a large proportion of the population in Fiji had seroconverted to ZIKV between 2013 and 2015, which is before cases were reported (Figure 1.9). *Kama et al.* (2019), found evidence of undetected circulation of ZIKV and CHIKV in Fiji using these data.

1.1.4 Mathematical modelling of arboviruses

Mathematical modelling of an infectious disease – shortened to 'mathematical modelling' from this point – is an abstract simplification of reality that uses mathematical language to describe the behaviour of a disease transmission system. The dynamics of disease transmission can be complex at an individual level and fields of study such as medicine, genomics and microbiology inform our understanding of these dynamics. At a population level, however, the dynamics often conform to simple processes which can be modelled mathematically. These models of infectious diseases include the mechanisms driving transmission in a population and can therefore be differentiated

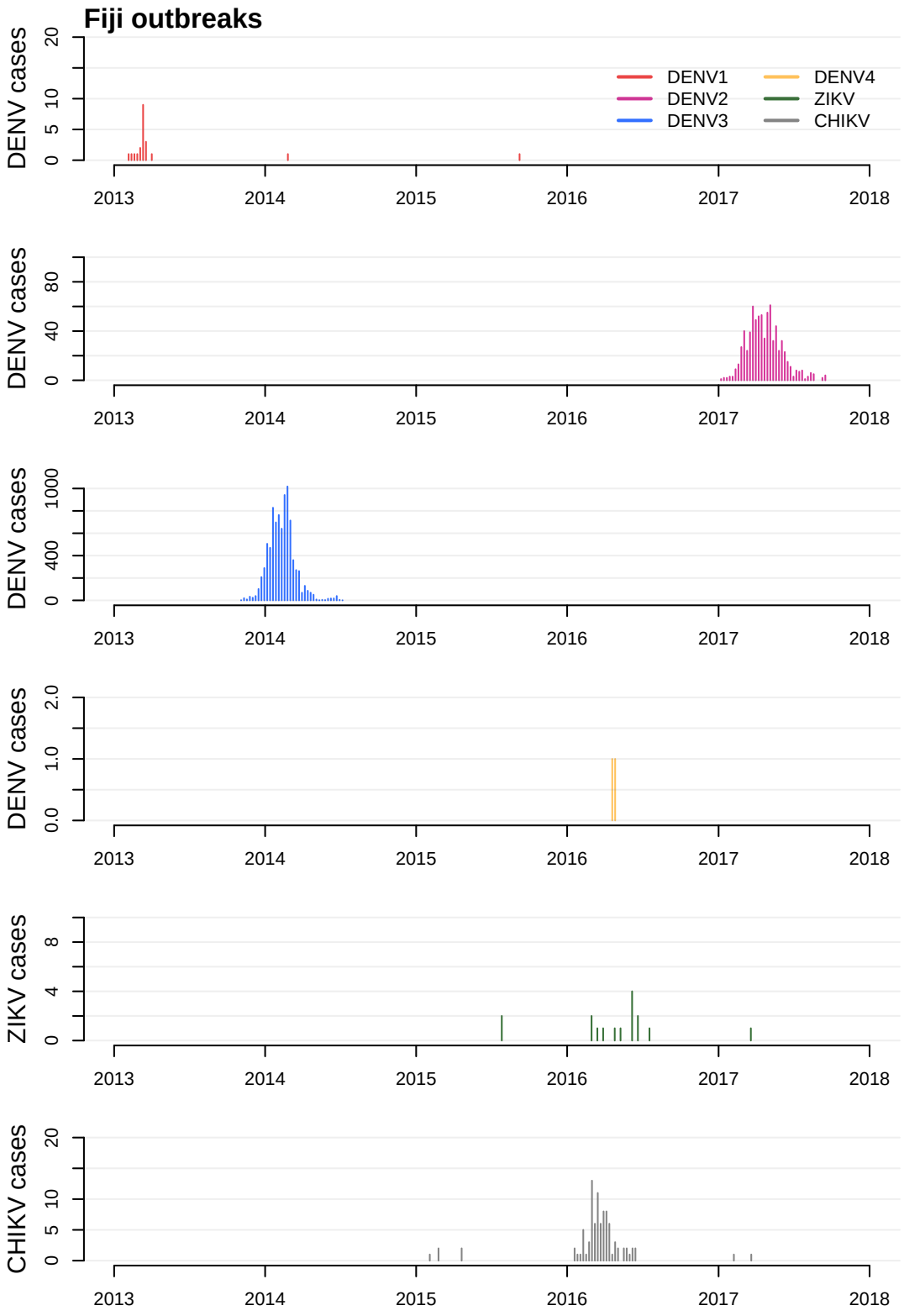

Figure 1.8: *Recent arbovirus outbreaks in Fiji between 2013 and 2018. Reported cases for DENV-3. Confirmed cases for DENV-1, DENV-2, DENV-4, CHIKV and ZIKV*

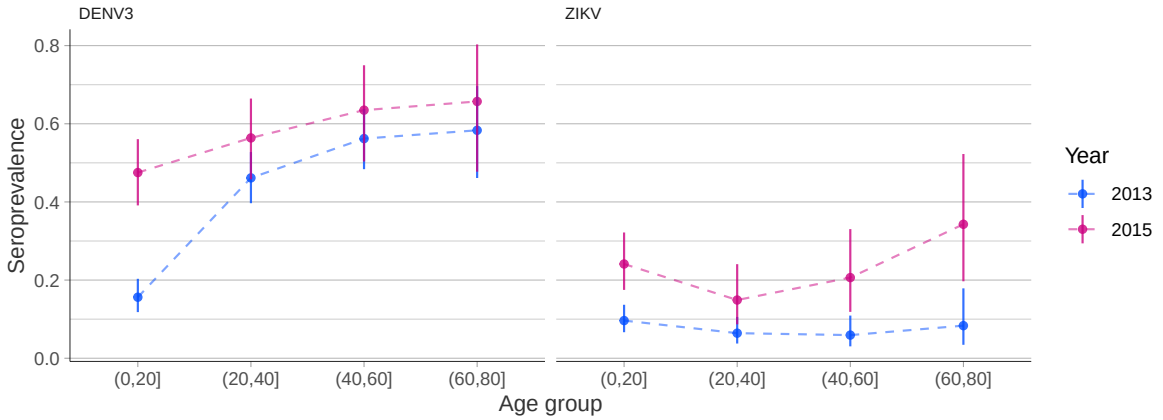

Figure 1.9: *Change in DENV-3 and ZIKV seroprevalence in Fiji between 2013 and 2015. Circles show estimated seroprevalence for ZIKV and DENV-3 in the corresponding age group. Vertical lines show 95% confidence intervals. The majority of the increase in seroprevalence between 2013 and 2015 was in the youngest age group, but for ZIKV there was an increase in seroprevalence across all age groups*

from 'statistical models' that describe the relationship between observed quantities and independent variables, common across the study of non-communicable diseases. By including these mechanisms explicitly in the model we can ask more interesting questions about an epidemic: When did the disease emerge? How quickly did it spread? When did transmission peak, and why did it end?

Models are simplified representations of reality and as such there is trade-off between the 'detail' and 'transparency' of the model. A model is usually simplified by making more assumptions and omitting details that are deemed unimportant. This can make the model easier to understand but conclusions from this model are only valid if these assumptions hold. The choice of model complexity and the balance of this trade-off will depend on the purpose of the model and what hypothesis is being tested.

A popular mathematical modelling approach is to use compartmental models. A population is divided up into compartments based on their infectious status. An example of compartmental modelling was first proposed by Ross and Hudson (*Ross and Hudson*, 1917) and expanded in the 1920s and 30s by Kermack and McKendrick (*Kermack et al.*, 1927, 1932, 1933). For a disease that confers lifelong immunity, a basic compartmental

model has three compartments: all individuals in a population (N) are either susceptible (S) to the virus, are infectious (I), or have recovered (R). The rate of transition between these compartments determines the dynamics of an epidemic in this model, which is typically expressed as a system of ordinary differential equations (Equations 1.1-1.3). These equations express the change in compartments for each time step t (where t is small).

$$\frac{dS}{dt} = -\lambda S = -\beta \frac{I}{N} S \tag{1.1}$$

$$\frac{dI}{dt} = \lambda S - \gamma I \tag{1.2}$$

$$\frac{dR}{1t} = \gamma I \tag{1.3}$$

Individuals move between the S and I compartment following infection at a rate known as the 'force of infection' (λ). The force of infection is the product of transmission probability (β) and the probability of contact with an infectious individual ($\frac{I}{N}$). Individuals remain infectious for a fixed period of time so transition to the R compartment occurs at a constant rate (γ) (Figure 1.10). This simple model makes several assumptions about how the disease spreads. Everyone mixes evenly and therefore has an equal probability of becoming infected, everyone is infectious for the same length of time and is equally infectious.

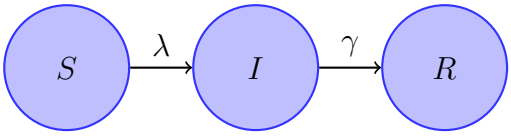

Figure 1.10: *Schematic of compartmental framework of an SIR model*

The parameters in this model can be used to calculate important epidemiological quantities. The basic reproduction number (R_0) is the average number of secondary infections generated by a typical infectious individual in an entirely susceptible population. In this basic SIR model R_0 is equal to the ratio $\frac{\beta}{\gamma}$ (*Keeling and Rohani*, 2011).

DENV and ZIKV are vector-borne diseases, viruses that are transmitted between humans by vectors. In the case of DENV and ZIKV the intermediary host is a mosquito. Modelling of vector-borne diseases is based on the work of Ronald Ross studying malaria in the early 20$^{\text{th}}$ century (*Ross*, 1908, 1911). After the Second World War George Macdonald continued the work of Ross and focused on applied theory to support the WHO Global Malaria Eradication Programme (*Macdonald*, 1956a,b, 1957; *Macdonald and Goeckel*, 1964; *WHO*, 1956). This class of models has been refined but still carries a common set of assumptions: mosquito bites are distributed randomly and evenly among single vertebrate host populations, mosquito mortality is independent of age, both the pathogen latent period and fraction of mosquitoes that blood feed on the host are constant and there is only one mosquito vector species (*Smith et al.*, 2012). A systematic review conducted in 2013 found that even recent models were still largely dependent on the same assumptions set out in Ross-Macdonald models (*Reiner et al.*, 2013).

Compartmental modelling of DENV has a long history (*Andraud et al.*, 2012; *Reiner et al.*, 2013). *Newton and Reiter* (1992), produced a compartment vector-host model to estimate the R_0 of DENV as 1.9. Other early modelling estimates for the DENV R_0 were 1.33 from serological data in Mexico (*Koopman et al.*, 1991) and 2.03 from an analysis of the early growth of a 1990-91 epidemic in Brazil (*Marques et al.*, 1994). By 1995 researchers were comparing model outputs with data to validate their simulations (*Focks et al.*, 1995). *Esteva and Vargas* (1998) developed a compartmental model for DENV to define the global stability of the endemic equilibrium and then extended it to include demographic changes (*Esteva and Vargas*, 1999).

As these models developed they were targeted at increasingly complex areas of DENV epidemiology. Compartmental models that included multiple serotypes were developed to analyse the effects of ADE and cross-protection between DENV serotypes. *Ferguson et al.* (1999), developed a DENV model with multiple serotypes to demonstrate that ADE acts to generate complex and persistent cyclical or chaotic epidemic behaviour. *Cummings et al.* (2005), used a two-serotype model to describe the effect of ADE on the evolutionary dynamics of DENV. *Adams et al.* (2006), used a compartmental two-serotype model for DENV transmission in Bangkok and found evidence that infection

with one serotype must be moderately cross-protective to recreate the observed epidemiological data. The importance of a temporary period of cross-serotype protection have been shown in models of DENV compared to data from Thailand (*Nagao and Koelle*, 2008; *Wearing and Rohani*, 2006). DENV models have advanced to the point of informing pivotal public health planning. The proven efficacy in phase III trials of a DENV vaccine, *Dengvaxia*, led to the licensing of the vaccine in several countries. Dynamic modelling of DENV and the effect of the vaccine informed WHO guidance on optimal use of the vaccine only in settings with high DENV endemicity (*Ferguson et al.*, 2016b; *Flasche et al.*, 2016).

Mathematical models can then be fitted to available data from infectious disease outbreaks. Such data can include surveillance data on the number, location and timing of cases as well as serological data on the proportion people infected. Outputs from mathematical models can then be formally compared to observed data under the assumption that these data follow a defined statistical distribution. Parameters from the model can then be estimated and compared between diseases, between different outbreaks and between locations. Further details are available in Chapter 2.

Modelling island outbreaks and models of DENV and ZIKV

Islands and remote settings have been a considerable source for epidemiological studies of infectious diseases since the mid 20$^{\text{th}}$ century (*Panum and Petersen*, 1940). The isolated nature of Pacific islands in particular led to several findings in the study of measles. *Black* (1966), found that measles could probably not persist in dispersed island communities with a population under 200,000. *Cliff and Haggett* (1984), used data from Iceland to demonstrate that measles spread in a hierarchical pattern depending on population size. *Gould et al.* (1971), studied the epidemiology of measles outbreaks in Ponane (now part of the Federated States of Micronesia) and *Rosen* (1962), studied a measles outbreak in 1951 in Tahiti using pre- and post-outbreak seroprevalence data.

Mathematical models of infectious diseases have similarly been used to great effect in island settings. *Camacho et al.* (2011), modelled an influenza outbreak with multiple

peaks on the remote island of Tristan da Cunha. The authors were able to characterise a rapid rate of reinfection in their model by using the remoteness of the island as a justification that the second wave of infections could not have been caused by reintroduction of the virus. The authors concluded that the second wave of infection was caused by either a delayed or deficient immune response to the primary wave of infections in the population. This insight into influenza transmission dynamics was facilitated by studying the 'natural experiment' of the remote island outbreak. Mathematical models have also been applied in island settings to design optimal pandemic preparedness strategies (*Nishiura et al.*, 2009) and to provide estimates of R_0 for emerging diseases (*Yakob and Clements*, 2013).

Small island outbreaks of DENV have been used to study the effect of seasonal climate variation on DENV transmission with statistical models including the association between El Niño southern oscillations and DENV incidence (*Hales et al.*, 1996) and a characterisation of 40 years of DENV outbreaks in New Caledonia (*Descloux et al.*, 2012). However, the use of compartmental models to study DENV outbreaks on small islands is limited. *Chowell et al.* (2013), used a compartmental model for a DENV outbreak on Easter island and estimated a very high R_0 for the outbreak assumed to be linked to the high level of susceptibility in the population. *Lourenço and Recker* (2014), modelled the first European DENV outbreak showing significant and prolonged autochthonous transmission in Madeira in 2012, and defined a period of high epidemic risk based on the climate in Madeira. *Rodrigues et al.* (2015), subsequently modelled the effect of control measures on this same outbreak. More recently *Funk et al.* (2016), used the dynamics of island outbreaks to compare DENV and ZIKV epidemics in island settings. The authors found greater similarity between the reproduction number of DENV and ZIKV in the same location, than between DENV outbreaks in separate locations, suggesting that location is pivotal to outbreak dynamics.

The initial spread of ZIKV through the Pacific led to several mathematical models of arboviruses in island settings. *Kucharski et al.* (2016), modelled the French Polynesia epidemic and estimated that the majority of French Polynesia was infected during the outbreak despite only 11.5% of the population reporting as cases (*Cao-Lormeau*

et al., 2014a). *Champagne et al.* (2016), compared two compartmental model structures to model ZIKV epidemics on several Pacific islands: Yap (Micronesia), Tahiti and Moorea (French Polynesia), and New Caledonia. They estimated a range of R_0 across these islands between 1.5 and 4.1, with smaller islands displaying higher and more variable values. *Nishiura et al.* (2016a), used a different modelling method to estimate R_0 for ZIKV based on the early exponential growth of the ZIKV epidemic in French Polynesia and estimated R_0 to between 1.8 and 2. *Riou et al.* (2017), analysed ZIKV and CHIKV outbreaks across the Pacific with a time-dependent SIR model and found similar transmission potential for both viruses in the same territory. *Lourenço et al.* (2018), were able to model an outbreak of ZIKV in Africa on the island of Cabo Verde and found similar characteristics to outbreaks in the Americas and Pacific. Finally, *Cousien et al.* (2019), used a household model with the ZIKV outbreak on the island of Martinique in the Caribbean to estimate that approximately one fifth of ZIKV infections occurred in the household setting.

Following these initial island outbreaks of ZIKV, the virus spread to large countries in the Americas. Here, mathematical models were important to characterise these emerging outbreaks, including initial estimates of R_0 (*Nishiura et al.*, 2016b; *Shutt et al.*, 2017) and of the contribution of sexual transmission to outbreak dynamics (*Gao et al.*, 2016; *Towers et al.*, 2016). Modelling also made a valuable contribution to outbreak response. *Ferguson et al.* (2016a), modelled the spread of ZIKV in the Americas to inform control strategies in real-time and *Perkins et al.* (2016), projected the number of child-bearing age women at risk of ZIKV infection. After the epidemic *Lourenço et al.* (2017), used a climate driven transmission model to characterise the determinants of high attack rates of ZIKV transmission in urban settings in Brazil. *Netto et al.* (2017), also used a compartmental model for the spread of ZIKV in one of the worst affected regions during the pandemic, Salvador in northeast Brazil. The authors used opportunistically sampled sera from blood donors and HIV registers to constrain their model alongside the available case data and estimated ZIKV R_0 was 2.1 (95% CI 1.8-2.5) at the onset of the outbreak.

In summary, mathematical modelling is a useful tool in analysing transmission dynam-

ics and is particularly valuable in island settings where outbreak periods, circulating viruses, and pre-outbreak immunity can be more clearly defined. Available data from Fiji shows regular DENV outbreaks of differing severity and silent circulation of ZIKV. This thesis includes an analysis of serological data and development of mathematical models of outbreak dynamics in the setting of Fiji and the wider Pacific.

1.2 Aims

The overall aim of this research is to better understand the transmission of DENV and ZIKV by using serological data and mathematical modelling. Specifically (i) to improve understanding of population immune dynamics following arbovirus outbreaks and (ii) identify the determinants of arbovirus transmission dynamics in island outbreaks.

1.2.1 Objectives

This aim will be met by fulfilling the following objectives:

1. Conduct a serological survey in Fiji, resampling as many participants of previous surveys in Central Division as possible

2. Analyse longitudinal serological data to determine the burden of ZIKV infection in Fiji between 2013 and 2017

3. Evaluate the population level immune response to ZIKV from serological data following outbreaks in Fiji and French Polynesia

4. Develop a mathematical model of arbovirus transmission and use it to explain different transmission dynamics of recent arbovirus outbreaks in Fiji

5. Inform control strategies for DENV outbreaks in Fiji by estimating the effect of vector control on DENV transmission

1.3 Thesis structure

This is a *research paper style thesis* consisting of four chapters written in the style of a journal article, preceded with this introduction chapter and followed by a discussion of the research. This opening chapter provides background on arboviruses, serological analysis of DENV and ZIKV and the principles of mathematical modelling to analyse arbovirus outbreak dynamics. The results chapters are as follows:

1. **Materials & Methods**. This chapter details methods used in this doctoral project. Methods are described for the data collection with a seroepidemiological survey in Fiji in 2017, serological testing on samples collected from this survey and mathematical modelling methods used in Chapters 5 and 6.

2. **A longitudinal seroepidemiological survey of arbovirus burden in Fiji**. This chapter details the serological survey I led in Fiji 2017. This chapter describes and discusses results about the burden of DENV, ZIKV and related arboviruses in Fiji between 2013 and 2017.

3. **Zika seroprevalence declines and neutralising antibodies wane in adults following outbreaks in French Polynesia and Fiji**. This is the only chapter that is currently published, in *eLife* in 2020 (*Henderson et al.*, 2020). This chapter presents an analysis of eight serological survey across Fiji and French Polynesia to analyse the long-term immune response to ZIKV in a population following an outbreak.

4. **Interactions between timing and transmissibility explain diverse flavivirus dynamics in Fiji**. This is the main modelling chapter of the thesis and has been submitted for publication. This chapter presents a mathematical model I developed to explain differing transmission dynamics of ZIKV and DENV in Fiji since 2013.

5. **Modelling dengue virus transmission in Fiji and assessing the contribution of vector control interventions in ending DENV epidemics**. The final chapter presents a model of DENV transmission that I used in real-time

in Fiji in 2017 to forecast the dynamics of a DENV outbreak. Post-outbreak, I used this model to estimate the contribution of vector control to the outbreak dynamics.

The thesis concludes with discussion of the findings, the strengths and limitations of the research and aims to put the results in context of the wider understanding of arbovirus outbreak dynamics.

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Chapter 2

Materials & Methods

This Chapter describes the methods used in this doctoral project: the design and implementation of a seroepidemiological study from 2017, the serological testing methods used to test for antibodies against specific viral infections and the mathematical model that is used to analyse trends in outbreak dynamics in Fiji between 2013 and 2017.

2.1 Data collection

2.1.1 Study design

Two previous serological studies have been conducted in Fiji. A nationally representative cross-sectional seroepidemiological study was conducted in 2013 (*Lau et al.*, 2016; *Watson et al.*, 2017). In 2015, participants from Central Division were contacted and another serum sample was collected. I led a third serological survey in the same area between May and June 2017 to recontact and sample participants from previous serological surveys who had given consent to being recontacted. A sample of longitudinal sera from the same individual is preferable to cross-sectional sampling because of the variation between individual's immune responses. By sampling repeatedly from the same individuals we were able to control for this within-host variation as well as pre-existing immunity. In addition, by successfully sampling a large proportion of these original participants I could ensure that our sample remained nationally representative and would therefore provide better information on population immune response to circulating arboviruses, compared to convenience sampling for instance.

This follow-up study, like the study performed in 2015 (*Kama et al.*, 2019; *Kucharski et al.*, 2018), restricted the geographic coverage from the whole of Fiji to Central Division only. This restriction was made because the 2013-14 DENV-3 outbreak was concentrated in Central Division. The reference laboratory for communicable diseases in Fiji is based at Mataika House in Suva, Central Division, so collected samples from Central Division could instantly be processed and stored. Central Division is the largest Division in Fiji and includes a broad distribution of rurality – how urbanised an area is. Sampling from Central Division could therefore potentially be generalised to the rest

of Fiji. We planned to compare existing pre-epidemic serum collected from volunteers in areas affected by arbovirus transmission with serum from another round of data collection.

Between September and December 2013, serum samples were collected from 2,000 randomly-selected people across Fiji for a combined typhoid and leptospirosis study by the Fijian Ministry of Health, LSHTM, the University of Queensland and World Health Organisation (LSHTM ethics ref 6344, Fiji national research ethics review committee ref 2013 04) (*Watson et al.*, 2017). 695 of the study participants were in Central Division and 455 of these have available data for arboviruses. Between October and November 2015 (LSHTM ethics ref 10207, Fiji national research ethics review committee ref 2015.111.C.D) (*Kucharski et al.*, 2018), 390 participants were followed up (56%). The 2017 study, which took place between May and June 2017, followed the same protocol as the 2015 project and aimed to revisit up to 400 of the original 2013 participants.

2.1.2 Sample size and exclusion criteria

We aimed to follow up 350 participants as this was approximately 50% of the original 2013 study participants in 2017. We assumed that approximately 15% of these paired samples would seroconvert between 2015 and 2017. Allowing for 5% seroreversion, assuming no cross-reactivity and a probability of type-1 error of 0.05, with a sample size of 350 we would be able to detect a 15% change in ZIKV seroprevalence between 2015 and 2017 with 88% power using McNemar's test, and a 20% change with 98% power.

Participants were eligible for inclusion if they were aged 12 months or older during the first study in 2013. The youngest possible participants for inclusion would be at least 3 by May 2017. Exclusion criteria were clotting disorders, such as haemophilia and other coagulopathies, concurrent medical anticoagulation such as through administration of warfarin or heparin, or the presence of severe underlying medical conditions or significant acute illness. Non-medical exclusion criteria were needlephobia or other

unwillingness to participate, inability to consent to treatment through lack of insight, understanding, and for children, the refusal of, or inability to attain parental consent. There were no additional exclusion criteria. These criteria were checked again in 2017 to confirm that the potential participant was still eligible.

2.1.3 Funding and ethical approval

The field work and serological analysis were funded by a grant from the Enhancing Research Activity in Epidemic Situations (ERAES) programme, funded by the Wellcome Trust. This grant covered all project costs, including consumables, staff, transport, shipping and laboratory testing.

Ethical approval was obtained from the LSHTM ethics committee in February 2017 (ref: 12007) conditional on local ethics approval. I submitted our ethics application in March 2017. I received a request for justification of our sampling method, storage of samples in Fiji and testing of samples outside of Fiji. I sent my response on 23 May 2017 and received ethical approval for the study on 29 May 2017 (ref: 2017.20.MC).

2.1.4 Field team

Field team – recruitment

Working with Dr. Adam Kucharski, we developed a budget based on the experience from the 2015 serological survey. We budgeted for recruitment of six operational field workers for a period of twenty-eight working days. We established two working field teams, each with a qualified phlebotomist and two field workers.

One field worker in each team would represent the team on field visits and was in charge of coordinating field visits, administering questionnaires and reporting on data and any problems in the field. Two such field workers were recruited through contacts of the surveillance department at the Ministry of Health. Jessica Paka and Amele

Ratevono were recruited to perform this role. Jessica and Amele both had a certificate in public health at Fiji National University (FNU). Mosese Ligani and Jonetani Bola were recruited as the second field workers for each team and were responsible for transport and maintenance of the vehicles during field work. Both had good knowledge of Suva and Central Division and were well recommended. Additionally, they were able to represent the field team effectively at iTaukei villages where it is important that a male performs rituals during a *sevusevu* ceremony to greet a village official. Finally, newly qualified phlebotomists Warren Fong and Manisha Prakash were recruited and were solely responsible for collecting venous blood samples and storage of samples until they could be processed at Mataika House the same day. I also budgeted for 112 hours of overtime laboratory work by an existing member of the laboratory staff at Mataika House, Taina Naivalu.

Field team – training and risk management

The six members of the field teams came to Mataika House for a full day of training and planning before the data collection began. I conducted training giving the team a background to the study and setting out the aims and objectives of this 2017 study. Formal training was then given in three key areas. Firstly, the plan to recontact participants which would be led by Jessica and Amele. Secondly, the process and importance of obtaining informed consent. Both Jessica and Amele were bilingual so could explain the purpose of the study and the process of data collection in English or iTaukei according to the preference of the participant. Finally, we covered the process of dealing with adverse events – most likely a sharps injury in this study – and detailed the standard operating procedures in the case of such events. The phlebotomists in the field teams reviewed the equipment that had been provided and additional pharmaceutical supplies were purchased to meet their requests.

I updated the standard operating procedures (SOPs) used in the previous serological surveys in Fiji for this study in 2017. These SOPs covered sharps injuries management and reporting, adverse event reporting, collection and transport of blood samples, obtaining informed consent, telephone follow up and processing and storage of blood

samples.

Field team – equipment

The majority of fieldwork supplies were purchased in the UK and I brought them to Fiji myself. We purchased enough phlebotomy and sample storage consumables for 400 samples. These included shielded needles, vacutainers, alcohol wipes and gel, first aid kits and hygienic products for phlebotomists. I also carried pipettes, cryovials, cryolabels, boxes and biohazard waste bags for the processing of samples at Mataika House.

2.1.5 Field work

Field work – plan

Potential participants (or the parents of child participants) were first contacted by telephone by a field worker, where phone details were provided. The field team explained the details of the proposed study explained and answered any questions from potential participants. Where a telephone number was not given or was no longer active, a visit was made to the village or house to seek permission for further involvement in the study. Address records, village governance systems, community nurses or community health workers, local knowledge and previous GPS mapping were also used to help locate participants.

Samples were collected in clusters in 2013. In Central Division these clusters all had 25 participants in the original 2013 study so in 2017, different clusters were targeted each day depending on their location. The majority of clusters were located in Suva so it was possible to visit several in a day. Others were very remote and took a whole day to visit (Figure 2.1). As a result, I kept track of samples collected and the age distribution of samples throughout the study and adapted where teams targeted their data collection in the limited available time.

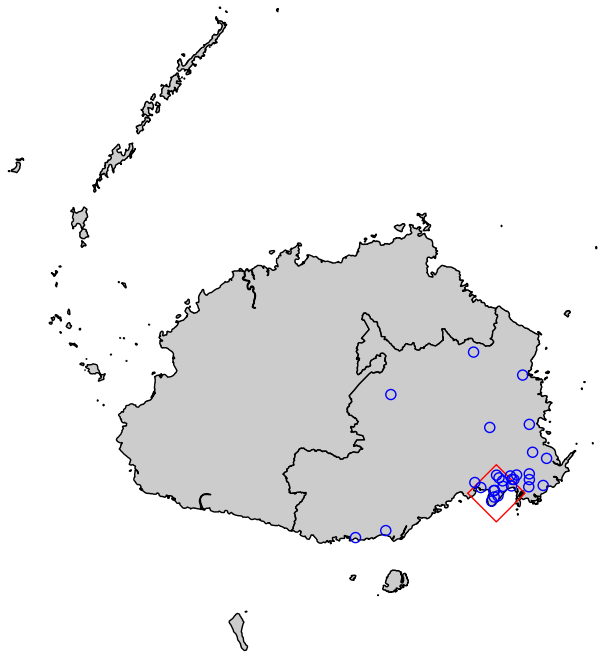

Figure 2.1: *Map of clusters of participants in serological surveys in Central Division, Fiji. Blue circles show the centroid of a cluster of 25 participants originally recruited in 2013. The red diamond shows the location of Suva, the capital and most populous city in Fiji*

Once contact had been made, a blood sample was collected and questionnaire completed at that time. In most cases however, permission was sought for a return visit at a convenient time to complete the data collection.

Field work – permissions and consent

I initially met with the Divisional Medical Officer Central (DMOC), Dr. Dave Whippy, to discuss plans for this study. I also presented the study plan to the National Taskforce for the Control of Outbreak Prone Diseases (NTCOPD) during my third week in Fiji while setting-up the study. These meetings along with continued support from established collaborators at the Ministry of Health ensured that we had administrative

support to conduct the study. The DMOC also helpfully supplied me with regional and local contacts across Central Division.

When visiting participants, essential information was provided in the form of written information sheets, which were retained by the participants. Information was also communicated by the field workers. Information sheets contained information on the purpose of the study and answered potential questions from participants. The study staff member who obtained the informed consent also signed the form.

For children age 12-17, study information was provided to the parent and the child. Written consent was obtained from both the parent and the child. If either lacked the capacity to give consent, or declined consent, then the child was not included in the study.

For children aged 1 to 11, written consent was obtained from the parent only, though information was provided to both. If the parent was unable to give consent, the child was not included in the study. Appropriate age-based professional judgement was used for children in this age range who indicated unwillingness or distress in participating in the study.

Several of the clusters of participants were located in traditional iTaukei villages. In these locations, local customs and traditions were respected and permission to enter a village was sought from a village official. This was informal acceptance in some locations but more typically it involved a *sevusevu* ceremony. A gift of dried *yaqana* roots was presented and the purpose of the study was presented. *Yaqana* is pounded into a powder and mixed with water to make a *kava* drink, a putative sedative drink which is has strong social significance in Fijian culture. Once permission was obtained from the village official, arrangements were made to come back to the village and sample as many participants as possible. These close knit village social structures often meant that many participants were located and could be sampled when we returned to the village.

Field work – implementation

We proceeded with the data collection as planned. The primary challenge was that people rarely keep the same phone number in Fiji beyond 12 months so most of our telephone records were obsolete. This increased the need to visit areas in person and work with local nurses to locate last known addresses of participants.

Another challenge was that names were often passed down over generations in the same family, especially down the male line, so cousins could often have the same name. This meant that field workers asked extra questions of participants to ensure that we were sampling the correct individual.

These serological studies were not originally designed to be longitudinal cohort studies. Permission from participants to be recontacted and followed up for further health research was obtained in both 2013 and 2015, however several participants wanted to learn about how their previous samples had helped inform health research. As a result, I outlined previous successful research with the field teams so they could communicate to participants that samples had been essential to the analysis of risk factors for typhoid fever (*Watson et al.*, 2017) and leptospirosis (*Lau et al.*, 2016) transmission in Fiji and quantifying dengue transmission during a large outbreak in 2013-14 (*Kucharski et al.*, 2018).

The majority of field visits were easily accessible and recruitment within Suva was most efficiently done on foot. Other clusters of participants could only be reached on rural roads so a four-wheeled drive vehicle was hired for the duration of the study. More complicated still were clusters that could only be accessed by boat. Local boat transport was hired to reach these sites as shown in Figure 2.2.

Recontacting participants was the largest challenge in this study and data collection was routine once contact had been made. The study purpose was outlined and the questionnaire completed before a blood sample was collected (details below). A short questionnaire asked for details of fever and rash and healthcare-seeking behaviour during the outbreak period.

Figure 2.2: *(Left) One of the study phlebotomists is led to a village field site in rural Central Division. (Right) One study team travels by boat to reach a remote cluster of participants to the East of Suva*

Completed questionnaires and fieldwork operational management paperwork were kept on paper in opaque folders or fold-over clipboards to protect confidentiality. Questionnaire data was entered using password-protected Microsoft Excel to a password protected computer and encrypted before external transfer.

Data will be kept for at least 10 years in line with LSHTM policy. Paper records will be kept at the national communicable disease surveillance centre at Mataika House. Electronic records will be kept at LSHTM, the National Data Repository at Mataika House. Mataika House records will be managed as per national surveillance data. Access to the full data set at LSHTM will be limited to the research team. Data are de-identified if shared with other researchers by removal of participant names and demographic information.

Field work – sample collection

Approximately 2ml of blood was collected using conventional clinical practices. A venous blood sample was collected using a needle & holder system or luer needle system, into the required vacutainer, as per manufacturer's directions and WHO best practice guidelines. All needles were disposed of in a designated 'sharps' bin. If the participant had veins that were difficult to palpate, or collapsed on the first fill attempt, the

phlebotomist had the option to use a 10ml syringe attached to a 23G or 25G needle.

After collection, the vacutainer was inverted gently five times to mix the blood with the tube content and left upright for 30 minutes to allow the clot to form.

Field work – sample management

Samples were transported to the laboratory at Mataika House, Suva, on the same day as collection. The sample was centrifuged for 10 to 15 minutes at 1000 to 1300 Relative Centrifugal Force (RCF) using a powered centrifuge. The samples were pipetted using aseptic technique into screw top cryovials, pre-labelled with the participant ID and participant initials according to the SOP. Two aliquots were pipetted in the following quantities: 1ml to Institut Louis Malardé (ILM), Tahiti and the remainder (approximately 1ml) to remain at Mataika House, Fiji.

Aliquoted samples were stored in freezer boxes at Mataika House before half of the samples were shipped to ILM in July 2017. The samples retained at Mataika House are to be stored for at least 10 years as a public health research serum bank. The information sheet informs participants that their samples and information may be used for other health research as determined by the Ministry of Health, as per the original survey.

2.1.6 Dissemination and follow up reporting

Preliminary results were shared with Dr. Mike Kama and his team at the Fiji Centre for Disease Control. I travelled with Dr. Adam Kucharski to Institut Louis Malardé to work directly with Dr. Van-Mai Cao-Lormeau, Dr. Maite Aubry and their team in French Polynesia. We spent two weeks combining data from Fiji and data from French Polynesia to analyse serological dynamics following ZIKV outbreaks which was later published (*Henderson et al.*, 2020) and is presented in chapter 4.

2.2 Serological testing

Serological analysis of prior DENV and ZIKV infection

There are three serological tests for previous infection with arboviruses discussed in this doctoral project: ELISA for detection of IgG antibodies, microsphere immunoassay (MIA) for IgG antibodies and plaque reduction neutralisation assays (PRNTs) for the detection of neutralising antibodies (NAbs) (Figure 2.3).

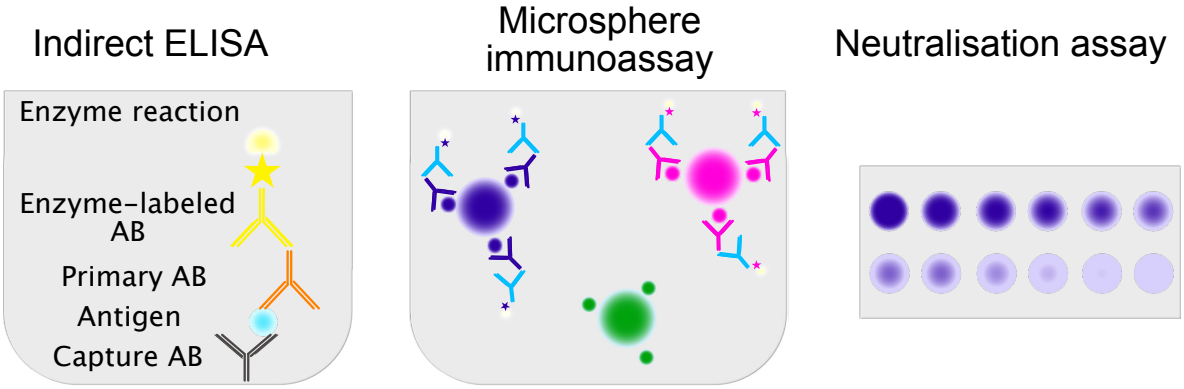

Figure 2.3: *Schematic of serological assays for detection of previous DENV and ZIKV infection used in this thesis. The ELISA test shows the process of generating a fluorescent signal if the patient's sera contains the primary antibody of interest. The higher the concentration of primary antibody in a serum sample, the stronger the fluorescent signal. The microsphere immunoassay follows a similar process but for several antigens simultaneously with colour coded fluorescent signals. The neutralisation assay uses a two-fold serial dilution of sera which is then incubated with the antigen of interest. Serum samples with higher concentrations of neutralising antibodies will prevent plaque formation at weaker dilutions*

ELISAs can detect for anti-DENV or -ZIKV IgG antibodies. Most antibodies generated following DENV or ZIKV infection are directed against the envelope (E) protein, which is composed of three domains: EDI, EDII and EDIII. EDIII mediates virus attachment to the cell membrane (*Beck et al.*, 2015; *Bhardwaj et al.*, 2001). For ELISAs, the EDIII protein for DENV-specific antigens is bound to anti-DENV IgG antibodies in a patient's serum, blood on filter paper, or saliva. These antibodies are then bound to monoclonal or polyclonal antibodies which are conjugated with an enzyme. This enzymic reaction can transform a non-coloured substrate into coloured products that can be read using

colorimetric readout (*Aubry et al.*, 2015; *Fernández and Vázquez*, 1990). In general, IgG ELISA lacks specificity within the flavivirus serocomplex group generating positive reactions for closely related flaviviruses (*Beck et al.*, 2015). This is especially true following secondary infections as newly produced antibodies have stronger avidity than antibodies produced months or years after infection (*Midgley et al.*, 2011; *Rothman*, 2011). *Theiler and Casals* (1958), demonstrated that a secondary flavivirus infection resulted in an increase in heterologous antibodies to other viruses of the same group.

Microsphere immunoassays (MIAs) use similar principles to the ELISA but are far more efficient and increasingly popular (*Anderson et al.*, 2011; *Beck et al.*, 2015; *Johnson et al.*, 2005; *van der Wal et al.*, 2012; *Wong et al.*, 2003, 2004, 2017; *Wynwood et al.*, 2015). The need to detect a specific colour in assays has complicated the ability to read multiple test results in a single sample volume. MIAs use technology based on the covalent bonding of antigen or antibody to microspheres or beads (*Mandy et al.*, 2001). By colour-coding the beads into several spectrally distinct sects, each bead can be coated with different recombinant antigens and it is possible to capture multiple binding antibodies in a single sample. This tool retains the ease and rapidity of ELISA with improved precision (*Beck et al.*, 2015). The binding of secondary antibodies and subsequent enzyme reactions follows, as before, except that the enzyme reactions are spectrally distinct for each of the antigens included in the assay. This technology allows for efficient testing of multiple viruses simultaneously as it only requires a small quantity of sample and is very quick (<3 hours compared to 3–6 days for flavivirus neutralisation tests) (*Beck et al.*, 2015).

For this doctoral project, the detection of IgG antibodies against ZIKV, DENV and CHIKV was performed using an MIA adapted from *Beck et al.* (2015). Sera were diluted 1/400 and incubated with a mix of microspheres coupled with recombinant antigens for CHIKV, DENV-1, -2, -3, -4, RRV and ZIKV. Recombinant antigens used in both assays comprised domain III of the envelope glycoprotein of ZIKV, DENV-1, DENV-2, DENV-3, or DENV-4 strains (respective GenBank accession no. KJ776791, AF226686.1, FM986654, FJ44740.1, FM986672.1). Antigens were produced using the Drosophila S2 expression system (Life Technologies, USA) as previously detailed (*Aubry*

et al., 2015). The median fluorescence intensity was read on a MagPix instrument (Bio-Rad Laboratories). The cut-off of the MIA was determined by colleagues at ILM using ROC curve analysis for all the antigens with positive and negative control sera. The positive control sera for DENV were collected within 3 months of an RT-PCR confirmed infection, or confirmed positive for all DENV serotypes by neutralisation test. The positive control sera for CHIKV and ZIKV were collected within one year of RT-PCR confirmed infection. The sensitivity and specificity of the MIA assay were respectively 100% and 100% for CHIKV, DENV-1, and DENV-3, 89.5% and 97.1% for DENV-2, 96.9% and 100% for DENV-4, and 79.6% and 94.9% for ZIKV (*Cao-Lormeau et al.*, 2016).

The third serological test studied in this thesis is the virus neutralisation test (VNT). These tests are often used as validation of results from quicker diagnostic methods such as ELISA and MIA (*Beck et al.*, 2015; *Dauphin and Zientara*, 2007). The purpose of a VNT is to test for the ability of a patient's sera to neutralise a virus. Plaque reduction neutralisation assays (PRNTs) are a form of VNT (*Roehrig et al.*, 2008; *Russell et al.*, 1967; *Schmidt et al.*, 1976) and are the gold standard for determining previous DENV exposure (*Raafat et al.*, 2019). Briefly, cell cultures are inoculated with serum that has been previously incubated with a specific antigen at serial dilutions (1:10, 1:20, ... , 1:1280). The cells are incubated for one week and if there are insufficient neutralising antibodies in the diluted sera the viral particles will enter and kill the cells creating a "plaque" of dead cells which can be counted (*Salje et al.*, 2014). The weakest dilution of sera that results in a 50% reduction in the number of plaques compared to a serum free virus was used to determine the PRNT value of a sample. The concentration of plaques can be measured by microscopic observation, fluorescent antibodies (with an ELISA) or specific dyes that react with infected cells depending on the virus (*Schmidt et al.*, 1976). This alternative process means the assay is less specific than ELISA based methods (*Beck et al.*, 2015). However, VNTs do provide a key measure of immunity to a virus given that they test the ability to neutralise the virus (*Salje et al.*, 2014), which also makes VNTs an excellent validation of previous ELISA results. *Katzelnick et al.* (2016), found that neutralising antibody titres against DENV correlated with protection from symptomatic infection in a cohort of Nicaraguan

children. *Venturi et al.* (2006), used PRNTs to monitor protective immunity against tick-borne encephalitis. PRNTs can also be used to analyse antibody dynamics in longitudinal sera (*Clapham et al.*, 2016). However, the variability in PRNT values is poorly understood and potentially important. *Salje et al.* (2014), recommend repeated testing of samples to obtain a measure of variability in the assay. The main limitation with this approach, and therefore this test, is that it is very time consuming and therefore expensive (*Beck et al.*, 2015; *Shan et al.*, 2017).

Detection of neutralising antibodies against ZIKV and each of the four DENV serotypes was performed for all serum samples in 2017 and a subset of samples in 2015 and 2013. Vero cells cultured on 96-well plates were inoculated with serial dilutions of each serum previously incubated with titrated ZIKV [PF13-251013-18], DENV-1 [PF15-080108-88], DENV-2 [PF96-300896-243/158], DENV-3 [PF90-300190-30/56] or DENV-4 [PF09-290509-104]. One week later, infected cells were detected by ELISA using primary mouse pan-flavivirus E mAb 4G2 which reacts with ZIKV E protein (*Hamel et al.*, 2015) and a secondary goat anti-mouse IgG HRP-conjugated antibody (Santa Cruz). The neutralising antibody titre was defined as the inverse of the latest serum dilution that inhibited the virus (*Cao-Lormeau et al.*, 2016).

2.3 Mathematical modelling

A deterministic compartmental SEIR model is used throughout this thesis and is introduced here. An SEIR model is an extension of the SIR model that includes a latency period for those that are 'exposed' or 'pre-infectious' (E). The intrinsic incubation period (IIP) is the duration between a human host being infected but before they are infectious. A key distinction in model structure when using compartmental models for vector-borne diseases is whether to explicitly model the mosquito population. A vector-host model is a compartmental model with additional compartments to include mosquito transmission dynamics explicitly, however this comes at the cost of estimating more parameter values in the model. *Pandey et al.* (2013), compared the performance of a vector-host model with a simple SIR model structure to estimate DENV incidence

data from Thailand. The authors found that both model structures fit the data well, but a comparison of model performance using Akaike's information criterion strongly selected the simpler SIR model. However, it may still be preferable to explicitly model the vector population, especially if the model includes interventions that are directly targeted at reducing mosquito density. In a systematic review of models of mosquito-borne pathogens, *Reiner et al.* (2013), found that 62% of models in the review explicitly modelled the mosquito population.

Two versions of an SEIR model are used in this thesis. In Chapter 5 an SEIR model with only a human population is used and described in full. Here, I will introduce the extension of this model which is used in Chapter 6 and includes a mosquito population. This model allows for two latent periods: the intrinsic incubation periods (IIP) (α_{H}) and the extrinsic incubation period (EIP) is the equivalent period in mosquitoes (α_{M}). The length of the extrinsic and intrinsic incubation period differ (*Chan and Johansson*, 2012) and the EIP is dependent on seasonal climate factors (*McLean et al.*, 1974; *Watts et al.*, 1987). The transmission rate from humans to mosquitoes, β_{M}, is equal to cp_{M} where c is the mean rate of bites per female mosquito per unit of time and p_{M} is the human-to-mosquito transmission probability. The transmission rate from mosquitoes to humans, $\beta_{\mathrm{H}} = mcp_{\mathrm{H}}$, where p_{M} is the mosquito-to-human transmission probability and m is the number of female mosquitoes per person (m) (*Chitnis et al.*, 2006; *Funk et al.*, 2016; *Manore et al.*, 2014; *Pandey et al.*, 2013). By explicitly modelling these rates separately we can allow for differences in transmission probability from human-to-mosquito and vice versa. In this model, mosquitoes are born and die in the model at a constant rate (δ) so the mosquito population size remains constant. These additional complexities require additional parameters, which in turn require more data to estimate accurately. This model is simplified by assuming that the total mosquito population size is unknown so the proportion of susceptible (s_{m}), exposed (e_{m}) and infectious (i_{m}) mosquitoes are used in the model, hence the lower case characters in the compartments. It is also assumed that mosquitoes do not recover from infection so there is no recovered compartment for mosquitoes.

The basic reproduction number from this model is equal to the product of the average

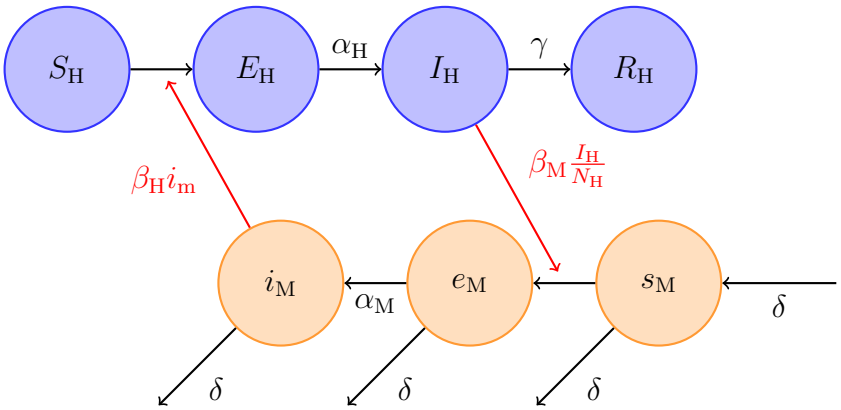

Figure 2.4: *Schematic of a vector-borne disease model. The disease spread between a human population (blue) and a vector population (orange). Humans move from being susceptible (S_H), to pre-infection (E_H), to infectious (I_H), then recover (R_H). The vector population follows the same process without a recovered compartment (s_M, e_M, i_M)*

number of mosquitoes infected by the typical infectious human and the average number of humans infected by the typical infectious mosquito (Equation 2.1) (*Diekmann et al.*, 2010; *Manore et al.*, 2014; *Van Den Driessche and Watmough*, 2002).

$$R_0 = \frac{\beta_M}{\gamma} \frac{\alpha_M}{\delta + \alpha_M} \frac{\beta_H}{\delta} \tag{2.1}$$

An important consideration in the modelling of DENV and ZIKV epidemics is the effect of seasonal changes in climate on the spread of these viruses. It has been well documented that temperature affects the vector biology of *Aedes* mosquitoes such that the transmission of viruses such as DENV and ZIKV changes (*Brady et al.*, 2014; *Lourenço and Recker*, 2014; *McLean et al.*, 1974; *Mordecai et al.*, 2017; *Watts et al.*, 1987; *Winokur et al.*, 2020). *Mordecai et al.* (2017), modelled the effect of temperature on mechanisms driving *Aedes aegypti* and *Aedes albopictus* transmission of DENV, ZIKV and CHIKV. The authors found non-linear relationships between temperature and several factors, including the extrinsic incubation period, mosquito lifespan and mosquito development rate. When combined, these effects show that transmission occurs between 18-34°C with maximal transmission occurring in a range from 26-29°C (*Mordecai et al.*, 2017).

Seasonal forcing is an important consideration in the modelling of a range of diseases and a variety of methods exist to vary transmission rates over time (*Altizer et al.*, 2006; *Bjørnstad*, 2018; *Keeling and Rohani*, 2011). In Fiji and the wider Pacific the temperature fluctuations over a year follow a sine wave-like pattern, therefore we can capture the corresponding fluctuations in transmission by using a sine function. This seasonal forcing function is characterised by a parameter for the amplitude (β_{amp}) and midpoint (β_{mid}) (Equation 2.2). Again, the model has become more complicated and a single transmission rate is not appropriate, but the model now better represents the reality of arbovirus transmission.

$$\beta(t) = \beta_0 \left(1 + \beta_{amp} \sin\left(2\pi \left(t + \beta_{mid}\right)\right)\right) \tag{2.2}$$

Several additional simplifying assumptions have been made in the model introduced here as well as those assumptions previously outlined in all Ross-Macdonald models. However, several of these assumptions are valid because of the advantages of the study setting. Epidemics in Fiji are typically self-limiting after introduction from external sources (*Cao-Lormeau et al.*, 2014; *Roth et al.*, 2014). This is likely because small, centralised and geographically isolated populations are less likely to sustain endemic transmission compared to a large populations (*Black*, 1966; *Keeling and Grenfell*, 1997). Greater heterogeneities in age structure, spatial dispersion and movement patterns in larger populations will decrease the probability of epidemic fade-out in the troughs between epidemics (*Grenfell et al.*, 1995; *Keeling and Grenfell*, 1997). Outbreaks of DENV in the Pacific have also typically been monotypic – caused by one virus or serotype of DENV – which simplifies models by removing the need to model several serotypes simultaneously (*Feng and Velasco-Hernández*, 1997; *Ferguson et al.*, 1999). Island settings also make it easier to characterise the level of susceptibility at the start of an outbreak (*Camacho et al.*, 2011).

The time frame also has implications for the structure of the model. This model is used for analysis of single outbreaks in this thesis and I do not include human births since the mean human lifespan is much longer than the outbreak duration. I have

also ignored disease induced mortality in the model because it is small (*Arima and Matsui*, 2011; *Burattini et al.*, 2008). I have assumed a simple homogeneously mixing human population with no age structure for simplicity. Age structure can be included by creating age-specific compartments in the model however this increases the number of parameters and complexity in the model (*Kucharski et al.*, 2018; *Pongsumpun and Tang*, 2003; *Supriatna et al.*, 2008). The small area and isolated nature of the Pacific islands meant that I ignored international travel during outbreaks, though this is an important consideration for studies over longer time periods (*Bhatt et al.*, 2013; *Messina et al.*, 2019). Finally, I have removed human-to-human transmission from this model because transmission of the arboviruses being studied is dominated by mosquito-borne transmission, even though sexual transmission of ZIKV is possible (*Althaus and Low*, 2016; *Foy et al.*, 2011; *Yakob et al.*, 2016).

Fitting mathematical models to data

A model output for a given set of parameter values can be compared to observed data to validate the model as well as provide estimates of unknown model parameters. We can then use these estimates to answer hypotheses or the parameters themselves might be of interest, for example estimating R_0. There are many frameworks under which models can be fit to data but this thesis will focus on Monte Carlo Markov Chains (MCMC) under a Bayesian framework. MCMC is an efficient method to sample from a posterior distribution when that distribution is unknown (*Chan*, 2013), which is often the case with epidemiological parameters of infectious disease transmission.

I want to generate samples of the parameter set of my model ($\theta = \{\beta, \gamma, ...\}$) from the posterior distribution $p(\theta|\text{data})$. Using Bayes theorem we know that this posterior distribution is proportionate to the product of the likelihood of the data given θ ($p(\text{data}|\theta)$) and prior information on θ ($p(\theta)$) (Equation 2.3). The posterior distribution ($p(\theta|\text{data})$) is a probability distribution that represents our uncertainty about θ after seeing the data. The posterior is not analytically tractable but the likelihood and prior distributions can be obtained in closed form.

$$p(\theta|\text{data}) \propto p(\text{data}|\theta)p(\theta) \tag{2.3}$$

The likelihood can also be flexible enough to combine multiple data sets. This is particularly valuable if data come from different sources and provide complimentary information, for example surveillance data of the number of cases during an outbreak and serological data of the proportion of the population infected (*Birrell et al.*, 2011; *Goubar et al.*, 2008). We know something about the observation process for these data and can define the probability of observing the data in data set 1 and data set 2 at each data point i, given the parameter set θ:

$$\log(p(\text{data}|\theta)) = \sum_i \log(p(\text{dataset1}_i|\theta)) + \sum_i \log(p(\text{dataset2}_i|\theta)) \tag{2.4}$$

Throughout this thesis surveillance data are assumed to follow a negative binomial distribution. The negative binomial (NB) distribution is a discrete probability distribution for count data that relaxes the assumption in the Poisson distribution that the mean and variance of the distribution are equal (*Lloyd-Smith*, 2007). The NB distribution has been used in several other disease modelling studies including severe acute respiratory syndrome (*Lloyd-Smith et al.*, 2005), Middle East respiratory syndrome-related coronavirus (*Kucharski and Althaus*, 2015) and DENV (*Padmanabha et al.*, 2012). The additional parameter in the NB distribution, the 'dispersion' parameter, gives greater flexibility to captures the skew in the transmission distribution because of individual variation in infectiousness. The NB distribution is preferable therefore to reflect both under- or over-reporting of case data. When fitting models to seroprevalence data I assumed that these data were binomially distributed as the result of n independent experiments with a binary outcome with probability $p = \frac{x}{n}$ where x is the positive results from a sample size n.

The prior information facilitates formal inclusion of information from previous studies in our estimation of the parameters. The prior, $p(\theta)$ quantifies our belief about the parameter via a probability distribution before comparing the model output to data. With the likelihood and prior defined, we can use Monte Carlo methods to draw de-

pendent samples from a Markov chain with $p(\theta|data)$ as its equilibrium distribution. There are several algorithms available to design a Markov chain with the required equilibrium distribution but I used the popular Metropolis-Hastings algorithm throughout this doctoral project (*Hastings*, 1970; *Metropolis et al.*, 1953).

The Metropolis-Hastings algorithm uses MCMC to sample from the posterior distribution. An initial value of θ is chosen ($\theta_0 = \theta_{t-1}$) as the 'current' sample. A 'candidate' sample, θ', is chosen randomly from a proposal distribution $g(\theta'|\theta_{t-1})$, for example a Gaussian distribution: $\theta' \sim N(\theta_{t-1}, \sigma)$. The next sample in the chain (θ_t) is selected as either the candidate θ' or current value θ_{t-1} with probability as defined in Equation 2.5. This process is repeated a large number of times to obtain a large sample of θ and in the long-run this chain will converge towards the target distribution.

$$P(\theta_t = \theta') = \min\left(1, \frac{P(\theta'|\text{data})/g(\theta'|\theta_{t-1})}{P(\theta_{t-1}|\text{data})/g(\theta_{t-1}|\theta')}\right) \tag{2.5}$$

The parameter set from arbovirus transmission models can be large so this process can be made more efficient by sampling all of θ from a multivariate Gaussian distribution. The performance of this MCMC sampler can be improved by adapting the proposal distribution. If the proposal distribution is too narrow the chain will be slow to reach the target distribution as it takes longer to explore the parameter space. If the proposal distribution is too broad then the chain will reject a lot of samples and 'stick' in the same place for many steps. *Roberts and Rosenthal* (2009), showed that mixing of the chain can be most efficient if the variance of the proposal distribution is tuned to a target acceptance rate of 0.234.

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Chapter 3

A longitudinal seroepidemiological survey of arboviruses in Fiji

Abstract

Mosquito-borne diseases such as dengue (DENV), Zika (ZIKV) and chikungunya (CHIKV) carry a large public health burden globally. However, understanding of these viruses is limited because a large proportion of infections are subclinical so are not detected by surveillance systems. Longitudinal serological data can therefore help our understanding of the quantity and timing of infections. In 2017 we collected 320 serum samples from a population-representative sample of participants from Central Division, Fiji, to combine with previous samples collected in 2013 and 2015. We found high levels of seroprevalence for all four dengue serotypes in 2017 but low levels of seroprevalence for ZIKV and CHIKV despite recent reports of transmission of these viruses. Analysis of serological data between 2013 and 2017 revealed a diverse range of serological patterns. CHIKV increased slightly 2015-17 in a predominantly naïve population. DENV-1 seroprevalence continued to increase despite an absence of recent case reports. DENV-3 and DENV-2 seroprevalence increased rapidly following an outbreak and ZIKV increased slightly then declined rapidly between 2013 and 2017. Given the challenge of accurately recording infections with these closely related viruses, longitudinal serological data reveals the diverse range of underlying outbreak dynamics.

3.1 Background

3.1.1 Arbovirus burden globally

This chapter presents details and results of a serological study of arbovirus burden in Fiji in 2017. Arboviruses such as dengue virus (DENV), Zika virus (ZIKV), chikungunya virus (CHIKV) and Ross River virus (RRV) present a large global health burden. Our understanding and ability to quantify how widely these viruses transmit, and interact with each other, can be improved through the collection of serological data.

DENV and ZIKV are two flaviviruses primarily transmitted by the *Aedes* genus of mosquito. The incidence of DENV transmission has increased 30-fold in the past 50 years (*World Health Organisation*, 2009). There is evidence that infection with one of the four DENV serotypes provides lifelong immunity for that serotype (*Halstead*, 1974) and temporary heterogeneous immunity against all serotypes (*Sabin*, 1952). An estimated 20-50% of cases are symptomatic (*Bhatt et al.*, 2013; *Endy et al.*, 2002; *Funk et al.*, 2016) so surveillance and modelling of the virus is complicated.

ZIKV is related to other flaviviruses such as DENV (*Hayes*, 2009; *Priyamvada et al.*, 2016). Symptoms of ZIKV infection include low grade fever, rash and conjunctivitis (*Heukelbach et al.*, 2016). ZIKV has additionally been associated with birth defects and neurological complications such as microcephaly (*Cauchemez et al.*, 2016; *Schuler-Faccini et al.*, 2016) and Guillain-Barré Syndrome (GBS) (*Cao-Lormeau et al.*, 2016). ZIKV spread throughout the Pacific between 2007 and 2016 (*Craig et al.*, 2016; *Musso and Gubler*, 2016) and the largest outbreak was in French Polynesia in 2013 (*Cao-Lormeau et al.*, 2014b). ZIKV spread to the Americas and was declared a Public Health Emergency of International Concern in 2016 (*World Health Organisation*, 2016). Between 2015 and 2017 there were fewer than one million confirmed or suspected ZIKV cases reported (*Pan American Health Organization*, 2018) but over 100 million people were estimated to have been infected (*Moore et al.*, 2019).

CHIKV can also be transmitted by the *Aedes* genus of mosquito, primarily *Aedes ae-*

gypti. Unlike ZIKV and DENV, which are flaviviruses, CHIKV is an alphavirus that causes an acute febrile syndrome and severe, debilitating rheumatic disorders in humans that may persist for months (*Wimalasiri-Yapa et al.*, 2019). Reports from the Americas demonstrate that all three of these arboviruses – CHIKV, DENV and ZIKV – are able to cocirculate in the same location (*Carrillo-Hernández et al.*, 2018).

Finally, RRV is an arbovirus and alphavirus like CHIKV. Infection with RRV may cause disease in humans, typically presenting as peripheral polyarthralgia or arthritis, sometimes with fever and rash (*Harley et al.*, 2001). There have been sporadic reports of RRV outbreaks in Pacific islands countries and territories (PICTs) along with evidence of silent circulation of RRV in French Polynesia (*Aubry et al.*, 2015b).

There is an urgent need to better understand arbovirus transmission dynamics and island populations present a unique opportunity to do this. Small populations are less likely to sustain endemic transmission so the timing of outbreaks can be well defined (*Black*, 1966; *Keeling and Grenfell*, 1997). Island populations are currently less likely to sustain endemic transmission. However it is important to understand whether these viruses will continue to pass through island populations as large but brief epidemics, or exhibit longer-term 'slow burn' dynamics and become endemic as DENV has in areas of Southeast Asia (*Bhatt et al.*, 2013; *Jentes et al.*, 2016; *Salje et al.*, 2019; *Shepard et al.*, 2016). In particular it is not well understood how infection with multiple arboviruses can affect the immune response to a novel but closely related virus in individuals and in populations.

To better understand the burden of different diseases in the Pacific there are multiple sources of data available. Surveillance data provides data on the number of people infected with a particular disease and when they presented with symptoms. A syndromic surveillance system is used in the Pacific to rapidly detect and assess infectious disease outbreaks (*Craig et al.*, 2016; *Kool et al.*, 2012). This system is implemented across twenty-one countries in the Pacific. Surveillance data provides important information on the cause, timing and size of disease outbreaks. However, surveillance data for these arboviruses are incomplete because some people will never show symptoms, some will never report at a health centre especially if symptoms are mild and data can be entered

incorrectly.

Individuals who are infected with these arboviruses develop specific and cross-reactive neutralising antibodies to the virus that can be detected years later (*Gubler*, 2004; *Halstead et al.*, 1983; *Imrie et al.*, 2007; *Tesh et al.*, 1975). Serological surveys can be used to detect antibodies against different viruses in serum samples and therefore characterise the proportion of the population that has likely been exposed to the virus at some point, regardless of whether the host experienced symptoms. These estimates can be used to estimate the true burden of viral infections after an arbovirus outbreak and to estimate the potential for that virus to emerge in a population (*Aubry et al.*, 2018; *Rosen*, 1958; *Salje et al.*, 2018; *Succo et al.*, 2018). Serological data can be used to monitor risks to public health by estimating population immunity, for example with ZIKV in Kenya where serum collected between 2007 and 2014 showed no evidence of prior exposure to ZIKV (*Kisuya et al.*, 2019). In the case of ZIKV the case definition has been shown to miss the majority of ZIKV infections. Evidence from longitudinal serological data collected in child participants in Managua, Nicaragua, showed evidence that the majority of ZIKV infections would not be recorded as ZIKV cases under the WHO case definition (*Burger-Calderon et al.*, 2020). This is a demonstration of the potential to study serological data to better understand the epidemiology of arboviruses.

3.1.2 Previous serological surveys of arboviruses in Fiji

This chapter presents results from a serological survey I led in 2017. This study built on two recent serological studies conducted in Fiji and aimed to sample repeated measurements from previous participants to estimate arbovirus burden and transmission dynamics in Fiji. In 2013 *Watson et al.* (2017) collected 1,781 samples from across the two main islands of Fiji with 695 of them collected from Central Division. They characterised the epidemiology of typhoid fever and *Lau et al.* (2016), used the samples to analyse risk factors for leptospirosis transmission in Fiji.

In 2013, the study team conducted a representative, clustered, cross-sectional seroepidemiological survey of the Fijian mainland. Population density sampling methods were

used and in Central Division samples were collected in clusters of twenty-five participants. The age of the sampled participants ranged from 1 to 85 years old. To ensure the sample was population representative nursing zones serving approximately 1,000 to 10,000 people were selected with probability proportional to population size (*Bennett et al.*, 1991). Random number generation using Ministry of Health administrative records was used. Households were randomly selected and an occupant aged $\geq$1 year was randomly selected. In Central Division, age-stratified sampling was used for representativeness across age groups. This original study was not focused on arboviruses but the serum samples collected were subsequently tested for evidence of previous infection with chikungunya virus (CHIKV), dengue viruses serotypes 1, 2, 3, 4 (DENV-1, -2, -3, -4), Ross River virus (RRV) and Zika virus (ZIKV).

A large DENV-3 outbreak began after this original serosurvey with suspected cases reported between October 2013 and August 2014. To better understand the outbreak dynamics a follow-up serological study was conducted in November 2015. 333 or the same participants were resampled in 2015 to collect a data set of pre- and post-outbreak serology. Between May and June 2017, I led a team of public health researchers and phlebotomists to collect paired samples from the same individuals sampled in 2013, or tripled samples if the individual was also sampled in 2015.

The two studies conducted in 2013 and 2015 add to a limited evidence base on post-outbreak seroprevalence for DENV (*Kucharski et al.*, 2018). There were two historic seroprevalence estimates available but these studies estimated dramatically different post-outbreak seroprevalence of 25% in 1971-3 following a DENV-2 epidemic (*Maguire et al.*, 1974) and 54% in 1989-90 after a predominantly DENV-1 epidemic (*Waterman et al.*, 1993) despite similar numbers of cases in both outbreaks, 3,413 and 3,686 respectively.

A modelling analysis of serum samples collected in 2015 showed evidence that herd immunity alone was not sufficient for a DENV-3 outbreak to end. *Kucharski et al.* (2018), found that seasonal variation and vector control measures contributed to the end of the 2013-14 DENV-3 outbreak. This demonstrates the potential for serological data to be used to infer transmission dynamics of a disease, as well as estimating the

burden of the disease.

Another key contribution from these previous serological surveys was the identification of low-level ZIKV circulation. ZIKV was first recorded in surveillance data in July 2015 (*Kama et al.*, 2019) and 16 cases were confirmed in total between 2015 and 2017 in Central Division. An outbreak of ZIKV was never declared in Fiji. However, there was evidence in serological data in 2015 that ZIKV transmission was widespread in Fiji because an estimated 21.9% of the population were seropositive for ZIKV in 2015 and phylogenetic analysis found evidence that ZIKV was introduced to Central Division in late 2013 (*Kama et al.*, 2019). Serological data can provide insight into outbreaks that are not possible with surveillance data alone.

3.1.3 Expanding uses of serology

Serological data are a vital tool in epidemiological research. When only a single sample is available for an individual, a threshold titre is often used as evidence of prior exposure or protection or both. For example seroprevalence estimates of ZIKV in Fiji in 2015 (*Kama et al.*, 2019) and RRV in French Polynesia between 2011 and 2013 (*Aubry et al.*, 2015b) both showed evidence of silent circulation of these viruses. *Salje et al.* (2016), reconstructed sixty years of CHIKV transmission in the Philippines from serological data. Age stratified serological data can be useful to analyse previous exposure in a location and if we assume that low seroprevalence is equivalent to high susceptibility in a certain age group, they can be used to detect potential future outbreaks (*Aubry et al.*, 2017).

To extend this, if multiple samples are available from the same individual it is possible to gain more insight from serological data than simply estimating previous exposure. When only one serum sample is available, a threshold titre is often used to define participants as seropositive or seronegative. These threshold values can be used to infer past exposure or protection or both (*Hay et al.*, 2020). With multiple samples from the same individuals, longitudinal serology can be used to estimate infection times or attack rates, as has been done for influenza (*Wu et al.*, 2014). These approaches have

been used successfully with arboviruses. *Salje et al.* (2018), used longitudinal data in a cohort from Thailand to detect sub-clinical infection of DENV. Longitudinal serology, when paired with knowledge of circulating viruses over the same period, can also help us better understand the complex cross-reactions of antibodies following flavivirus infections (*Montoya et al.*, 2018). Generalised tools have been developed to enable analysis of longitudinal serological data to better understand the immunology and epidemiology of immune responses following infection (*Hay et al.*, 2020).

3.1.4 2017 serological study outline

The primary outcome of the 2017 study was to characterise ZIKV transmission by estimating seroprevalence and analysing changes in neutralising antibodies, but secondary outcomes were prevalence and titres against other arboviruses. Before we collected samples in 2017 we knew that there were 16 confirmed cases of locally acquired ZIKV in Central Division, Fiji, between 2015 and 2016. We also had estimated that 21.9% of the population were seropositive by multiplex immunoassay for ZIKV in samples collected in November 2015 (*Kama et al.*, 2019). The serological data indicated that ZIKV was circulating before cases were detected by surveillance systems. Additionally, at the time it appeared that major ZIKV epidemics on islands – such as in French Polynesia in 2013-14 (*Cao-Lormeau et al.*, 2014b) and Micronesia 2007 (*Duffy et al.*, 2009) – infected the majority of the population and hence there was sufficient herd immunity to prevent outbreaks in the near future (*Kucharski et al.*, 2016). The situation in Fiji however was less clear. Data from 2015 suggested that there had not been a large ZIKV outbreak in Fiji yet. However, these results could have been consistent with a large epidemic that was beginning when data were collected in 2015, or low level ZIKV circulation over multiple years. We wanted to test more serum samples to characterise ZIKV transmission over this period.

Representative pre- and post-epidemic paired data are extremely rare for ZIKV, so our study presents a unique opportunity to investigate this pathogen – and its interaction with related arboviruses – at multiple time points during an outbreak. Our study

aimed to answer three main public health questions about ZIKV and other circulating arboviruses. Firstly, what was the extent of ZIKV infection in Fiji and association with other arboviruses? Paired serology would show what proportion of the population are likely to have been infected between 2015-17 and how infection was distributed spatially. Additionally, we could estimate risk factors for ZIKV infection and potential association with serological evidence of other arbovirus infections, such as DENV.

Secondly, what proportion of ZIKV infections were reported and were they related to other health complications? Sample collection would be accompanied by a health questionnaire. This would make it possible to estimate the proportion of cases that were asymptomatic, as well as health-seeking behaviour such as visiting their doctor or healthcare centre.

Finally, what is the level of immunity in the population and what is the potential for ZIKV to transition to an endemic state? Serological analysis would show how many people remain susceptible to ZIKV infection and whether a threshold of herd immunity has been reached. If the level of immunity in the population is too low to provide herd immunity then it is possible that the effective reproduction number for ZIKV in Fiji equals one and transmission could have become endemic.

3.2 Materials & Methods

Data collected in 2013 (*Lau et al.*, 2016; *Watson et al.*, 2017) and 2015 (*Kama et al.*, 2019) were combined with a third seroepidemiological survey in 2017 (*Henderson et al.*, 2020). The data collection in 2017 has been described in detail in Chapter 2. Briefly, participants from previous serological surveys in Central Division were recruited for a third round of data collection between May and July 2017.

Serum samples were tested at Institut Louis Malardé (ILM) in French Polynesia. Testing has previously been described in several publications (*Aubry et al.*, 2015a, 2017; *Beck et al.*, 2015; *Cao-Lormeau et al.*, 2016; *Henderson et al.*, 2020; *Kama et al.*, 2019; *Kucharski et al.*, 2018; *Watson et al.*, 2017) and is described in Section 2.2. Briefly, all

320 serum samples collected in 2017 were tested for detection of immunoglobin class G (IgG) antibodies against CHIKV, DENV, RRV, and ZIKV using a recombinant-antigen based microsphere immunoassay (MIA).

3.2.1 Data analysis and serological modelling

I combined all data available from the three serological surveys in 2013, 2015 and 2017. In total, over the three studies we collected 12,850 measurements of antibodies against ZIKV, the four DENV serotypes, CHIKV and RRV. Seroprevalence was calculated using `prop.test` in `R`. Tests for association for categorical variables were initially performed with a χ^2 test.

Explanatory variables with evidence of an association with the response variable from the χ^2 test were identified as potential risk factors. I wanted to analyse risk factors for arbovirus seroprevalence in 2017. A multivariable model was developed from univariable risk factors with p values of less than 0.25, after-regrouping sparse cells for numerical stability, using a backward step-wise approach, removing variables with $p > 0.1$ from a likelihood-ratio test (package `lmtest` (*Hothorn et al.*, 2019)), with deletion of observations with missing data.

To analyse non-linear relationships between seroprevalence (as measured by MIA) and age (as measured by year of birth) I fit a Generalised Additive Model (GAM) (*Hastie and Tibshirani*, 1986; *Wood*, 2017). I defined an explanatory variable X as the year of birth and I defined serostatus as measured by MIA as the dependent variable Y in the model, as follows:

$$g\left(E\left(Y\right)\right) = \alpha + s_1\left(X\right) \tag{3.1}$$

Where $E(Y)$ denotes the expected value, and $g(Y)$ denotes the link function, in this case the `logit` function because of the binary outcome. The term $s_1(X)$ defines a non-parametric function to model the non-linear relationship between X and Y and α is the intercept value when $X = 0$. I used the `mgcv` package in `R` so smooth functions

were represented using penalised regression splines (*Wood*, 2019).

3.3 Results

3.3.1 Study population

We collected 320 blood samples in 2017 from individuals in Central Division, Fiji, that had been previously sampled in 2013. Our sample size decreased between 2013 and 2017, from 455 samples collected and tested in 2013, to 333 in 2015 and 320 in 2017 (Table 3.1). This presented us with a large data set to analyse arbovirus dynamics in Fiji. To ensure comparability across the three data sets we wanted to validate that our samples were similarly representative of the general population as they had been in 2013.

We were particularly interested in the age distribution of the samples between surveys. Age is strongly related to exposure to arboviruses and we expect seroprevalence to increase with age. We therefore wanted each sample to be representative of the national population. The sampling methods used in 2013 ensured that the collected sample closely reflected the age distribution from census data (Figure 3.1). The samples collected in 2015 and 2017 both included a higher proportion of children between 5 and 20 than are in the general population, suggesting that this age group was easier to recontact and sample in follow up surveys. The sample collected in 2017 had a similar age distribution to the 2013 study and 2007 census data for adults older than 20 (Figure 3.1).

As well as age, we wanted to collect a sample that was comparable across other demographic variables. Table 3.1 shows a breakdown of the study population in each sampling year by key demographic variables and Figure 3.2 shows these data graphically for four key variables: sex, rurality, ethnicity and occupation. Broadly, the three samples collected in 2013, 2015 and 2017 have similar distributions for age, sex, household size, ethnicity. In 2017 43.8% of the sample were aged under 20, 57% were female

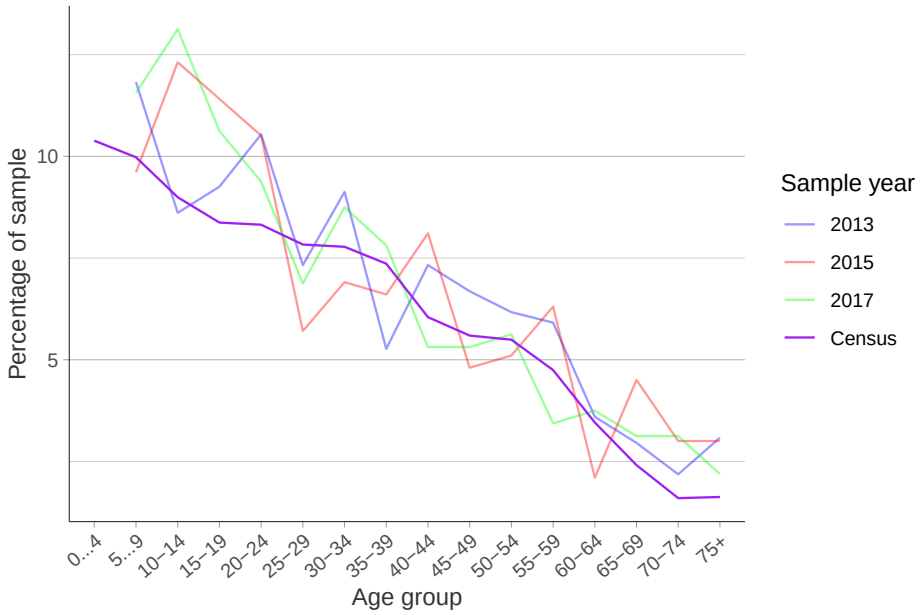

Figure 3.1: *Age distribution of Fijian population from 2007 census data compared to the age distribution of serological surveys conducted in Central Division in 2013, 2015 and 2017*

and 43% male, 42% of households had 4 or fewer occupants and 83.4% of the sample were iTaukei ethnicity.

The main occupation of participants in the 2017 sample was similar to the previous surveys in Fiji, although the proportion of our 2017 sample that were in out-of-home work environments (neither at school or full time home keepers) did decrease (Figure 3.2D). 50% of study participants buried their garbage while 44% had it collected and approximately one third (31%) of participants travelled regularly, 4-6 days per week (Table 3.1).

Table 3.1: *Characteristics of the serological surveys conducted in Central Division, Fiji, in 2013, 2015 and 2017*

Variable	Level	2013	2015	2017
Total		455 [100%]	333 [100%]	320 [100%]
Age (in 2017)	(5,10]	56 [12.3%]	32 [9.61%]	37 [11.6%]
	(10,15]	42 [9.23%]	41 [12.3%]	42 [13.1%]
	(15,20]	47 [10.3%]	38 [11.4%]	34 [10.6%]
	(20,40]	145 [31.9%]	99 [29.7%]	105 [32.8%]
	(40,60]	108 [23.7%]	81 [24.3%]	63 [19.7%]
	(60+)	57 [12.5%]	42 [12.6%]	39 [12.2%]
Sex	Male	207 [45.5%]	143 [42.9%]	139 [43.4%]
	Female	248 [54.5%]	190 [57.1%]	181 [56.6%]
Rurality	Peri-Urban	94 [20.7%]	77 [23.1%]	62 [19.4%]
	Rural	157 [34.5%]	113 [33.9%]	135 [42.2%]
	Urban	204 [44.8%]	143 [42.9%]	123 [38.4%]
Household	(0,4]	227 [49.9%]	154 [46.2%]	135 [42.2%]
	(4,8]	205 [45.1%]	159 [47.7%]	157 [49.1%]
	(8,12]	22 [4.84%]	18 [5.41%]	21 [6.56%]
	(12,16]	1 [0.22%]	.	.
	Missing	.	2 [0.601%]	7 [2.19%]
Ethnicity	Indo-Fijian	67 [14.7%]	56 [16.8%]	49 [15.3%]
	iTaukei	378 [83.1%]	269 [80.8%]	267 [83.4%]
	Other	10 [2.2%]	8 [2.4%]	4 [1.25%]
Job	Farming	34 [7.47%]	21 [6.31%]	28 [8.75%]
	Housewife / husband	126 [27.7%]	95 [28.5%]	94 [29.4%]

Cont.

Table 3.1: *Characteristics of the serological surveys conducted in Central Division, Fiji, in 2013, 2015 and 2017*

Variable	Level	2013	2015	2017
	Other	20 [4.4%]	14 [4.2%]	14 [4.38%]
	Pre-School Child	50 [11%]	27 [8.11%]	32 [10%]
	Office Worker	17 [3.74%]	12 [3.6%]	9 [2.81%]
	Student	121 [26.6%]	102 [30.6%]	95 [29.7%]
	Retired	28 [6.15%]	18 [5.41%]	13 [4.06%]
	Skilled Manual Worker	35 [7.69%]	24 [7.21%]	21 [6.56%]
	Unemployed	22 [4.84%]	19 [5.71%]	13 [4.06%]
	Missing	2 [0.44%]	1 [0.3%]	1 [0.312%]
Home material	Concrete / Brick	209 [45.9%]	143 [42.9%]	105 [32.8%]
	Corrugated Iron	134 [29.5%]	105 [31.5%]	121 [37.8%]
	Other	5 [1.1%]	5 [1.5%]	6 [1.88%]
	Wood	105 [23.1%]	80 [24%]	88 [27.5%]
	Missing	2 [0.44%]	.	.
Garbage	Buried	197 [43.3%]	141 [42.3%]	161 [50.3%]
	Collected	236 [51.9%]	169 [50.8%]	142 [44.4%]
	Other	22 [4.84%]	23 [6.91%]	17 [5.31%]
Travel	Every Day	5 [1.1%]	5 [1.5%]	7 [2.19%]
	4 To 6 Days Per Week	147 [32.3%]	110 [33%]	100 [31.2%]
	1 To 3 Days Per Week	78 [17.1%]	56 [16.8%]	54 [16.9%]
	Once A Month Or More	141 [31%]	96 [28.8%]	96 [30%]
	Less Than Once A Month	72 [15.8%]	55 [16.5%]	52 [16.2%]
	Never	9 [1.98%]	9 [2.7%]	8 [2.5%]
	Missing	3 [0.659%]	2 [0.601%]	3 [0.938%]

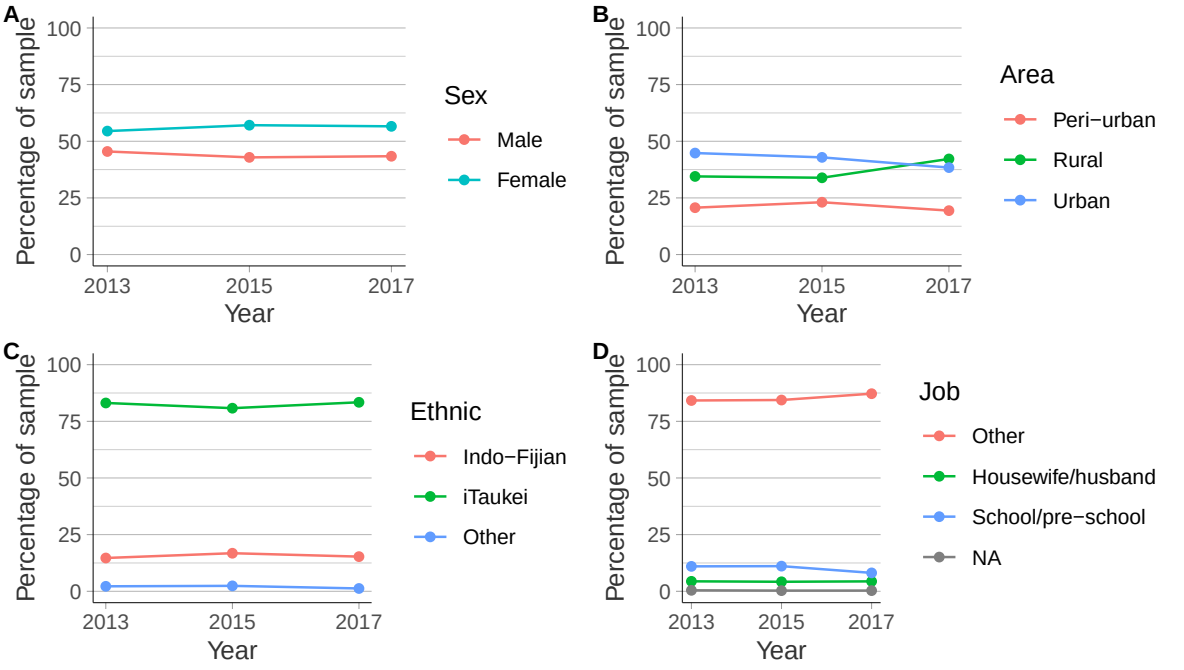

Figure 3.2: *Characteristics of serological survey samples in Central Division, Fiji. A, sex distribution in samples between 2013 and 2017. B, rurality. C, ethnicity. D, occupation*

The most notable deviation from previous sampling proportions in our 2017 sample was with respect to rurality. The proportion of the 2017 sample from rural locations increased to 42% in 2017 from previous levels in 2015 (34%) and 2013 (35%). Follow up success rates were higher in rural areas compared to urban areas in 2017. 157 of the original participants from 2013 lived in rural areas and in 2017 we resampled 86% (135) of these participants. In peri-urban and urban areas, that percentage decreased to 66% and 60.3% respectively. Figure 3.3 shows the follow-up success in rural areas compared to the capital Suva. We typically resampled a higher proportion of participants in clusters away from the capital and in more remote areas.

We also wanted to monitor the reason for loss to follow up in our study. We attempted to contact all participants from the 2013 survey in each subsequent survey and did not replace participants in our follow-up survey if they were lost to follow up. We collected data on the reason a participant from 2013 was not sampled in follow-up studies in 2015 and 2017. In 2015, 144 people were not resampled and the main reason for failing to sample an individual was that they had moved home or could not be found. By 2017, it was increasingly hard to find participants and 215 participants were not

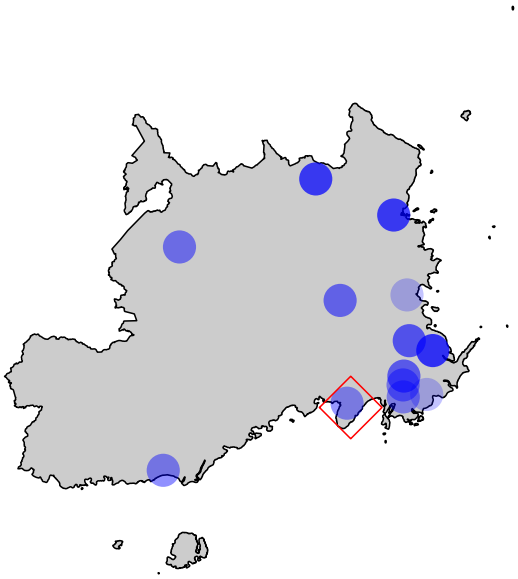

Figure 3.3: *Follow up success rate in 2017 across Central Division. Dots are located at the centroid of a cluster of 25 participants originally sampled in 2013. The opaqueness of the dots indicated the percentage of that cluster successfully resampled in 2017. Darker indicates a higher percentage were successfully resampled. All clusters in Suva (red diamond) have been grouped for simplicity. We typically resampled a higher percentage (darker blue) of participants in rural clusters away from the capital*

resampled. It was also more difficult to ascertain whether the participant had moved home (Figure 3.4). Loss to follow-up in our cohort due to death was small. However, a larger proportion of potential participants refused to participate in the 2017 study than had done in 2015.

3.3.2 Flavivirus seroprevalence in Fiji in 2017

We estimated that the most prevalent virus in Fiji was DENV-1 with an estimated 74% (95% CI: 68.9-78.8%) of the population seropositive. While there have been no reported outbreaks of DENV-1 in Fiji between 2013 and 2017, this high seroprevalence could be a result of the large DENV-1 epidemic between 2001 and 2004 (*Singh et al.*, 2005). Seroprevalence against other DENV serotypes was below 50% for each. Only 25% (95% CI: 21-30.8%) were seropositive against DENV-2, despite widespread transmission in

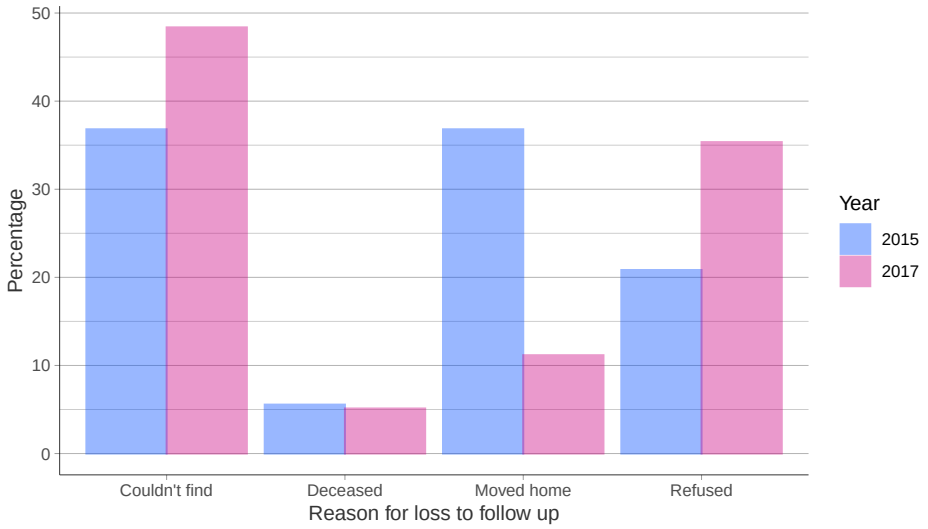

Figure 3.4: *Reasons for loss to follow up in serological surveys in 2015 and 2017*

early 2017. Seroprevalence against DENV-3 and DENV-4 was estimated to be 45% (DENV-3 95% CI: 39.8-50.9%) (DENV-4 95% CI: 39.5-50.6%), however there were no reports of DENV-4 outbreaks in Fiji between 2013 and 2017 but there was a large DENV-3 outbreak in 2013-14 (*Cao-Lormeau et al.*, 2014a). Seroprevalence against CHIKV (12.5%; 95% CI: 9.4-17%) and ZIKV (12.5%; 95% CI: 9.1-16.6%) was low in 2017 and seroprevalence against Ross River virus was 39% (95% CI: 33.7-44.6%), consistent with endemic circulation of RRV in Fiji (*Aubry et al.*, 2019a) (Table 3.2).

I investigated the relationship between demographic data collected from questionnaires in 2013 and arbovirus seroprevalence in 2017. I simplified analysis by focusing on seropositivity to at least one DENV serotype instead of trying to analyse trends in seroprevalence across all arboviruses that were tested. Overall, 289 of the 320 participants (90.3%; 95% CI: 86.5-93.3%) were seropositive to at least one DENV serotype in 2017. I used a χ^2 test for uniform relationship between categorical variables (age, sex, rurality etc.) and seroprevalence to at least one DENV serotype in 2017. I found strong evidence that seroprevalence to at least one DENV serotype was different across groups of age, rurality, occupation and garbage removal method (Table 3.2). The relative contribution of these factors to risk of arbovirus infection is not known from these data and there is likely to be strong confounding between groups. For example, younger participants were more likely to be at school, and garbage removal method depends

on the urbanisation of the area. Seroprevalence to at least one DENV serotype increased with age from 51.4% (95% CI: 34.4-68.1%) in those between 5 and 10 years old, to 97.4% (95% CI: 86.5-100%) in those over 60. Participants in rural areas had lower seroprevalence for at least one DENV serotype compared to those living in urban or peri-urban settings. There was weak evidence that seroprevalence to at least one DENV serotype varied by ethnicity with Indo-Fijian seroprevalence to at least one DENV serotype higher than for iTaukei Fijians ($p = 0.1$). Seroprevalence to at least one DENV serotype varied by job, but this is likely confounded by age since it was highest for retired participants (100%; 95% CI: 75.3-100%) and lowest for students (69.5%; 95% CI: 59.2-78.5%) and pre-school children (50%; 95% CI: 31.9-68.1%). Finally, seroprevalence varied by garbage collection method and was highest for those that had garbage collected (90.1%; 95% CI: 84-94.5%) versus buried (70.2%; 95% CI: 62.5-77.1%). Garbage collection points are typically open areas at the end of residential streets and could plausibly lead to increased mosquito breeding grounds making arbovirus infection more likely. There was no evidence of a difference in seroprevalence to at least one DENV serotype by sex, home material or household size (Table 3.2 & Figure 3.5).

Table 3.2: *Arbovirus seroprevalence in Fiji in 2017. Number seropositive defined by MIA [percentage of sample]. CHIKV, chikungunya virus. DENV-1,-2,-3,-4, dengue virus serotypes 1,2,3,4. ZIKV, Zika virus. RRV, Ross River Virus.* ≥ 1 *DENV, seropositive to at least one DENV serotype). p,* χ^2 *test comparing seroprevalence to at least one DENV serotype across all levels of that variable (the null hypothesis is that seroprevalence is the same across all levels in that variable. Smaller p values therefore present stronger evidence against this null hypothesis and evidence that there is an association between that variable and seroprevalence to at least one DENV serotype)*

Variable	Level	N	CHIKV	DENV1	DENV2	DENV3	DENV4	RRV	ZIKV	≥ 1 DENV	p
Total	Total	320	41 [12.8]	237 [74.1]	82 [25.6]	145 [45.3]	144 [45]	125 [39.1]	40 [12.5]	289 [90.3]	-
Age	(5,10]	37	1 [2.7]	14 [37.8]	9 [24.3]	15 [40.5]	9 [24.3]	10 [27]	5 [13.5]	19 [51.4]	
	(10,15]	42	10 [23.8]	22 [52.4]	10 [23.8]	14 [33.3]	17 [40.5]	10 [23.8]	10 [23.8]	27 [64.3]	
	(15,20]	34	6 [17.6]	24 [70.6]	5 [14.7]	15 [44.1]	17 [50]	10 [29.4]	9 [26.5]	26 [76.5]	***
	(20,40]	105	17 [16.2]	83 [79]	24 [22.9]	48 [45.7]	53 [50.5]	38 [36.2]	4 [3.81]	87 [82.9]	
	(40,60]	63	4 [6.35]	56 [88.9]	20 [31.7]	32 [50.8]	29 [46]	36 [57.1]	5 [7.94]	59 [93.7]	
	(60+)	39	3 [7.69]	38 [97.4]	14 [35.9]	21 [53.8]	19 [48.7]	21 [53.8]	7 [17.9]	38 [97.4]	
Sex	Male	139	21 [15.1]	101 [72.7]	35 [25.2]	65 [46.8]	60 [43.2]	54 [38.8]	18 [12.9]	107 [77]	0.3
	Female	181	20 [11]	136 [75.1]	47 [26]	80 [44.2]	84 [46.4]	71 [39.2]	22 [12.2]	149 [82.3]	

Cont

Table 3.2: *Arbovirus seroprevalence in Fiji in 2017. Number seropositive defined by MIA [percentage of sample]. CHIKV, chikungunya virus. DENV-1,-2,-3,-4, dengue virus serotypes 1,2,3,4. ZIKV, Zika virus. RRV, Ross River Virus.* ≥ 1 *DENV, seropositive to at least one DENV serotype). p,* χ^2 *test comparing seroprevalence to at least one DENV serotype across all levels of that variable (the null hypothesis is that seroprevalence is the same across all levels in that variable. Smaller p values therefore present stronger evidence against this null hypothesis and evidence that there is an association between that variable and seroprevalence to at least one DENV serotype)*

Variable	Level	N	CHIKV	DENV1	DENV2	DENV3	DENV4	RRV	ZIKV	≥ 1 DENV	p
Rurality	Peri-Urban	62	12 [19.4]	52 [83.9]	20 [32.3]	38 [61.3]	39 [62.9]	28 [45.2]	11 [17.7]	53 [85.5]	
	Rural	135	1 [0.741]	81 [60]	23 [17]	38 [28.1]	39 [28.9]	65 [48.1]	12 [8.89]	89 [65.9]	***
	Urban	123	28 [22.8]	104 [84.6]	39 [31.7]	69 [56.1]	66 [53.7]	32 [26]	17 [13.8]	114 [92.7]	
Household	(0,4]	135	14 [10.4]	106 [78.5]	36 [26.7]	63 [46.7]	61 [45.2]	57 [42.2]	23 [17]	112 [83]	
	(4,8]	157	25 [15.9]	111 [70.7]	39 [24.8]	70 [44.6]	71 [45.2]	57 [36.3]	16 [10.2]	120 [76.4]	0.46
	(8,12]	21	2 [9.52]	15 [71.4]	6 [28.6]	8 [38.1]	9 [42.9]	8 [38.1]	0 [0]	18 [85.7]	
	Missing	7	0 [0]	5 [71.4]	1 [14.3]	4 [57.1]	3 [42.9]	3 [42.9]	1 [14.3]	6 [85.7]	
Ethnicity	Indo-Fijian	49	15 [30.6]	42 [85.7]	15 [30.6]	27 [55.1]	27 [55.1]	10 [20.4]	7 [14.3]	44 [89.8]	
	iTaukei	267	26 [9.74]	191 [71.5]	66 [24.7]	115 [43.1]	114 [42.7]	114 [42.7]	32 [12]	208 [77.9]	0.1
	Other	4	0 [0]	4 [100]	1 [25]	3 [75]	3 [75]	1 [25]	1 [25]	4 [100]	

Cont

Table 3.2: *Arbovirus seroprevalence in Fiji in 2017. Number seropositive defined by MIA [percentage of sample]. CHIKV, chikungunya virus. DENV-1,-2,-3,-4, dengue virus serotypes 1,2,3,4. ZIKV, Zika virus. RRV, Ross River Virus.* ≥ 1 *DENV, seropositive to at least one DENV serotype). p,* χ^2 *test comparing seroprevalence to at least one DENV serotype across all levels of that variable (the null hypothesis is that seroprevalence is the same across all levels in that variable. Smaller p values therefore present stronger evidence against this null hypothesis and evidence that there is an association between that variable and seroprevalence to at least one DENV serotype)*

Variable	Level	N	CHIKV	DENV1	DENV2	DENV3	DENV4	RRV	ZIKV	≥ 1 DENV	p
Job	Farming	28	2 [7.14]	26 [92.9]	6 [21.4]	12 [42.9]	13 [46.4]	19 [67.9]	2 [7.14]	26 [92.9]	
	Housewife / husband	94	9 [9.57]	81 [86.2]	29 [30.9]	44 [46.8]	50 [53.2]	49 [52.1]	6 [6.38]	84 [89.4]	
	Other	14	4 [28.6]	13 [92.9]	3 [21.4]	7 [50]	6 [42.9]	3 [21.4]	3 [21.4]	13 [92.9]	
	Pre-School Child	32	1 [3.12]	12 [37.5]	8 [25]	12 [37.5]	8 [25]	7 [21.9]	5 [15.6]	16 [50]	***
	Office Worker	9	0 [0]	6 [66.7]	2 [22.2]	5 [55.6]	5 [55.6]	2 [22.2]	0 [0]	8 [88.9]	
	Student	95	18 [18.9]	56 [58.9]	16 [16.8]	37 [38.9]	38 [40]	24 [25.3]	19 [20]	66 [69.5]	
	Retired	13	0 [0]	13 [100]	6 [46.2]	8 [61.5]	7 [53.8]	6 [46.2]	4 [30.8]	13 [100]	
	Skilled Manual Worker	21	3 [14.3]	17 [81]	8 [38.1]	13 [61.9]	10 [47.6]	9 [42.9]	1 [4.76]	17 [81]	
	Unemployed	13	4 [30.8]	12 [92.3]	4 [30.8]	7 [53.8]	7 [53.8]	5 [38.5]	0 [0]	12 [92.3]	
	Missing	1	0 [0]	1 [100]	0 [0]	0 [0]	0 [0]	1 [100]	0 [0]	1 [100]	

Cont

Table 3.2: *Arbovirus seroprevalence in Fiji in 2017. Number seropositive defined by MIA [percentage of sample]. CHIKV, chikungunya virus. DENV-1,-2,-3,-4, dengue virus serotypes 1,2,3,4. ZIKV, Zika virus. RRV, Ross River Virus.* ≥ 1 *DENV, seropositive to at least one DENV serotype). p,* χ^2 *test comparing seroprevalence to at least one DENV serotype across all levels of that variable (the null hypothesis is that seroprevalence is the same across all levels in that variable. Smaller p values therefore present stronger evidence against this null hypothesis and evidence that there is an association between that variable and seroprevalence to at least one DENV serotype)*

Variable	Level	N	CHIKV	DENV1	DENV2	DENV3	DENV4	RRV	ZIKV	≥ 1 DENV	p
Home	Concrete / Brick	105	16 [15.2]	82 [78.1]	32 [30.5]	57 [54.3]	57 [54.3]	39 [37.1]	11 [10.5]	89 [84.8]	
	Corrugated Iron	121	14 [11.6]	88 [72.7]	26 [21.5]	44 [36.4]	43 [35.5]	52 [43]	12 [9.92]	93 [76.9]	0.41
	Other	6	0 [0]	3 [50]	2 [33.3]	1 [16.7]	1 [16.7]	2 [33.3]	1 [16.7]	4 [66.7]	
	Wood	88	11 [12.5]	64 [72.7]	22 [25]	43 [48.9]	43 [48.9]	32 [36.4]	16 [18.2]	70 [79.5]	
Garbage	Buried	161	4 [2.48]	105 [65.2]	34 [21.1]	54 [33.5]	56 [34.8]	82 [50.9]	18 [11.2]	113 [70.2]	
	Collected	142	36 [25.4]	118 [83.1]	44 [31]	82 [57.7]	80 [56.3]	38 [26.8]	21 [14.8]	128 [90.1]	***
	Other	17	1 [5.88]	14 [82.4]	4 [23.5]	9 [52.9]	8 [47.1]	5 [29.4]	1 [5.88]	15 [88.2]	

$p = \chi^2$ test

*** $p < 0.001$

** $p < 0.01$

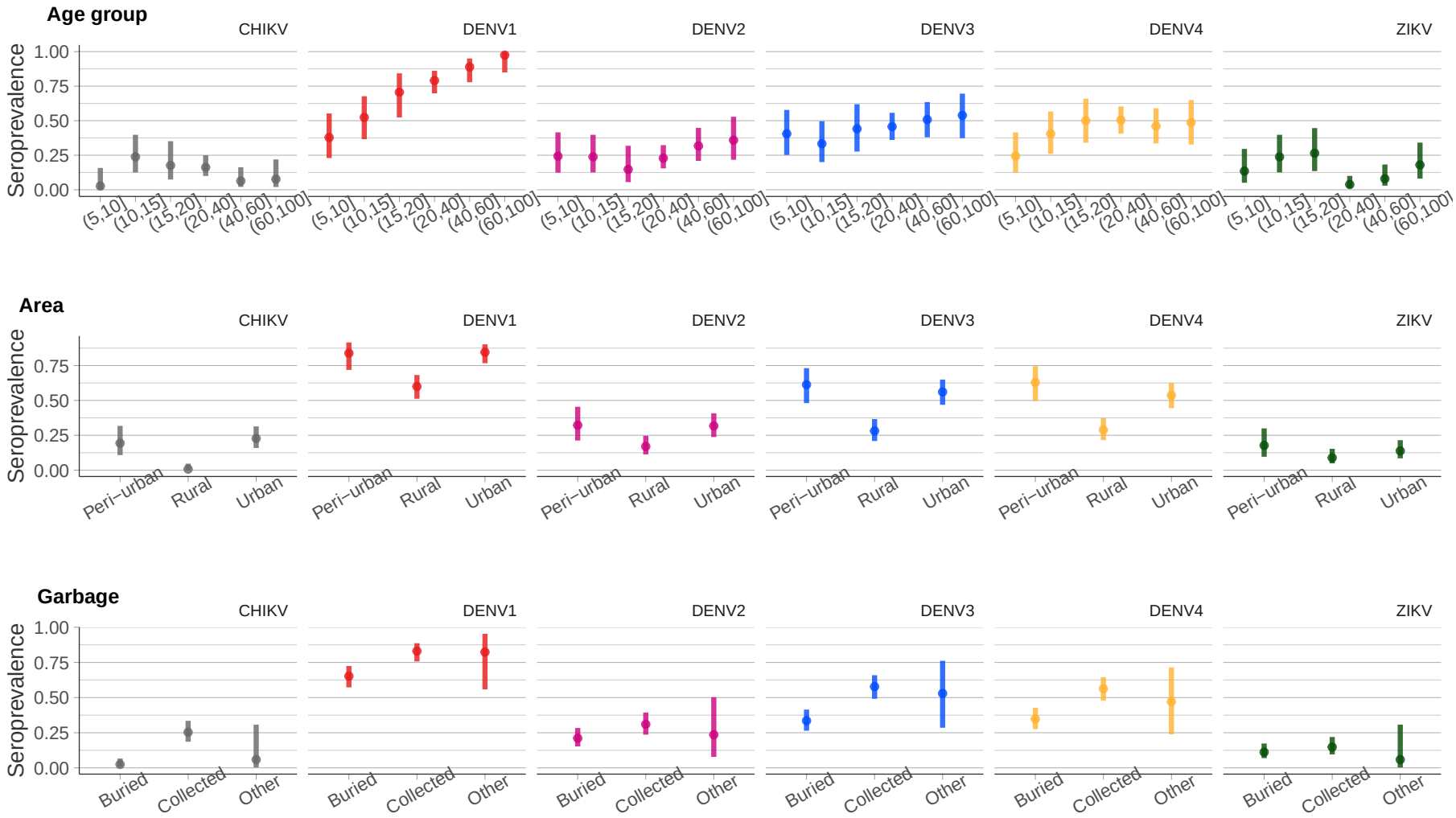

Figure 3.5: *Arbovirus seroprevalence in 2017 in Central Division, Fiji, by demographic and environmental factors. Points, estimated seroprevalence. Lines, 95% confidence intervals.*

We conducted a short questionnaire when sampling participants in 2017 and asked about health seeking behaviour and recent ZIKV-like symptoms. Participants were asked if they experienced a fever or a rash in the previous two years and whether they visited a doctor because of the symptom. One aim of our study was to test whether ZIKV-like symptoms and health seeking behaviour were related with ZIKV infection. We had 202 samples in 2017 that were paired with a 2015 sample. 40 of these 202 sampled participants reported no fever in the previous two years but only 2 reported a rash in the past two years. There were too few seroconversions between 2015 and 2017 – only 7 out of the 202 paired samples seroconverted from negative to positive – to investigate the association between health-seeking behaviour and risk of ZIKV infection.

I wanted to assess whether certain demographic variables increased the probability of and individual being seropositive. I focused on the most prevalent virus in our study, DENV-1. I fit univariable logistic regressions and rejected variables with $p > 0.5$ from an F-test. I built the final model in a backwards step-wise approach and removed variables with $p < 0.1$ from a likelihood ratio test with that variable removed. The initial full variable set included age group, the number of DENV positive serotypes in 2013, rurality, garbage collection method, home material, ethnicity, primary occupation, household size and sex. The final model is shown in Table 3.3 and shows strong evidence in the adjusted model that seroprevalence increases in smaller households, more urban areas and with age, fully adjusted for each other. Compared to participants younger than 20, those aged between 20 and 40 had 3.9 times higher odds of seropositivity (95% CI: 2-8) and those aged 40 and above had 12 times higher odds of seropositivity (95% CI: 5.2-35). Age had the largest effect on the odds of DENV-1 seropositivity in 2017, but even after adjusting for age there was strong evidence that those in rural areas had lower odds of seropositivity. Compared to residents of urban and peri-urban areas, those in rural areas had 78% lower odds of DENV-1 seropositivity (95% CI: 61-88%), adjusted for age and households size. There was weak evidence that larger households (4 or more household members) had 0.63 (95% CI: 0.34-1.1) times the odds of DENV-1 seropositivity compared to smaller households, adjusted for age and rurality (Table 3.3).

Table 3.3: *Risk factors for DENV-1 seroprevalence in 2017. Odds ratios from a simple logistic regression and fully adjusted model are shown*

Variable	Level	OR (95% CI)	Adjusted OR (95% CI)	p
Age	(0,20]	Ref	Ref	NA
	(20,40]	3.4 (1.9-6.5)	3.9 (2-8)	***
	(40+)	6.6 (2.9-18)	12 (5.2-35)	***
Rurality	Urban/peri-urban	Ref	Ref	NA
	Rural	0.28 (0.16-0.47)	0.22 (0.12-0.39)	***
Household	(0-4]	Ref	Ref	NA
	(4+)	0.66 (0.39-1.1)	0.63 (0.34-1.1)	0.13

*** $p < 0.0001$

3.3.3 Sensitivity of cutoff values for arbovirus seroprevalence in Fiji in 2017

The seroprevalence results presented so far are estimated by converting continuous measurements of relative fluorescence units (RFU) into positive or negative values according to a cutoff value. This value was calculated by laboratory colleagues using an ROC analysis. To assess the sensitivity of our findings in relation to the choice of cutoff I analysed the distribution of MIA values in the 2017 serosurvey and recalculated seroprevalence according to various cutoff values.

Figure 3.6 shows the distribution of RFU values for each virus included in the MIA in 2017 separated by serostatus. Values above the vertical dashed line were classified as positive and evidence of a previous infection. These data are also presented as 'S' shaped curves by ranking the participants by their MIA value for each virus. A robust assay would clearly define values as either positive or negative. Ideally, the histograms would show a bimodal distribution and there would be few values close to the cutoff value. Our assay was robust when classifying values as positive or negative for CHIKV as there is very little data near the cutoff value. This is less true for DENV-2 and DENV-4 where the cutoff value cuts the data in an area of high density on the histogram. This means

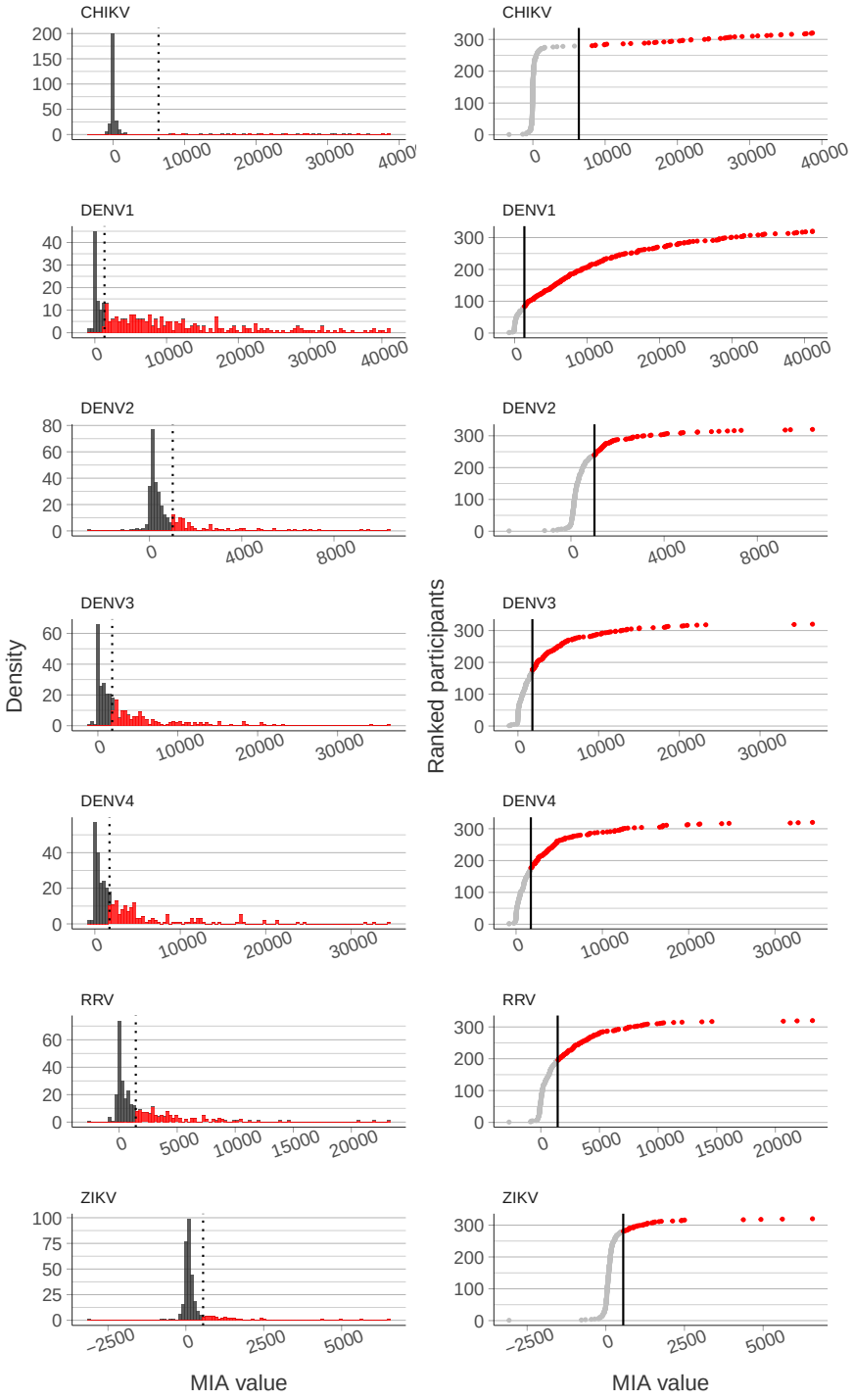

Figure 3.6: *Arbovirus MIA RFU values in 2017. Left-hand column shows histograms of the MIA RFU values for each virus with the cutoff value for seropositivity. Red values were classified as positive. The right-hand column shows the same data ranked by value. Cutoff value is shown as a dotted vertical line and red values were classified as seropositive*

that changing the cutoff value slightly would reclassify a lot of observations as positive or negative and change our estimated seroprevalence.

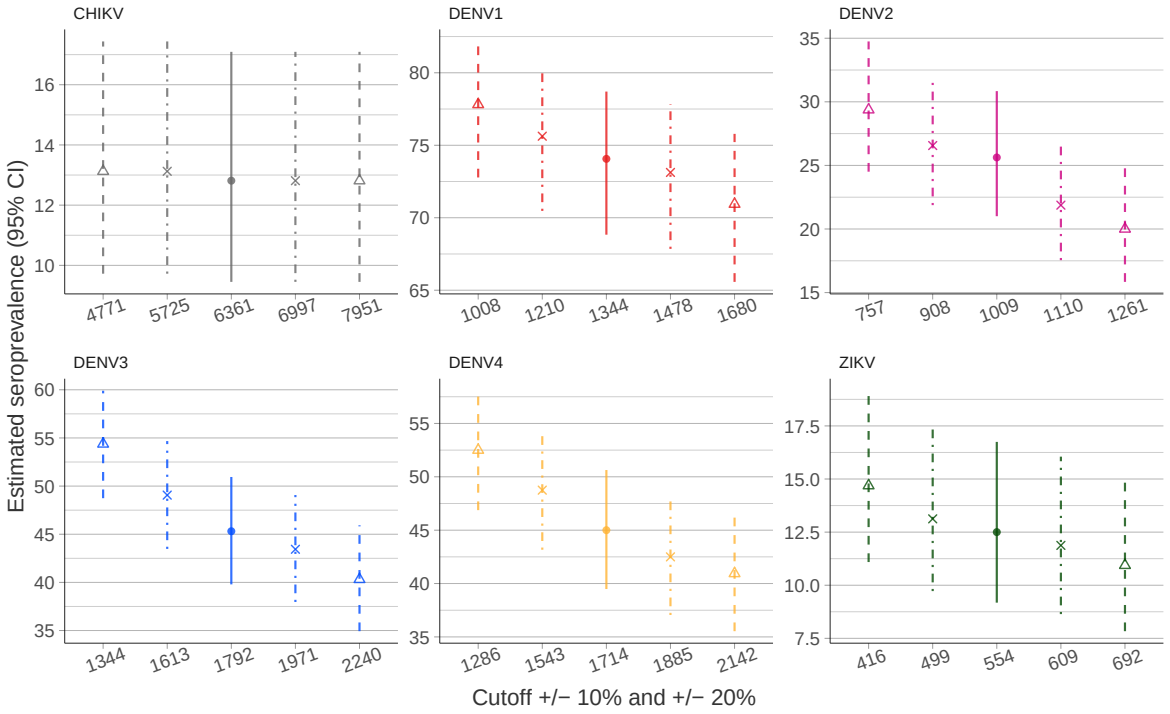

Figure 3.7: *Arbovirus seroprevalence (2017) by variable cutoff values. The centre dot and solid line shows the estimate for 2017 seroprevalence and 95% CI. Crosses and dot-dash vertical lines show seroprevalence estimates if the cutoff was ± 10%. Triangles and dash lines show seroprevalence estimates if the cutoff was ± 25%.*

To test the sensitivity of our estimates of seroprevalence to the choice of cutoff value I recalculated seroprevalence if the cutoff in 2017 was ± 10% or ± 25%. As seen in Figure 3.6 the assay is robust to movements in the cutoff for CHIKV as the estimated seroprevalence ranges between 12.8% and 13.1% across a ± 25% change in cutoff value (Figure 3.7). For CHIKV, if the cutoff value was 10% higher, the estimated seroprevalence would have been 13.1% and if it was 10% lower it would have been 12.8%, a relative difference of 2.4%. This relative difference in seroprevalence estimates with a ± 10% change in cutoff value was also low for DENV-1 at 3.3% but was higher for DENV-3, -4, and ZIKV at 11.5%, 12.8% and 9.5% respectively. However, the virus which was most sensitive to the choice of cutoff was DENV-2 where a ± 10% change in the cutoff value changed estimated seroprevalence from 22% to 29%, a relative differ-

ence of 17.6%. This analysis shows that some viruses are more sensitive to the choice of cutoff value than others.

3.3.4 Trends in seroprevalence for outbreaks in Fiji

I used the cross-sectional serological data from 2017 to investigate the relationship between seroprevalence and the arbovirus outbreaks an individual might have been exposed to in Fiji. I wanted to assess whether our serological data sensibly recaptures past outbreaks. To model this non-linear relationship I fit a generalised additive model (GAM) to seroprevalence data for CHIKV, DENV-1, -2, -3, -4 and ZIKV in 2017 with an explanatory variable of year of birth with five smooth terms. Figure 3.8 shows the serostatus of individuals in the 2017 study by year of birth and the probability of seropositivity for each of the viruses from the GAM. The age-specific pattern of seropositivity broadly reflects the pattern of observed DENV outbreaks and hence potential exposure to the virus. The probability of DENV-1 seropositivity is lower for those born after 2003, the last known outbreak of this virus in Fiji. Seropositivity for DENV-3 in 2013-14 and DENV-2 in 2017 – the two DENV viruses that caused the most recent epidemics – are consistently high across all age groups.

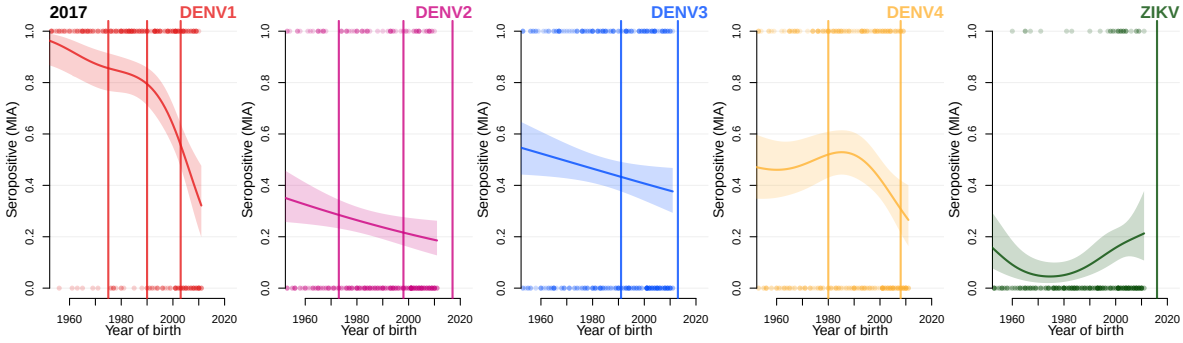

Figure 3.8: *Arbovirus seroprevalence (as measured by MIA) by year of birth and known outbreaks in Fiji, samples from 2017. Coloured dots, show serostatus by year of birth. Coloured line and region, predicted serostatus from a GAM fitted to individual serostatus for each virus. Vertical lines indicate timing of known outbreaks of that virus*

This chapter has so far presented analysis of the 2017 data set as a cross-sectional survey. However, a major strength of this study is that we collected longitudinal sera

from the same individuals so can investigate changes in seroprevalence and antibody response against arboviruses over time.

These longitudinal serological data are particularly valuable when combined with knowledge from surveillance data of when certain viruses had confirmed transmission or outbreaks between 2013 and 2017. There were four notable arbovirus outbreaks over the study period. There was a large DENV-3 epidemic that began in late 2013 and ended by July 2014 (*Cao-Lormeau et al.*, 2014a; *Kucharski et al.*, 2018). A DENV-2 outbreak began in January 2017 and spread until September 2017 (Ministry of Health data). There was low level confirmed local transmission of ZIKV between 2015 and 2017 (*Kama et al.*, 2019). Finally, there was an outbreak of CHIKV in 2016 (*Aubry et al.*, 2019b; *Kama et al.*, 2019).

Figure 3.9 shows the confirmed and suspected cases from surveillance data for all DENV viruses, CHIKV and ZIKV, as well as the seroprevalence at each serological survey in 2013, 2015 and 2017. The seroprevalence estimates connected by dotted lines uses all available data at each survey. The seroprevalence estimates connected by solid lines are from the subset of our study with observations at each time point ($n = 189$). DENV-2 and CHIKV show expected dynamics in the seroprevalence data with low pre-outbreak seroprevalence that increases rapidly between 2015 and 2017. This is true also for DENV-3 between 2013 and 2015 but estimated seroprevalence declined from 55% (95% CI: 49.4-60.4%) to 45.3% (95% CI: 39.8-50.9%) after the outbreak. The ZIKV outbreak shows the most unusual dynamics. Estimated ZIKV seroprevalence increased rapidly from 7.7% (95% CI: 6-9.9%) to 22% (95% CI: 17.7-26.8%) between 2013 and 2015. However, there were only 2 cases confirmed in surveillance data during this period. ZIKV seroprevalence then declined sharply between 2015 and 2017. There were very few reported cases of DENV-1 or DENV-4, however the seroprevalence estimates did fluctuate over this period. DENV-1 increased from 68.4% (95% CI: 65-71.6%) in 2013 to 74.1% (95% CI: 68.8-78.7%) in 2017. DENV-4 increased significantly between 2015 and 2017 from 38.7% (95% CI: 33.5-44.2%) to 45% (95% CI: 39.5-50.6%).

In Section 3.3.3 I analysed the effect of changing the cutoff value on seroprevalence estimates in 2017. This showed that seroprevalence estimates for some viruses were

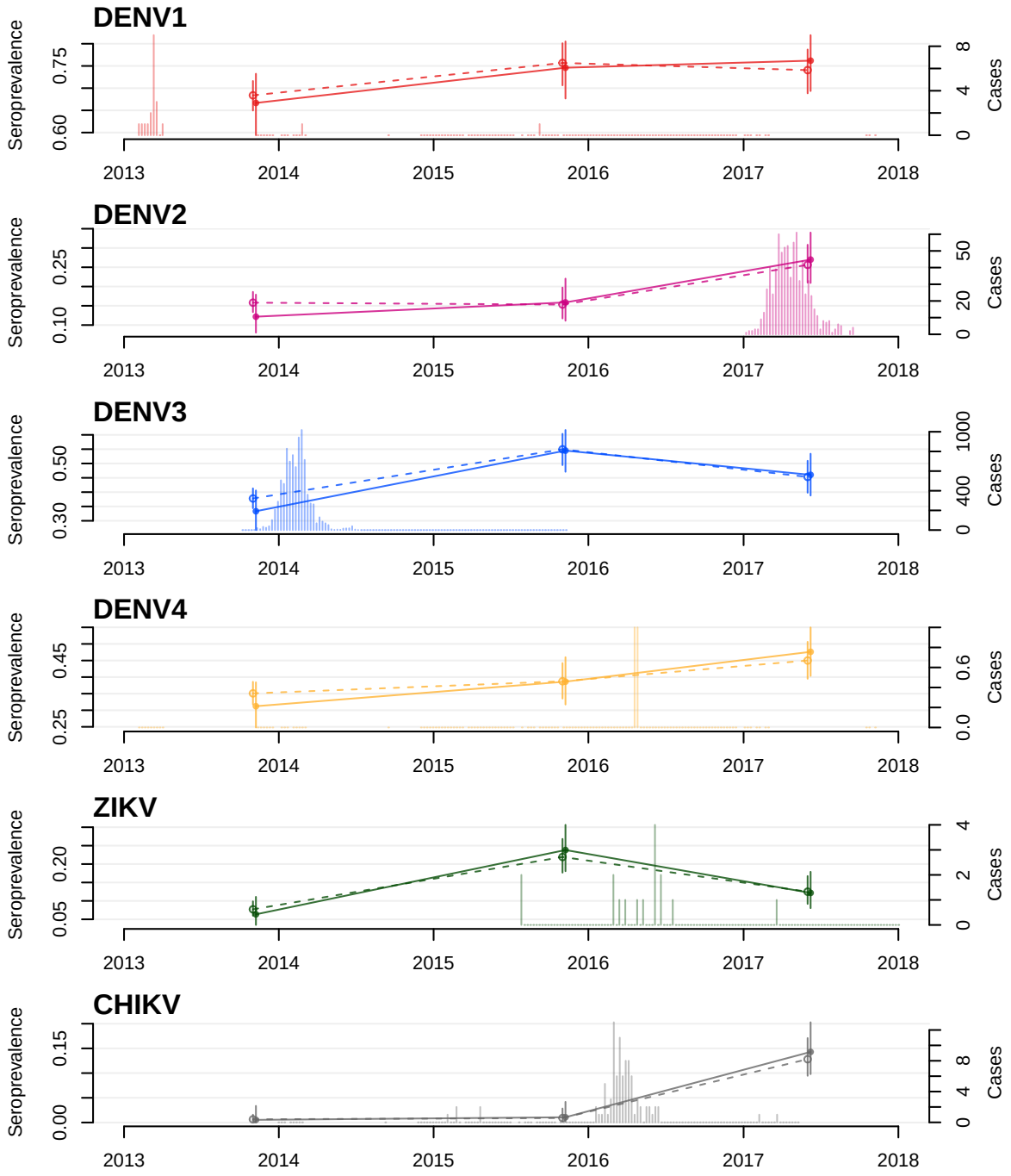

Figure 3.9: *Seroprevalence for circulating arboviruses in Fiji between 2013 and 2017. Dots and lines, estimated seroprevalence and 95% confidence interval. Dotted lines connect seroprevalence estimates using all available data at each time point. Solid lines connect seroprevalence from the same subset of the study with observations at every time point. Vertical lines in the background show weekly cases of the corresponding virus (Confirmed and suspected cases for DENV-3 and DENV-2. PCR confirmed cases for DENV-1, DENV-4, ZIKV and CHIKV)*

more sensitive to the cutoff value than others. Here I present those same estimates alongside estimates from 2013 and 2015 to assess whether the choice of cutoff values affects our interpretation of the trend in seroprevalence between 2013 and 2017. Figure 3.10 shows the variable 2017 estimates ($\pm$ 10 or $\pm$ 25% change in cutoff value) alongside the 2013 and 2015 estimates. While the 2017 estimates can range considerably if the cutoff had been $\pm$ 25%, the effect on our conclusions about the trend in seroprevalence would have been minimal. For example, ZIKV seroprevalence estimates ranged between 10-15% if the cutoff was shifted $\pm$ 25%. However, even this highest estimate still shows a significant decrease from the 2015 estimate. One notable exception to this is DENV-3 where a 25% lower cutoff value would have shown very little change in seroprevalence between 2015 and 2017.

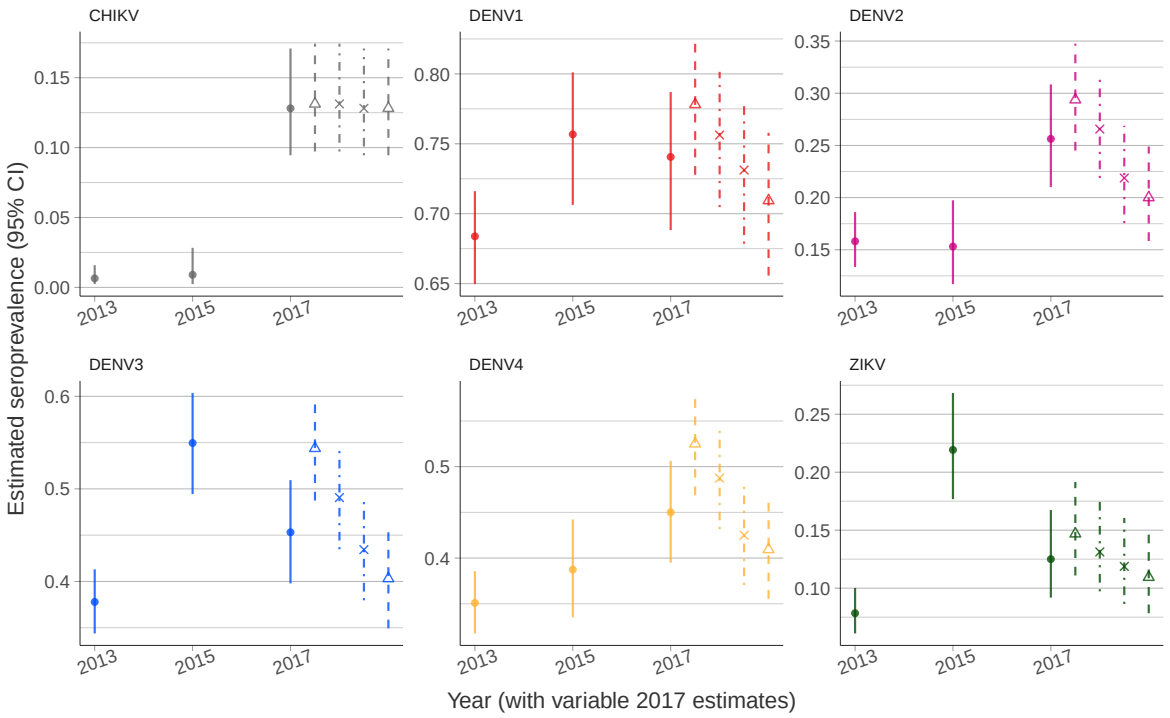

Figure 3.10: *Sensitivity analysis of estimated seroprevalence in 2017. Dots, estimated seroprevalence. Vertical lines, 95% confidence interval. Triangles and dashed vertical lines, estimated 2017 seroprevalence if cutoff was $\pm$ 25%. Crosses and dot-dash vertical lines, estimated 2017 seroprevalence if cutoff was $\pm$ 10%*

3.3.5 Change in raw MIA values between 2013 and 2017

The diverse range in seroprevalence dynamics is observable in the raw MIA values as well as the summary seroprevalence estimates. As explained above, dividing the data into positive and negative binary values according to a specific cutoff is sensitive to cutoff value used. I analysed the changes in the raw continuous data between the three serological surveys and found consistent trends in serological dynamics as in the binary seroprevalence data.

Figure 3.11 shows the raw MIA relative fluorescence units (RFU) values for DENV-1, -2, -3, -4, CHIKV and ZIKV in 2013, 2015 and 2017. To analyse whether changes between surveys were significant I performed two separate paired t-tests to compare the change in RFU between 2013 and 2015 ($n = 312$), then 2015 to 2017 ($n = 189$) (Table 3.4).

The pattern in changes in raw MIA RFU is similar to that seen in the changes in summary seroprevalence estimates between surveys. The mean RFU is highest for DENV-1 in 2013 and very low for ZIKV, CHIKV, and DENV-2. There was very strong evidence ($p < 0.001$) of an increase in mean RFU between 2013 and 2015 for DENV-1, -3, -4 and ZIKV. There was very strong evidence ($p < 0.001$) of a continued increase between 2015 and 2017 for DENV-1, -4 and an increase of CHIKV RFU. Meanwhile, there was evidence ($p < 0.01$) of a decrease of ZIKV RFU between 2015 and 2017. Another notable observation from this analysis is that, while the headline seroprevalence estimate for DENV-3 appeared to decline between 2015 and 2017 from 55% 55% (95% CI: 49.4-60.4%) to 45.3% (95% CI: 39.8-50.9%) (Figure 3.9), there was no evidence of a change in mean DENV-3 RFU when analysing the raw MIA values over the same time period (Table 3.4). This is consistent with an analysis of neutralising DENV-3 antibodies presented in Chapter 4.

Figure 3.12 shows the results from these paired t-tests alongside the summary seroprevalence estimates from all three surveys using the original cutoff values. This demonstrates that the observed trend in estimated seroprevalence between all three surveys is broadly consistent with the changes in raw MIA RFU values. With the exception of DENV-1,

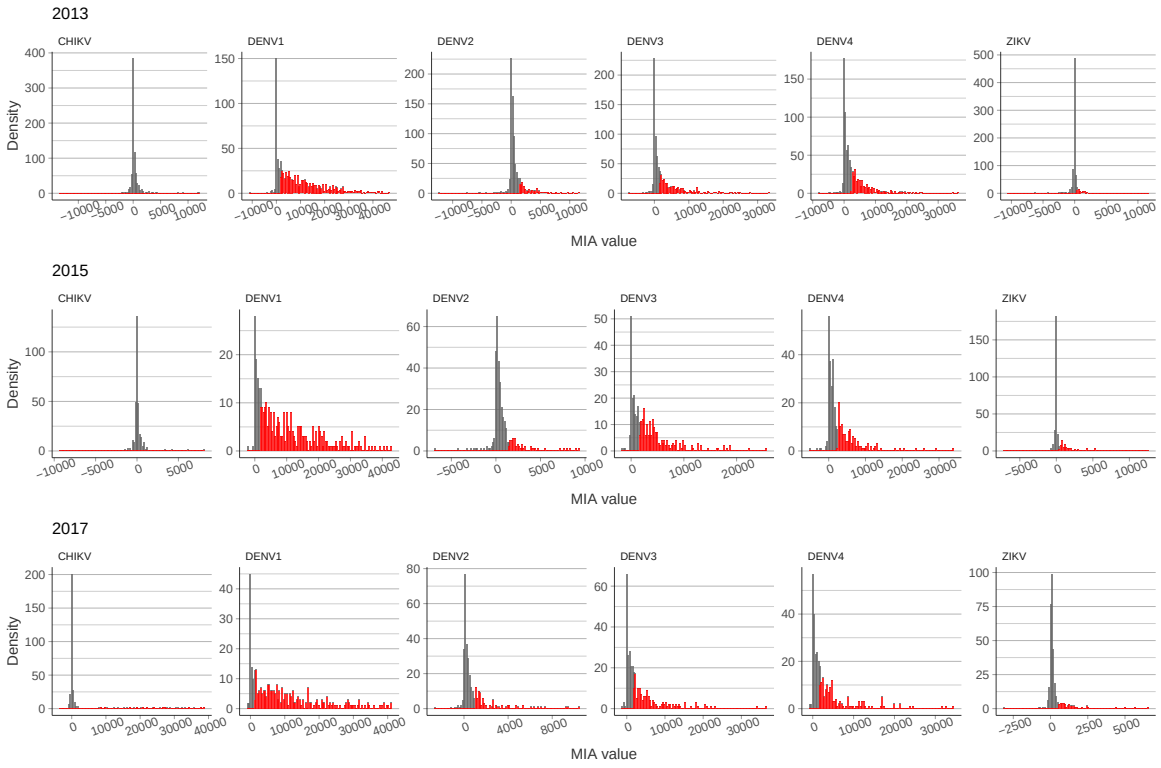

Figure 3.11: *Arbovirus MIA RFU values between 2013 and 2017. Histograms of the MIA RFU values for each virus with the cutoff value for seropositivity. Red values were classified as positive. The right-hand column shows the same data ranked by value*

which has the largest raw MIA RFU values (Figure 3.11), the changes in seroprevalence are matched by similar dynamics in the raw MIA RFU values. DENV-1 raw values however, are so large that even a moderate increase in raw MIA RFU did not change seroprevalence in 2017, and therefore infections in the raw values may have been missed. Elsewhere, the decrease in ZIKV seroprevalence between 2015 and 2017 is consistent with a negative change in raw MIA RFU. Large relative increases in seroprevalence between 2015 and 2017 for CHIKV, DENV-2 and DENV-4 are also observed in the raw MIA RFU changes.

3.4 Discussion

We successfully resampled 320 participants from a seroepidemiological survey conducted in 2013 in Fiji and tested samples for evidence of previous infection with a range of

Table 3.4: *Arbovirus seroprevalence in Fiji between 2013 and 2017 as measured by RFU and changes in RFU between surveys. The mean RFU value and standard deviation in 2013 is shown alongside estimated change in RFU value between surveys (2013-2015 and 2015-2017). Estimated change in value and 95% confidence intervals were calculated with a paired t-test*

Virus	Mean MIA value 2013 [std. dev.]	Mean difference 2013-2015 (99% CI) [n=312]	Mean difference 2015-2017 (99% CI) [n=189]
DENV1	8839 [9840]	2268 (1382 – 3154)**	2580 (690 – 4471)**
DENV2	658 [1543]	126 (-1 – 252)	447 (46 – 848)*
DENV3	2574 [4348]	1227 (797 – 1657)**	93 (-748 – 933)
DENV4	2809 [4371]	650 (222 – 1078)**	2163 (833 – 3494)**
ZIKV	-25 [973]	390 (189 – 592)**	-287 (-537 – -37)*
CHIKV	170 [1285]	-94 (-196 – 9)	2041 (1004 – 3078)**
			* $p < 0.01$, ** $p < 0.001$

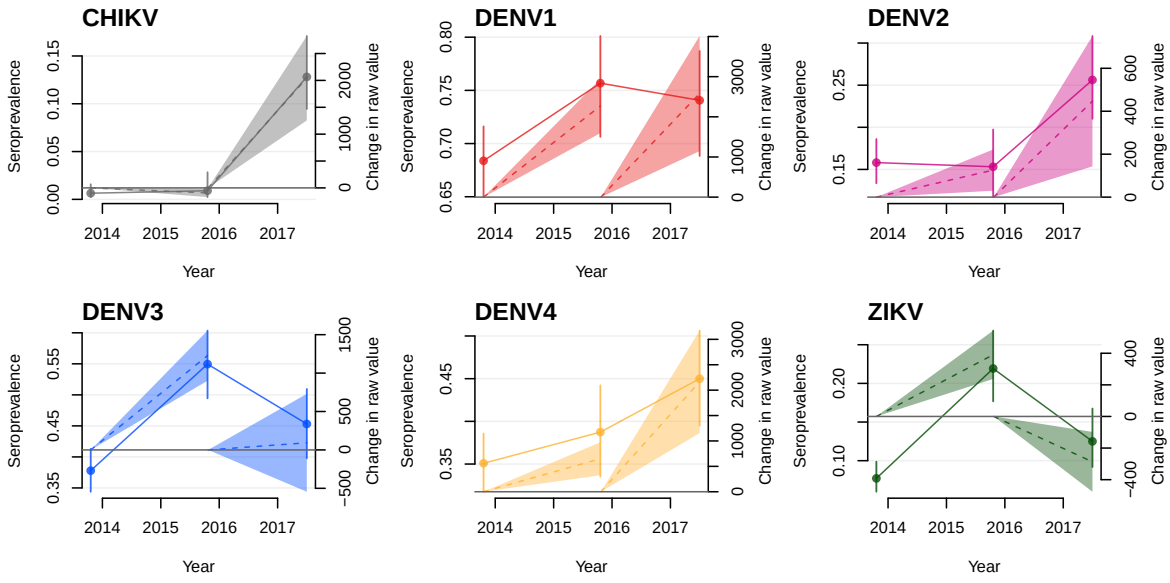

Figure 3.12: *Comparison of estimated seroprevalence and raw values from the MIA. For each virus the left-hand y-axis shows the estimated seroprevalence in each survey (vertical lines show 95% CI). The right-hand y-axis shows the estimated change in MIA RFU value (as in Table 3.4). The shaded region connects the 95% CI of the estimated change in MIA RFU value from a baseline of 0 in 2013 or 2015. Grey horizontal line indicates no change in the MIA RFU value between surveys*

arboviruses. This original study used population representative sampling and, by following a systematic recontacting approach in 2017, our sample still represented the

population in terms of age distribution and had a similar breakdown to the sample in 2013 in terms of ethnicity, sex and household size, but not by rurality. This serological survey in 2017 found evidence that approximately 90% (95% CI: 86.5-93.3%) of the sample were seropositive (as measured by MIA) to at least one DENV serotype (Table 3.2). Seroprevalence was highest for DENV-1 (74.1%; 95% CI: 68.9-78.8%) and lowest for CHIKV (12.5%; 95% CI: 9.4-17%) and ZIKV (12.5%; 95% CI: 9.1-16.6%) despite reported recent outbreaks of these viruses (*Kama et al.*, 2019). We found evidence that arbovirus seroprevalence differed across age groups, by rurality where rural populations had a lower seroprevalence for all viruses, by occupation, method of garbage collection and home material. We modelled age-specific seroprevalence – as measured by MIA in 2017 – and found that our data reflected the sequence of observed outbreaks of ZIKV and DENV in Fiji (Figure 3.8).

We combined data collected in 2017 with previous arbovirus seroprevalence estimates from the same participants in 2013 (*Lau et al.*, 2016; *Watson et al.*, 2017) and 2015 (*Kama et al.*, 2019; *Kucharski et al.*, 2018). We compared serological dynamics before and after known outbreaks of DENV-3 between 2013-14 (*Cao-Lormeau et al.*, 2014a; *Kucharski et al.*, 2018), ZIKV between 2015-17 (*Kama et al.*, 2019), CHIKV between 2015-17 (*Aubry et al.*, 2019b; *Kama et al.*, 2019) and DENV-2 in 2017 (*Fijivillage*, 2017). Seroprevalence, as measured by MIA, increased following confirmed outbreaks of each of these viruses (Figure 3.9). However, serological dynamics were different for these outbreaks and this is reflected in the seroprevalence estimates at each time point. The largest outbreak was DENV-3 in 2013-14 when seroprevalence increased from 38% (95% CI: 34.4-41.3%) to 55% (95% CI: 49.4-60.4%) between 2013 and 2015. In contrast, CHIKV seroprevalence increased from 0.6% (95% CI: 0.2-1.5%) in 2015 to 12.5% (95% CI: 9.4-17%) in 2017. The longer-term serological dynamics following an outbreak differed as well. Seroprevalence for DENV-3 remained high in 2017 at 45% (95% CI: 40-50.9%), whereas ZIKV declined rapidly from 22% (95% CI: 17.7-26.8%) in 2015 to 12.5% (95% CI: 9.1-16.6%) in 2017. This trend will be explored in greater detail in Chapter 4.

This study was susceptible to selection bias because of our sampling design. Partic-

ipants in the original 2013 study were never recruited to a longitudinal study and subsequent sampling was performed opportunistically. As a result, we relied on last known addresses and contact details to recruit participants to this study in 2017. Some participants are more likely to be contacted under this framework. Recontacting and sampling took place during typical business hours and some evening and weekend work was required, which meant that those who worked at home were more likely to be found and sampled. This method also meant that we sampled very few young children. The youngest participant in the study in 2013 was 2 years old, so was 6 by the time samples were collected in 2017. One limitation of this is that we could not look at serological patterns in younger children, who can only have experienced certain outbreaks. School-age children and teenagers are also more likely to be at home during our visits which helps explain their over-representation in our sample (Figure 3.1). If these groups are systemically more or less likely to have been exposed to arboviruses then this could introduce non-random bias to our results. We aimed to mitigate this bias by collecting a sample as similar to the 2013 baseline as possible. Across a range of demographic and lifestyle factors we were able to achieve this (Figure 3.2). One notable exception was rurality, the type of urban area that participants lived. Participants in rural locations constituted 35% of the 2013 sample, 34% of the 2015 sample but this increased to 42% of the 2017 sample. We also found evidence that rurality was associated with lower odds of ZIKV seroprevalence in 2017 (Table 3.3). To estimate the importance of this change we can compare the unadjusted ZIKV seroprevalence in 2017 of 12.5% (95% CI: 9.1-16.6%) with the adjusted seroprevalence, using the rurality distribution from the 2013 sample as weights, which was 12.3% (95% CI: 8.8-16.8%). While this change in rurality distribution is not ideal it does not appear to have greatly affected results from 2017. As further evidence for this, we also found similar seroprevalence dynamics for known outbreaks of arboviruses using a restricted data set – participants with repeated measurements from 2013, 2015 and 2017 – as in the unrestricted samples (Figure 3.9). It is therefore reasonable to assume that our sample is representative of the population, as the 2013 sample was. With respect to sample size we did collect fewer than our target of 350 participants in 2017. However, with our sample size of 320 and allowing for the observed 3.5% seroconversion we were able to detect a reduction

in ZIKV seroprevalence from 21.7% to 12.5% with 85.9% power and 95% sensitivity.

This was the third study of the same participants and the challenge to recontact and resample participants became more difficult with each follow-up survey. As a result, methods that recruited the maximum number of people in an efficient manner were preferred to ensure that the sample size was sufficient to make valid inferences. Rurality is one such example. Rural communities required a more cautious recontacting method to respect local customs. Contact was made with a village representative, gifts were offered to thank the village for welcoming us and their previous contributions to our research. If permission for resampling in the village was granted then the study team would revisit the next day and a large proportion of that cluster from the 2013 study would be gathered in a central location in the village to contribute to our 2017 study. This meant that resampling of rural clusters was more successful than in urban and peri-urban areas (Figure 3.3). This change in rurality distribution in 2017 is a potential weakness in the study since we were analysing transmission of viruses mostly spread by urban-based mosquito species such as *Aedes aegypti*. As discussed above however, our findings were valid across the unrestricted and restricted data sets so this logistical benefit for our study is unlikely to have introduced bias to our results. Repeating analysis in the restricted population sampled at three time points can help validate results, as we did here. More broadly, this study design of opportunistic follow-up appears to have a limit on how many repeat visits are possible without introducing non-random bias to results and design of future studies should be mindful of this.

We designed our study to be as non-invasive as possible which reduced the extensiveness of our 2017 data set, but was necessary in light of ethical consideration. Participants had consented to be contacted for future health research but were not recruited to a longitudinal study with pre-specified repeat visits. We therefore designed our questionnaire to be as short as possible so our visit with participants could be as brief as possible. As a result of this abbreviated questionnaire we did not recollect demographic or lifestyle information that was collected in 2013. We therefore assumed throughout this study that values collected in 2013 were still true and valid in 2017. Certain demographic variables such as sex are unlikely to change frequently. Details about a

participant's home material or garbage would change if they moved home but this in turn would mean they were less likely to be part of our 2017 study because they would have been harder to locate and resample. Another data quality issue is missing data on household details when participants were resampled in centralised locations in rural villages. In these settings it was more difficult to ascertain whether the household had air conditioning or mosquito breeding sites. We had aimed to analyse these data as potential risk factors for arbovirus seroconversion but due to these data quality issues we removed this analysis.

We used data from an MIA and defined a cutoff for seropositivity using positive and negative control sera analysed by ROC curve. The sensitivity and specificity of the MIA assay were respectively 100% and 100% for DENV-1, 89.5% and 97.1% for DENV-2, 100% and 100% for DENV-3, 96.9% and 100% for DENV-4, and 79.6% and 94.9% for ZIKV (*Henderson et al.*, 2020). The lower sensitivity for ZIKV in particular suggests that some ZIKV seropositive participants could have been falsely defined as negative in our MIA results. These sensitivity and specificity values were calculated using control sera that were collected shortly after symptomatic arbovirus infections (*Cao-Lormeau et al.*, 2016; *Henderson et al.*, 2020). These control sera are therefore more likely to have a strong antibody response to infection than in the general population where arbovirus infections may be asymptomatic (*Haby et al.*, 2018; *Moro et al.*, 2010). The sensitivity and specificity of the assay using general population sera may therefore be lower than the values quoted here. Observed increases in DENV-1 and DENV-4 seroprevalence over the study period when these viruses were not widely detected could be evidence of cross-reaction in the assay. The increase in DENV-4 seroprevalence between 2015 and 2017 in particular could be a result of cross-reaction since samples were collected at the end of an outbreak of closely related DENV-2 (*Katzelnick et al.*, 2015).

Separating the values from our MIA into positive and negative serostatus enabled us to succinctly summarise the findings from this seroepidemiological survey. However, separating serological data into dichotomous data has limitations, especially around the value used to cut data into positive or negative values. In this chapter I have presented sensitivity analyses of the distribution of the raw MIA values, the effect of changing the

cutoff value, and an analysis of the change in raw MIA values between surveys. This work demonstrated that the MIA performed more robustly and was less sensitive to the choice of cutoff for CHIKV than for DENV and ZIKV. However, although seroprevalence estimates varied when the cutoff was shifted by ± 25% for DENV and ZIKV, it did not materially change the conclusions of the trend in seroprevalence between 2013 and 2017 (Figure 3.10). In addition, the trend in seroprevalence estimates matched the changes in raw MIA RFU values between 2013 and 2017. The large increases in estimated seroprevalence for ZIKV and DENV-3 between 2013 and 2015, and the decrease of ZIKV seroprevalence 2015-17, were also found with a paired t-test comparing raw MIA values. In summary, although dichotomising serological data has limitations, in this study the dichotomous and continuous versions of the MIA data were complimentary, and findings were broadly robust to the choice of cutoff value.

Longitudinal serological studies such as this study can help us better understand immunity dynamics over time but there are limitations to generalising these results to protective immunity. We do not know the direct relationship between a specific titre value or serostatus and susceptibility to further infection. In the case of DENV, it has been shown that higher values of neutralising antibodies correlate with protection from symptomatic infection (*Katzelnick et al.*, 2016). Studies have also shown that infection with one DENV serotype can lead to a cross-reactive immune response against other serotypes (*Calisher et al.*, 1989; *Guzmán and Kourí*, 2002; *Mansfield et al.*, 2011; *Scott et al.*, 1983; *Wahala and de Silva*, 2011). It has also been shown that prior DENV infection could provide a cross-protective effect against symptomatic ZIKV infection (*Barba-Spaeth et al.*, 2016; *Gordon et al.*, 2019; *Rodriguez-Barraquer et al.*, 2019). We found evidence that 12.5% (95% CI: 9.1-16.6%) of our study population had a previous ZIKV infection but there were only 16 reported cases of ZIKV, which suggests that most infections in Fiji were asymptomatic, possibly because of the high seroprevalence for other DENV serotypes. This also shows evidence against the idea that ZIKV immunopathogenesis is enhanced in the setting of high seroprevalence of DENV antibodies (*Andrade and Harris*, 2018; *Dejnirattisai et al.*, 2016; *Paul et al.*, 2016).

We collected 320 samples in 2017 to complete a population representative, longitudinal

survey with serological data from two complimentary assays. Population representative sampling is preferable to convenience sampling from populations such as blood donors (*Netto et al.*, 2017) as it enables us to generalise conclusions from our sample to the wider population. Our study now benefits from longitudinal sera at three time points for 189 study participants. Data from joined sera observations before and after a variety of arbovirus outbreaks are rare and facilitated a comparison of serological response to different outbreaks. Our study demonstrates the value of maintaining a sustainable biobank following cross-sectional seroepidemiological surveys. It was not foreseen in 2013 that those data would be valuable for studying the population immune response to a ZIKV epidemic but because of the banking of samples and testing for multiple viruses we were able to maximise the value of samples collected in 2013.

We were able to investigate ZIKV dynamics from a case study in the Pacific where transmission occurred before a global ZIKV pandemic was declared in 2016 (*World Health Organisation*, 2016). This study aimed to answer three public health questions focused on ZIKV transmission. Firstly, what was the extent of ZIKV infection in Fiji and association with other arboviruses? We identified a lack of ZIKV spreading, especially in comparison to other related arboviruses such as DENV-1 and DENV-3 (Table 3.2), and in comparison to international studies of ZIKV seroprevalence (*Flamand et al.*, 2019; *Netto et al.*, 2017; *Zambrana et al.*, 2018).

Secondly, what proportion of ZIKV infections were reported and were they related to other health complications? We expected more seroconversions between 2015 and 2017 on the assumption that ZIKV and DENV share similar immune responses following an outbreak, an assumption with precedent given the antigenic similarity of these viruses (*Priyamvada et al.*, 2016). However, we found very few seroconversions so we dropped this analysis because of a lack of power. Other studies have also shown that ZIKV case detection may have been poorly defined (*Burger-Calderon et al.*, 2020) and case counts underestimated the burden of the ZIKV pandemic (*Moore et al.*, 2019).

Finally, what is the current level of immunity and what is the potential for ZIKV to transition to an endemic state? We estimated that a low proportion of Fiji is seropositive for ZIKV and potentially susceptible to infection. These dynamics and the implications

for ZIKV herd immunity are explored in more detail in Chapters 4 and 5.

The data introduced in this chapter are used throughout the thesis to facilitate analysis of arbovirus outbreaks in Fiji. In Chapter 4 I use these serological data to characterise the long-term antibody response to ZIKV following outbreaks in Fiji and French Polynesia. In Chapter 5 I combined these serological data with surveillance and molecular data to estimate unobserved transmission dynamics of ZIKV. Finally, in Chapter 6, I use pre-outbreak serology to improve a real-time forecasting model of a DENV outbreak and estimate the effect of vector control on transmission.

In this study we collected a large sample of sera in 2017 and combined with previously collected data, have a longitudinal serological data set to investigate trends in arbovirus transmission. In this chapter I have presented a summary of what we have found and how we were able to benefit from our study design. There are limitations that have stopped us from exploring trends further but this remains a valuable data set for the study of arbovirus transmission. We were able to explore the burden of arbovirus transmission in Fiji by extending beyond seroprevalence and investigating antibody dynamics. However, as the rest of this thesis will demonstrate, the value of these data are maximised when combined with other data sources from Fiji and the wider Pacific. Combined with mathematical modelling, these data can be used to investigate long-term immune responses to ZIKV, unobserved transmission of ZIKV and improve real-time forecasting of DENV outbreaks.

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Chapter 4

Zika seroprevalence declines and neutralising antibodies wane in adults following outbreaks in French Polynesia and Fiji

4.1 Bridging section

This study, published in January 2020, presents an analysis of data from eight serological surveys conducted at different time points during Zika virus (ZIKV) outbreaks in French Polynesia and Fiji.

Serological data are crucial in the study of flaviviruses in humans and in animals. Infection with dengue virus (DENV) or ZIKV in humans can be asymptomatic and, as a result, confirmation of flavivirus infections is mostly based on rapid serological tests such as enzyme-linked immunosorbent assays (ELISAs) (*Beck et al.*, 2015). Multiplex immunoassays (MIAs) (*Aubry et al.*, 2017; *Beck et al.*, 2015) and plaque reduction neutralisation assays (PRNTs) (*Collins et al.*, 2017) can be used to test for antibodies against specific flaviviruses to show evidence of previous infection and estimate the level of immunity in a population. Serological data has a well-established role in improving understanding of transmission dynamics of other diseases, such as influenza (*Wu et al.*, 2011), malaria (*Helb et al.*, 2015), chikungunya virus (*Salje et al.*, 2016) and DENV (*Katzelnick et al.*, 2017; *Salje et al.*, 2018). The rapid emergence of ZIKV between 2013 and 2016 means that most early seroprevalence estimates were from convenience sampling (*Gake et al.*, 2017; *Gallian et al.*, 2017; *Lozier et al.*, 2018; *Netto et al.*, 2017). Convenience sampling is well documented to provide flawed estimates of the burden of disease, in Human Immunodeficiency Virus for example (*Boerma et al.*, 2003). We compared seroprevalence estimates from population-representative cross-sectional studies in French Polynesia and longitudinal seroepidemiological data in Fiji.

The collection of longitudinal serological data from the same individuals allows for more detailed analysis of population level and within-individual immunological responses to viruses. The previous chapter in this thesis outlined the study population in Fiji of a longitudinal seroepidemiological study. Samples collected in this study are positioned around known periods of ZIKV circulation in Fiji. Samples were initially collected in November 2013 (*Lau et al.*, 2016; *Watson et al.*, 2017). Chapter 5 will show evidence that ZIKV began circulating in late 2014 and the follow up serological sample was collected in November 2015. Community transmission of ZIKV cases were confirmed in

2015 and 2016 and our final serological sample was collected in June 2017. Analysing these serological data enabled us to study long-term immunological responses to these outbreaks which are poorly understood, especially in the case of ZIKV.

This chapter describes a study of serological data from Fiji and French Polynesia and the level of ZIKV-specific immunity in the population. Samples collected were analysed by collaborators at Institut Louis Malardé (ILM) in Tahiti, French Polynesia. This is the only published paper in this thesis. I share first authorship with Dr. Maite Aubry (ILM) who led the serological analysis. I led the statistical analysis, wrote the `R` code to analyse data and wrote the initial draft manuscript that was submitted to journals and published as a pre-print on *BioRxiv* in January 2019. The version presented in here is the published version in *eLife* (*Henderson et al.*, 2020). The first draft was conditionally accepted by *eLife* in June 2019 while I was on a placement at Epicentre in Paris. I was unable to work on the revisions so Dr. Adam Kucharski led on these. In the appendix to this chapter I have described the statistical methods used in more detail than presented with the original publication.

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First Name(s)	Alasdair		
Surname/Family Name	Henderson		
Thesis Title	Mathematical Modelling of Arbovirus Outbreak Dynamics in Fiji and the Wider Pacific		
Primary Supervisor	Dr. Adam Kucharski		

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SECTION E

Student Signature	Alasdair Henderson
Date	15/05/2020

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Date	15/05/2020

Abstract

It has been commonly assumed that Zika virus (ZIKV) infection confers long-term protection against reinfection, preventing ZIKV from re-emerging in previously affected areas for several years. However, the long-term immune response to ZIKV following an outbreak remains poorly documented. We compared results from eight serological surveys before and after known ZIKV outbreaks in French Polynesia and Fiji, including cross-sectional and longitudinal studies. We found evidence of a decline in seroprevalence in both countries over a two-year period following first reported ZIKV transmission. This decline was concentrated in adults, while high seroprevalence persisted in children. In the Fiji cohort, there was also a significant decline in neutralising antibody titres against ZIKV, but not against dengue viruses that circulated during the same period.

4.2 Introduction

Zika virus (ZIKV), a flavivirus primarily transmitted to humans by *Aedes* mosquitoes, was first reported in the Pacific region on Yap island (Federated States of Micronesia) in 2007 (*Duffy et al.*, 2009). Six years later, there was a large ZIKV outbreak in French Polynesia (*Cao-Lormeau et al.*, 2014b) where an estimated 11.5% of the population visited healthcare facilities with clinical symptoms suggestive of ZIKV infection (*Kucharski et al.*, 2016). Since then the virus has spread across the Pacific region (*Musso et al.*, 2014), including to Fiji where cases of ZIKV infection were first detected in July 2015 (*World Health Organisation Western Pacific Region*, 2017). The same year, cases of ZIKV infection in Latin America were reported for the first time (*Zammarchi et al.*, 2015). From February 1 to November 18, 2016, due to its rapid spread and association with birth defects, microcephaly in newborns and Guillain-Barré syndrome in adults (*Cao-Lormeau et al.*, 2016) the WHO declared ZIKV a Public Health Emergency of International Concern (*World Health Organisation*, 2016). At the end of 2016, outbreaks had declined in most of the countries recently affected (*O'Reilly et al.*, 2018). However, ZIKV was still circulating in 2018 in several countries, including Fiji and Tonga in the Pacific region (*World Health Organisation*, 2018).

In countries with known ZIKV outbreaks, the few serological surveys that have been published found a high level of ZIKV seroprevalence following the outbreak. In French Polynesia, a population-representative cross-sectional serological survey at the end of the outbreak in 2014 found a seroprevalence of 49% (*Aubry et al.*, 2017). In Martinique, a study of blood donors showed a post-outbreak seroprevalence of 42% in 2015 (*Gallian et al.*, 2017). In Salvador, Northeastern Brazil, a serosurvey in 2016 of prospectively sampled individuals including microcephaly and non-microcephaly pregnancies, HIV-infected patients, tuberculosis patients, and university staff, found a post-outbreak seroprevalence of 63% (*Netto et al.*, 2017). Another study in Salvador, conducted in a long-term health cohort, also found a post-outbreak seroprevalence of 63% (*Rodriguez-Barraquer et al.*, 2019). Finally, in paediatric and household cohort studies in Managua, Nicaragua, ZIKV seroprevalence was estimated to be 46% in households following the outbreak in 2016 (*Zambrana et al.*, 2018).

It has been suggested that infection with ZIKV confers immunity that lasts several years; if so, the high level of seroprevalence in affected countries may reflect sufficient herd immunity for the current ZIKV epidemic to be over in many locations, with the virus unable to re-emerge for decades to come (*Ferguson et al.*, 2016; *Kucharski et al.*, 2016; *Netto et al.*, 2017; *O'Reilly et al.*, 2018). Recent evidence suggests that neutralising antibodies can distinguish between ZIKV and dengue virus (DENV) – a closely related flavivirus – and that the immune response following ZIKV infection can persist over a year (*Griffin et al.*, 2019; *Montoya et al.*, 2018). It has also been suggested that primary ZIKV infection may confer protective immunity (*Osuna et al.*, 2016). However, ZIKV serosurveys conducted at the end of the outbreak in French Polynesia and 18 months later found a drop in seroprevalence in the Society Islands, the archipelago where over 85% of the inhabitants of French Polynesia reside (*Aubry et al.*, 2017). Therefore, the long-term antibody response following a ZIKV outbreak remains unclear.

Here, we explore short- and long-term seroprevalence against ZIKV as well as neutralising responses against ZIKV following two ZIKV outbreaks in the Pacific region. We compared results from five serological surveys in the Society Islands, French Polynesia, over a seven-year period, and three serial serological surveys in the same cohort of individuals in Central Division, Fiji, over a four-year period. These surveys span the pre- and post- outbreak period in each country, allowing us to examine temporal changes in antibody responses following a ZIKV outbreak.

4.3 Materials & Methods

4.3.1 Study location and participants

French Polynesia

Four separate ZIKV serosurveys were previously conducted in the Society Islands (Table 4.2). As reported previously (*Aubry et al.*, 2015a, 2017), a first serosurvey ($n = 593$)

was conducted in adult blood donors recruited between July 2011 and October 2013, before the ZIKV outbreak that occurred between October 2013 and April 2014 (*Cao-Lormeau et al.*, 2014b). Two population-representative serosurveys were conducted among the general population, firstly between February and March 2014 ($n = 196$), and then between September and November 2015 ($n = 700$). The two studies in the general population both spanned a range of adult age groups (Table 4.1). An additional serosurvey was conducted among schoolchildren between May and June 2014 ($n = 476$). Finally, a fifth serosurvey was conducted among schoolchildren in the Society Islands in June 2018 ($n = 457$) using the same protocol as in 2014 (*Aubry et al.*, 2017).

Table 4.1: *Age distribution of study population in French Polynesia. Overall population distribution shown, along with total samples collected in each age group in 2014 and 2015 serosurveys*

Age range	Population estimate (2017)	Samples in 2014 study	Samples in 2015 study
0–9	42,770	0	0
10–19	43,705	3	22
20–29	48,914	10	135
30–39	42,144	5	131
40–49	40,886	8	119
50–59	34,478	15	128
60–69	21,099	2	85
70–79	10,481	5	46
80–89	3,773	0	9
90+	416	0	0

Fiji

Three serosurveys were conducted in Fiji (Table 4.2). Individuals were first recruited into a population-representative community-based typhoid/leptospirosis seroprevalence study between September and November 2013 (*Watson et al.*, 2017) ($n = 1,787$), before autochthonous transmission of ZIKV was first detected in July 2015 (*World Health Organisation Western Pacific Region*, 2017). Briefly, nursing zones were randomly selected, from which one individual from 25 households in a randomly selected com-

munity was recruited. Participants who had consented to being contacted again for health research were subsequently recruited in November 2015 in 23 communities in Central Division through last known addresses, phone numbers and the assistance of local nurses ($n = 327$) (*Kama et al.*, 2019). A third follow-up serosurvey was conducted in June 2017 using the same protocol as in 2015 ($n = 321$) (*Kucharski et al.*, 2018a). Follow-up surveys were only performed in Central Division, which was the focus of a DENV-3 outbreak in 2013–14 (*Kucharski et al.*, 2018a). Only blood samples serially collected from the same participants ($n = 189$) in 2013, 2015 and 2017 were analysed in the main results presented in this study.

4.3.2 Informed consent and ethics approvals

French Polynesia

The five serosurveys were approved by the Ethics Committee of French Polynesia (ref 61/CEPF 08/29/2013, 60/CEPF 06/27/2013, 74/CEPF 05/04/2018, and 75/CEPF 05/04/2018).

Fiji

The original 2013 study, and the 2015 and 2017 follow up studies were approved by the Fiji National Research Ethics Review Committee (ref 2013–03, 2015.111.C.D, 2017.20.MC) and the London School of Hygiene and Tropical Medicine Observational Research Ethics Committee (ref 6344, 10207, 12037).

4.3.3 Serological analysis

French Polynesia

Serum samples collected from blood donors between July 2011 and October 2013 and samples collected from the general population and schoolchildren in 2014 were all tested

for presence of IgG antibodies against ZIKV and each of the four DENV serotypes using a recombinant antigen-based indirect ELISA as reported previously (*Aubry et al.*, 2015a, 2017). Samples collected from the general population in 2015 and from schoolchildren in 2018 were tested by microsphere immunoassay (MIA) using the same recombinant antigens as for the ELISA (*Aubry et al.*, 2017; *Cao-Lormeau et al.*, 2016; *Kama et al.*, 2019). Recombinant antigens used in both assays comprised domain III of the envelope glycoprotein of ZIKV, DENV-1, DENV-2, DENV-3, or DENV-4 strains (respective GenBank accession no. KJ776791, AF226686.1, FM986654, FJ44740.1, FM986672.1) and were produced using the Drosophila S2 expression system (Life Technologies, USA) as previously detailed (*Aubry et al.*, 2015b). Serostatus was defined by a cut-off determined using positive and negative control sera analysed by ROC curve. The sensitivity and specificity of the MIA assay were respectively 100% and 100% for DENV-1, 89.5% and 97.1% for DENV-2, 100% and 100% for DENV-3, 96.9% and 100% for DENV-4, and 79.6% and 94.9% for ZIKV. The positive control sera for ZIKV was collected 13 months after RT-PCR confirmed infection. In the serosurvey conducted among the general population of the 5 archipelagos in French Polynesia in 2014 (*Aubry et al.*, 2017), 196 samples were tested using both ELISA and MIA: among the 97 serum samples that tested positive for anti-ZIKV IgG by ELISA, 78 (80%) were also found positive by MIA; and among the 99 serum samples that tested negative for anti-ZIKV IgG by ELISA, 70 (71%) were also found negative by MIA. This produced a value of Cohen's $\kappa = 0.51$ (*Aubry et al.*, 2017).

Fiji

All serum samples collected in Fiji were tested using MIA to detect IgG antibodies against ZIKV and all four DENV serotypes as previously reported (*Aubry et al.*, 2017; *Cao-Lormeau et al.*, 2016; *Kucharski et al.*, 2018a). To follow the evolution of antibody titres at the individual level, a subset of samples collected from the same individuals in 2013, 2015 and 2017 were tested for the presence of neutralising antibodies against ZIKV and each of the four DENV serotypes using a neutralisation assay as previously described (*Cao-Lormeau et al.*, 2016). ZIKV log titres followed a bimodal distribution,

which supported the use of a log titre of ≥ 2 as a cutoff for seropositivity (Figure 4.1).

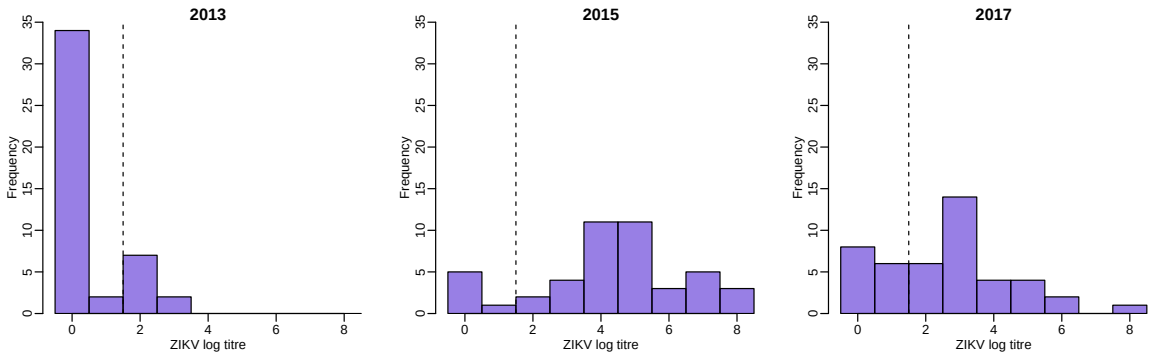

Figure 4.1: *Distribution of ZIKV neutralisation titres in the Fiji serosurveys. Results shown for 45 participants who had samples available from 2013, 2015, and 2017. Dashed line shows the threshold used to define seropositivity*

Of the 9/45 participants who were seropositive to ZIKV by neutralisation assay in 2013, all were seropositive to at least one DENV serotype (Figure 4.2). To assess the potential for cross-reactive antibody responses, we examined the correlation between changes in log titre to different viruses between 2013 and 2015. As well as the 45 participants who had three samples from 2013, 2015, and 2017, we also had an additional 24 participants from the same cohort for whom we had sufficient serum from 2013 and 2015 to test by neutralisation assay (i.e. 69 paired samples in total). Among the 20/69 participants that tested seronegative against all five viruses in 2013 and were re-tested in 2015, there was no evidence of an association between changes in ZIKV titre and changes in titre against any of the DENV serotypes, suggesting that the changes in ZIKV titre were unlikely to be strongly influenced by DENV cross-reaction (Figure 4.3). However, the 49/69 participants who had a pre-2013 DENV exposure and a large rise against ZIKV between 2013–15 tended to exhibit a smaller rise against DENV viruses (Figure 4.4).

A previous study, which tested serological samples from Fiji across three divisions (*Kama et al.*, 2019), found that of the samples reactive by MIA, 66/83 (79.5%) exhibited neutralising activity for ZIKV ($\kappa = 0.71$) and 109/112 (97.3%) for DENV ($\kappa = 0.80$). In this study, we tested what proportion of samples for the 45 participants in the full data set (i.e. 135 samples in total) that were seropositive or seronegative by MIA had the same result by the neutralisation assay. We found that 54/68 (79.4%) samples that

were positive to ZIKV by MIA were also positive in the neutralisation assay, and 42/67 (62.7%) who were seronegative were also negative by neutralisation assay ($\kappa = 0.42$). We also calculated the proportion positive by neutralisation assay that had the same result by MIA. We found that 54/79 (68.4%) samples that were positive to ZIKV in the neutralisation assay were also positive by MIA, and 42/56 (75%) who were seronegative were also negative by MIA.

4.3.4 Statistical analysis

For data from Fiji, where serial samples were collected from the same individual, changes in seroprevalence between studies were tested using McNemar's test. In French Polynesia, chi-squared tests were performed to test for evidence of a change in seroprevalence between two cross-sectional surveys. Changes in mean log titre between groups were analysed using a t-test. To analyse the potentially non-linear relationship between DENV neutralisation titres and seroprevalence by MIA and neutralisation test (Figure 4.13), we used a generalized additive model via the `mcgv` package in R (*Wood*, 2019). The model was of the form $g(E(y)) = b + f(x)$, where y was the binary outcome variable, x was the predictor (i.e. titre), g was the link function, b was the intercept, and f was a smooth function represented by a penalized regression spline. Mean DENV titre was calculated as the mean of log titres against the four DENV serotypes for each participant. All data and code used in the analysis are available at: `https://github.com/a-henderson91/zika-sero-pacific/`.

4.4 Results

In French Polynesia, seroprevalence of IgG antibodies against domain III of the ZIKV envelope glycoprotein in blood donors recruited before October 2013 was <1% (0.3%-2%), which confirmed that the virus had not previously circulated in the population (Table 4.2). Analysis of samples collected in the general population of the Society Islands of French Polynesia after the emergence of ZIKV showed a decrease in ZIKV

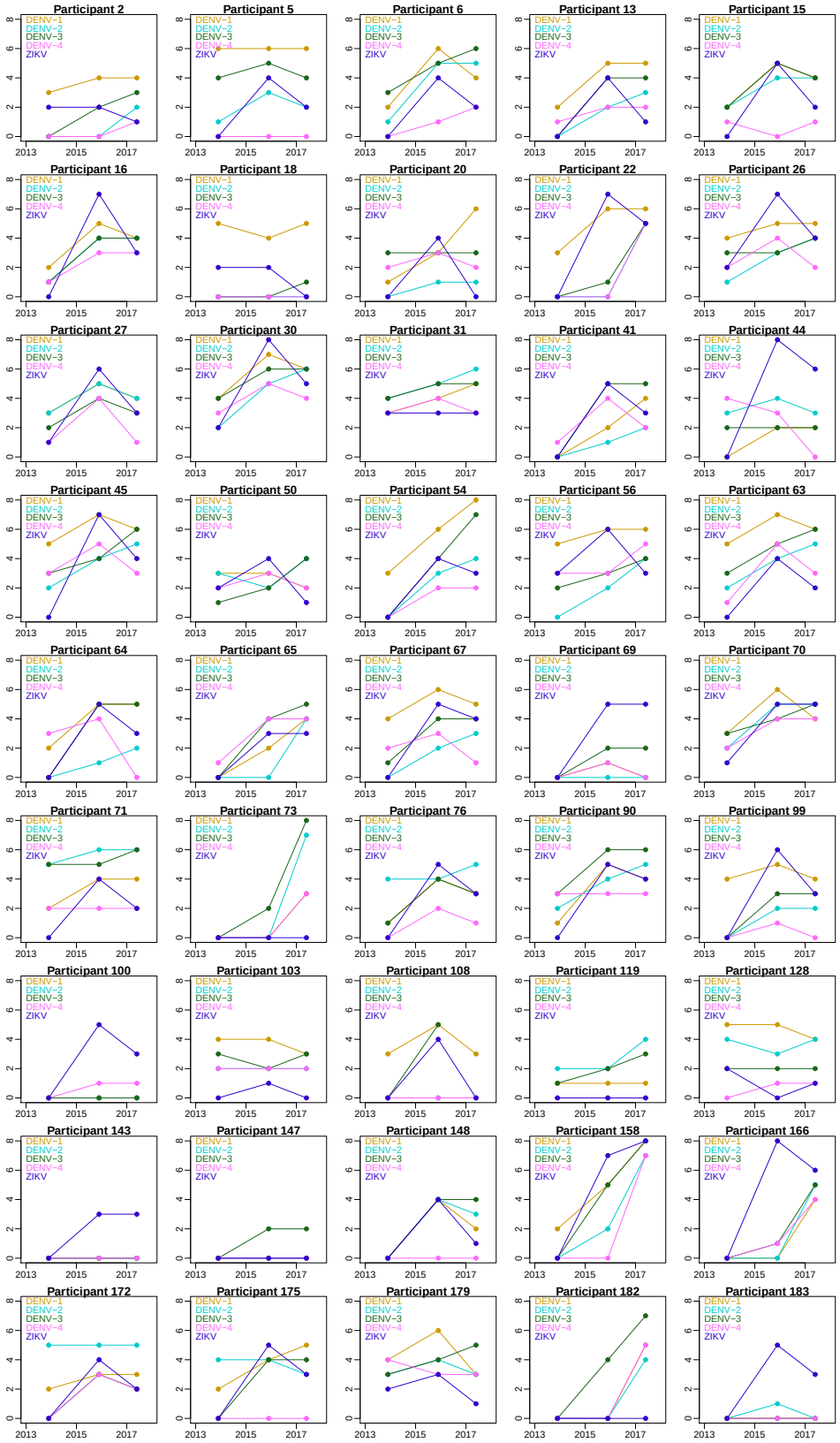

Figure 4.2: *Individual-level neutralisation log titres against the four DENV serotypes and ZIKV in Fiji. Points show assay results in the 2013, 2015 and 2017 sample collections for each participant, coloured by virus (n=45)*

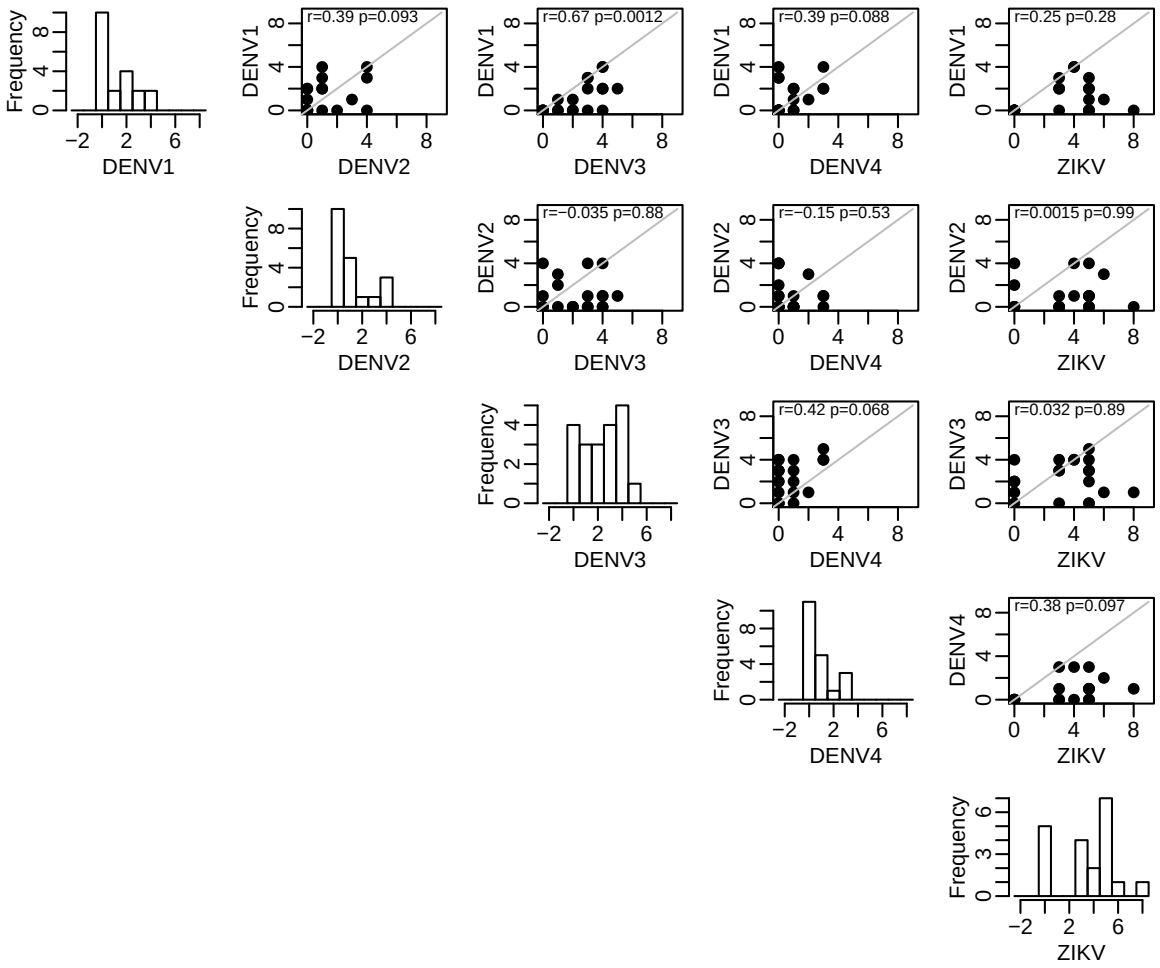

Figure 4.3: *Correlation between rise in DENV and ZIKV neutralisation log titres between 2013-2015 for participants who were initially seronegative (i.e. log titre < 2) to all five viruses in 2013 (n=20). There is significant correlation between DENV-1 and DENV-3 viruses (top row, p=0.0012), suggesting likely cross-reactive responses. However, changes in ZIKV titres were not associated with responses to any of DENV viruses (far right column), which strongly indicates that the ZIKV results were genuine infections*

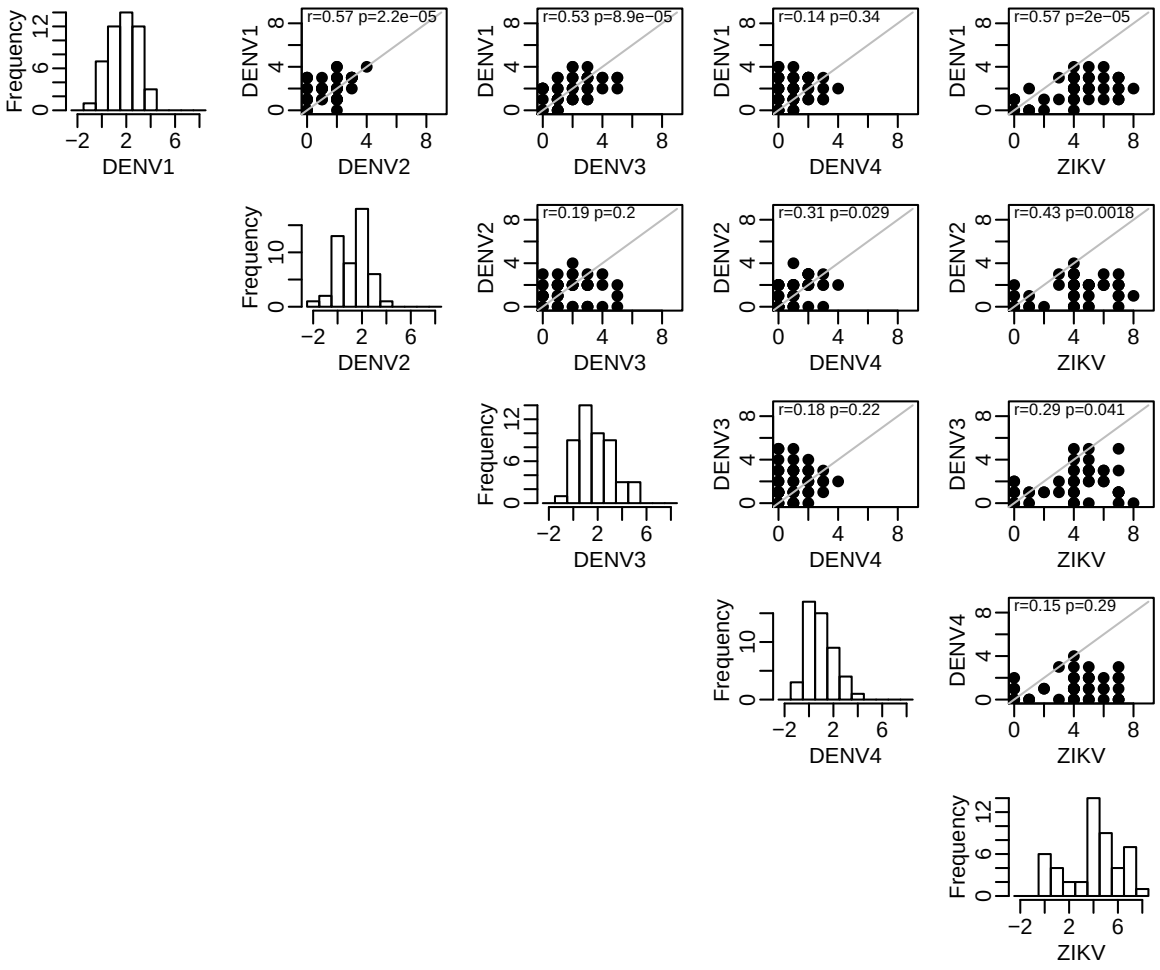

Figure 4.4: *Correlation between change in DENV and ZIKV log titres between 2013-2015 for participants who were initially seropositive (i.e. log titre ≥ 2) to at least one DENV virus in 2013 (n=49). There is significant correlation between ZIKV and other DENV viruses, suggesting likely cross-reactive responses. However, there was limited circulation of viruses such as DENV-1 and DENV-2 during 2013–15 (Figures 4.6, 4.7), suggesting that it was infection with ZIKV that generated a cross-reactive response against these viruses, rather than the other way around*

seroprevalence from 37% (26%-47%) to 22% (16%-28%) between February-March 2014 and September-November 2015 (chi-squared test, $p = 0.03$). In Fiji, analysis of the serum samples serially collected from a cohort of participants in the Central Division showed an increase in ZIKV seroprevalence from 6.3% (3.3%-11%) in October-November 2013 to 24% (18%-31%) in November 2015 (chi-squared test, $p < 0.0001$), and then a decrease to 12% (7.9%-18%) by June 2017 (chi-squared test, $p = 0.005$). In this cohort, based on IgG results tested by microsphere immunoassay (MIA), 6 of the 189 participants seroconverted (from negative to positive) and 28 seroreverted (from positive to negative) to ZIKV between 2015 and 2017 (McNemar's test, $p = 0.0003$).

Table 4.2: *Seroprevalence of ZIKV among participants in five serological surveys in French Polynesia and three serological surveys in Fiji, conducted between July 2011 and June 2018*

Date	Country	Population and assay used	Age Range (median)	Total no. seropositive/total no. tested	Seroprevalence % [95% CI]
French Polynesia - General Population					
Jul 2011-Oct 2013	Society Islands, French Polynesia	Blood donors, ELISA	18-75 (36)	5/593	0.8 [0.3-2.0]
Nov 2013	*First confirmed local transmission of ZIKV in French Polynesia*				
Feb-Mar 2014	Society Islands, French Polynesia	General, ELISA	13-77 (47)	18/49	37 [26-46]*
Sep-Nov 2015	Society Islands, French Polynesia	General, MIA	4-88 (43)	154/700	22 [16-28]*
French Polynesia - schoolchildren					
May-Jun 2014	Society Islands, French Polynesia	School children, ELISA	6-16 (11)	312/476	66 [60-71]*
Jun 2018	Society Islands, French Polynesia	School children, MIA	6-16 (11)	291/457	64 [58-69]*
Fiji					
Oct-Nov 2013	Central Division, Fiji	General, MIA	2-78 (27)	12/189	6.3 [3.3-11]
Jul 2015	*First confirmed local transmission of ZIKV in Fiji*				
Nov 2015	Central Division, Fiji	General, MIA	4-80 (26)	45/189	24 [18-31]
Jun-2017	Central Division, Fiji	General, MIA	6-82 (28)	23/189	12 [7.9-18]

* CIs were calculated taking into account the cluster sampling design (*Aubry et al.*, 2017) and using Fisher exact test
MIA - microsphere immunnoassay

To investigate possible factors influencing the decline in seroprevalence, we compared the seroprevalence profiles in children (defined as ≤16 years) and adults (>16 years)

in both settings (Table 4.2 and Figure 4.5). In French Polynesia, although ZIKV seroprevalence declined in the general population from the Society Islands over 18 months, there was no evidence of a significant decline in seroprevalence in two serosurveys conducted four years apart in schoolchildren aged 6 to 16 years, with 66% (60%-71%) positive in 2014 and 64% (58%-69%) in 2018 (chi-squared test, $p = 0.6$) (Table 4.2). When stratifying the general population from the Society Islands by age ($\leq$16 years and >16 years), there was a decline in adults in the two consecutive cross-sectional studies conducted in 2014 and 2015, from 35.4% (22.2%-50.5%) to 21.3% (18.2%-24.5%) (Figure 4.5). A decline in adults was still observed, albeit with larger uncertainty, when the two data sets were standardised according to the age distribution of the population, with age-adjusted seroprevalence decreasing from 32.0% (16.7%–62.1%) to 26.0% (20.1%–33.9%) (Table 4.3).

Table 4.3: *Age-adjusted seroprevalence by MIA in participants aged over 16 in the general population of the Society Islands in French Polynesia, based on serosurveys conducted in 2014 ($n = 48$) and 2015 ($n = 672$)*

Virus	2014 seroprevalence (95% CI)	2014 age-adjusted seroprevalence (95% CI)	2015 seroprevalence (95% CI)	2015 age-adjusted seroprevalence (95% CI)
DENV1	85 (72-94)	83 (55-100)	80 (77-83)	80 (71-91)
DENV2	48 (33-62)	50 (28-87)	19 (16-22)	21 (15-21)
DENV3	75 (60-86)	72 (47-100)	56 (52-60)	55 (48-64)
DENV4	63 (47-76)	65 (40-100)	42 (38-46)	45 (38-54)
ZIKV	35 (22-50)	32 (16-62)	21 (18-25)	26 (20-34)

* chi-squared test comparing 2014 bootstrap estimates with 2015 results

In Fiji, in the subset of individuals who were aged over 16 years ($n = 122$), there was a decrease in seroprevalence by MIA from 24% (17%-33%) in 2015 to 7.3% (3.4%-13%) 2017 (Figure 4.5). There were two seroconversions in the collected samples over this period but 23 seroreversions (McNemar's test, $p < 0.0001$) (Table 4.4). In contrast seroprevalence in participants aged 16 and under ($n = 67$) remained relatively stable over this period (Figure 4.5), with four seroconversions and five seroreversions (McNemar's test, $p = 1$) (Table 4.4).

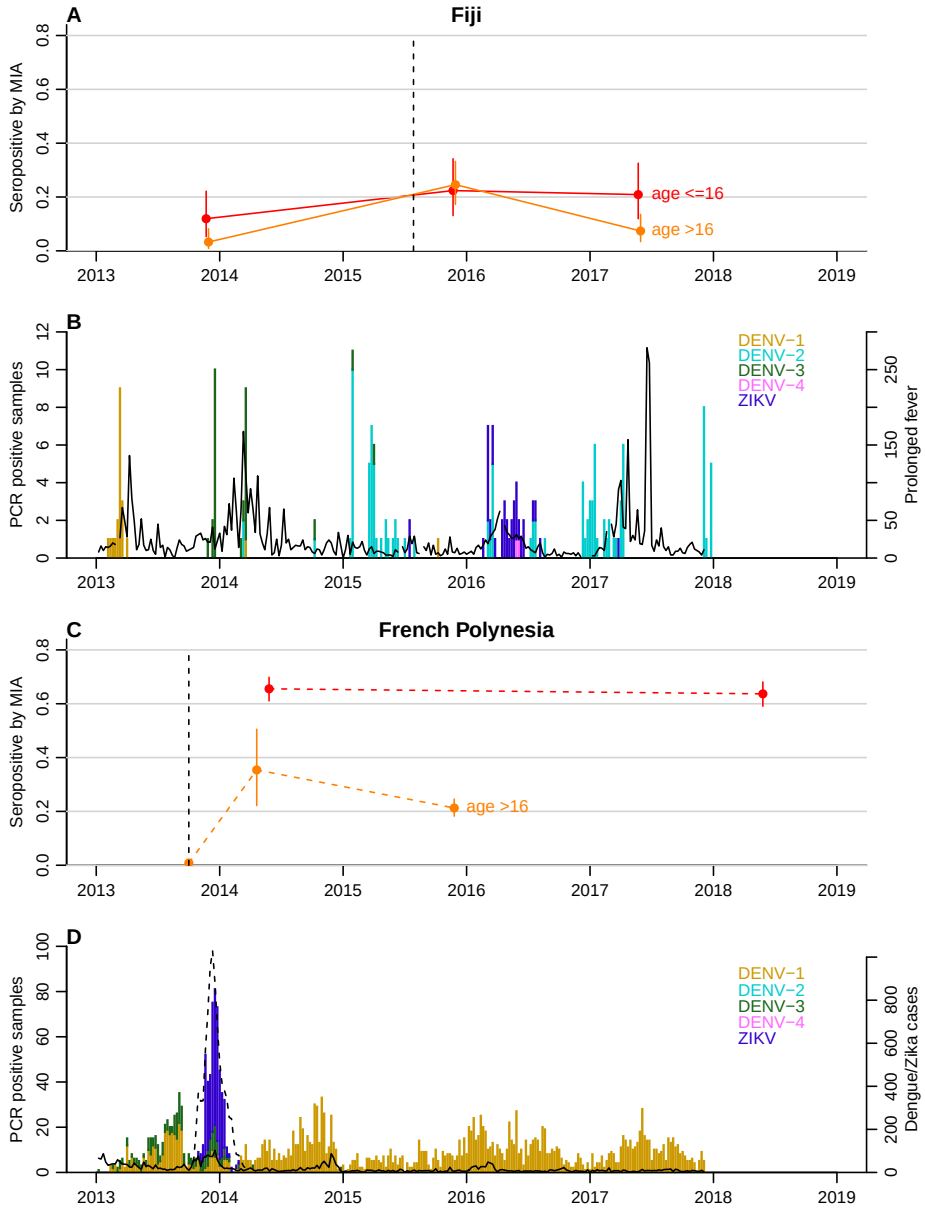

Figure 4.5: *Dynamics of ZIKV seroprevalence following outbreaks in Fiji and French Polynesia. A) Seroprevalence by MIA in Fiji. Red, seroprevalence and 95% confidence intervals for children (aged ≤16 years). Orange, seroprevalence and 95% confidence intervals for adults (aged >16 years). Solid lines, trends in data collected from the same individuals. Dotted line indicates the first confirmed ZIKV case.* *(Continued on the following page)*

Figure 4.5: *caption (continued): B) Epidemiological dynamics in Fiji between 2013 and 2018. Coloured bars show number of PCR-confirmed samples of different DENV serotypes and ZIKV in Fiji; black lines show reported prolonged fever in Fiji from the Pacific Syndromic Surveillance System (World Health Organisation, 2018). There was a major outbreak of DENV-3 outbreak in 2013–14 (Kucharski et al., 2018a) with a smaller DENV-2 outbreak in early 2017 (Aubry et al., 2019). C) Seroprevalence by MIA in French Polynesia. Dashed lines, trends in seroprevalence between population representative cross-sectional surveys. Note that the pre-outbreak samples were collected between July 2011 and October 2013; for brevity, the latest possible collection date is used in the plot. D) Epidemiological dynamics in French Polynesia between 2013 and 2018. Solid black line shows reported symptomatic dengue cases; dashed lines showed reported symptomatic Zika cases. In French Polynesia, between the sampling periods, there were no reported DENV outbreaks for serotypes 2,3,4, and there was hyper-endemic DENV-1 circulation. In April 2019, a DENV-2 outbreak was declared, the first since 1997 (Aubry et al., 2019)*

In order to assess whether the decline in ZIKV seroprevalence was also observed for other circulating flaviviruses, the MIA seroprevalence pattern against each of the four DENV serotypes was analysed in both countries, by age group (Figures 4.6, 4.7, 4.8, 4.9). In Fiji, seroprevalence for DENV-1, DENV-2 and DENV-4 increased in participants in both age groups between 2013 and 2017. DENV-3 seroprevalence also increased in both age groups between 2013 and 2015 following an outbreak in 2013–14 (*Kucharski et al.*, 2018a) and then declined in 2017 from 44% (32%-57%) to 40% (28%-52%) in children (McNemar's test, $p = 0.6$) and from 59% (50%-68%) to 49% (40%-58%) in adults (McNemar's test, $p = 0.01$) (Figure 4.8). In French Polynesia between 2014 and 2018, seroprevalence in children aged under 16 years showed no evidence of a change for DENV-1 and DENV-2 (chi-squared test, $p = 0.1917$ and $p = 1$, respectively) (Figures 4.6, 4.7) and decreased for DENV-3 and DENV-4 (chi-squared test, $p < 0.0001$ and $p = 0.0085$, respectively) (Figures 4.8, 4.9). In adult participants from the general population, seroprevalence for all four DENV serotypes declined between 2014 and 2015.

The age-adjusted values for seroprevalence by MIA for the four DENV serotypes were

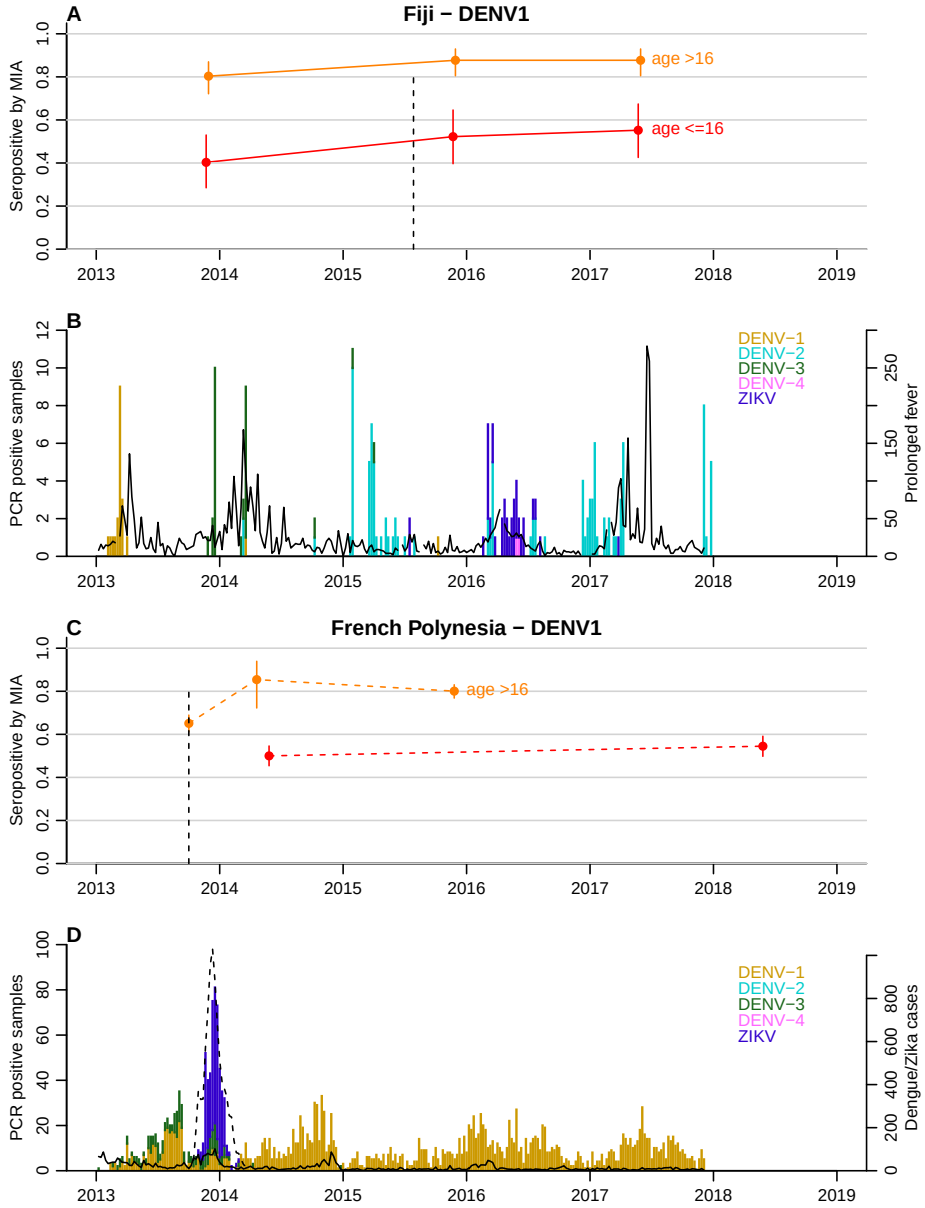

Figure 4.6: *Seroprevalence against DENV-1 in Fiji and French Polynesia, by age group. Figure colour scheme and data characteristics are same as in Figure 4.5*

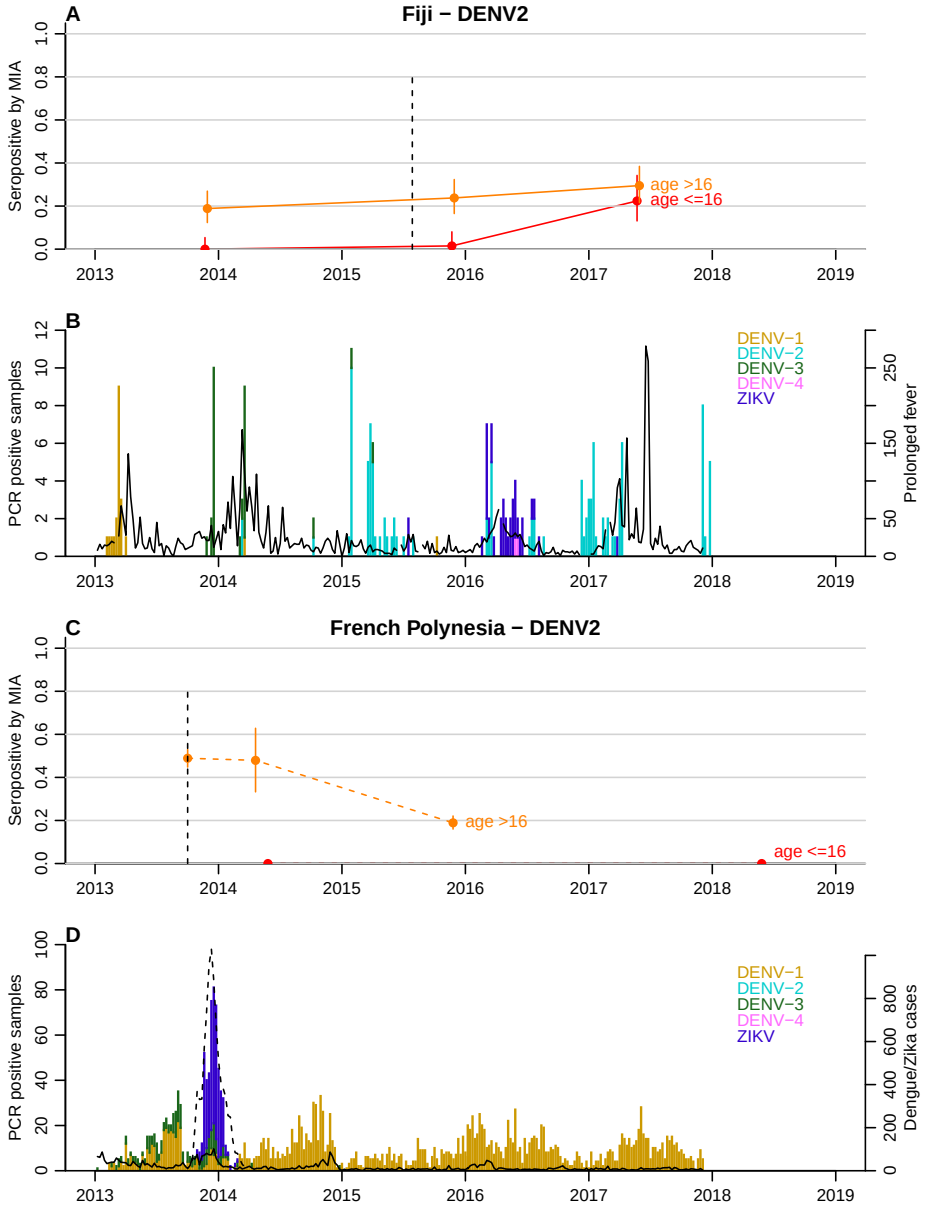

Figure 4.7: *Seroprevalence against DENV-2 in Fiji and French Polynesia, by age group. Figure colour scheme and data characteristics are same as in Figure 4.5*

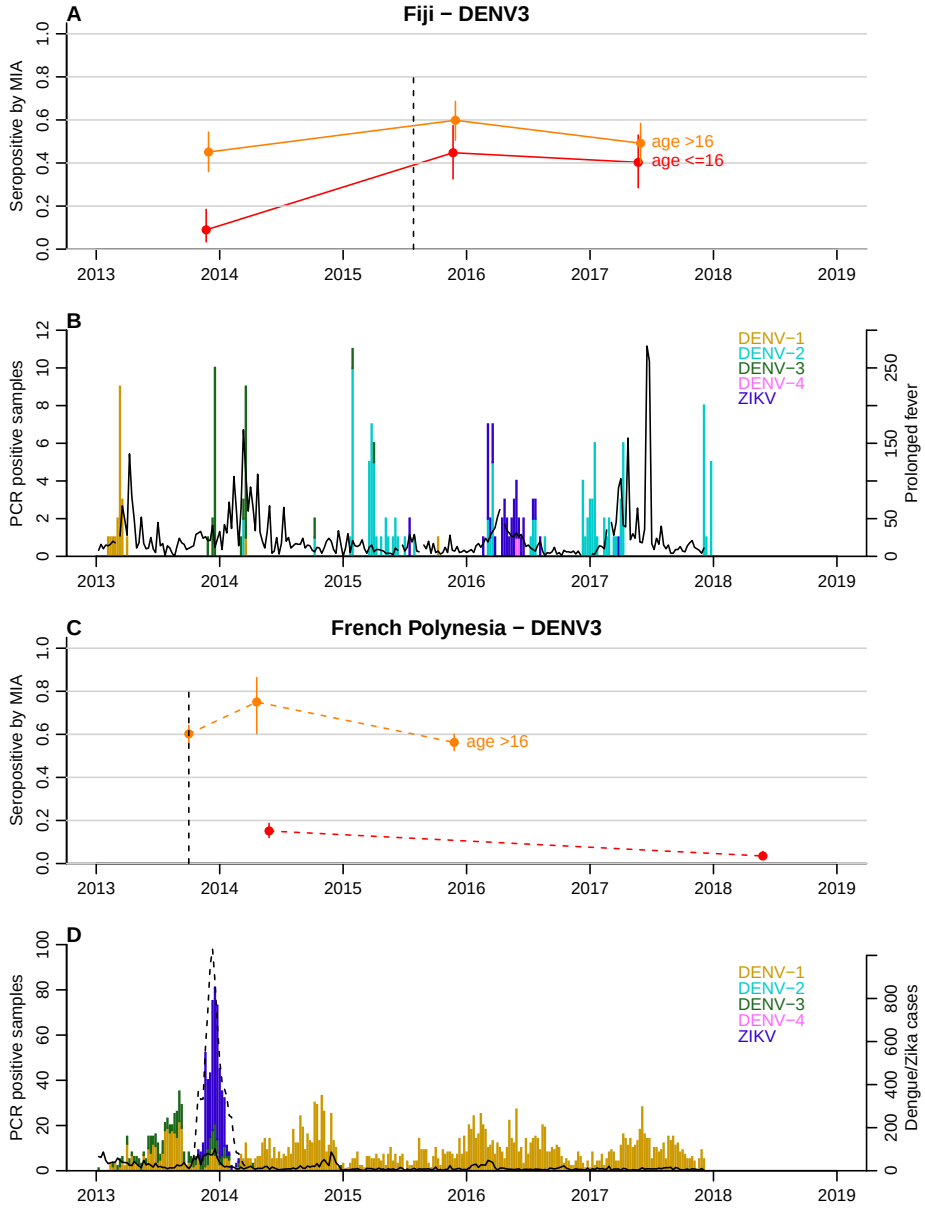

Figure 4.8: *Seroprevalence against DENV-3 in Fiji and French Polynesia, by age group. Figure colour scheme and data characteristics are same as in Figure 4.5*

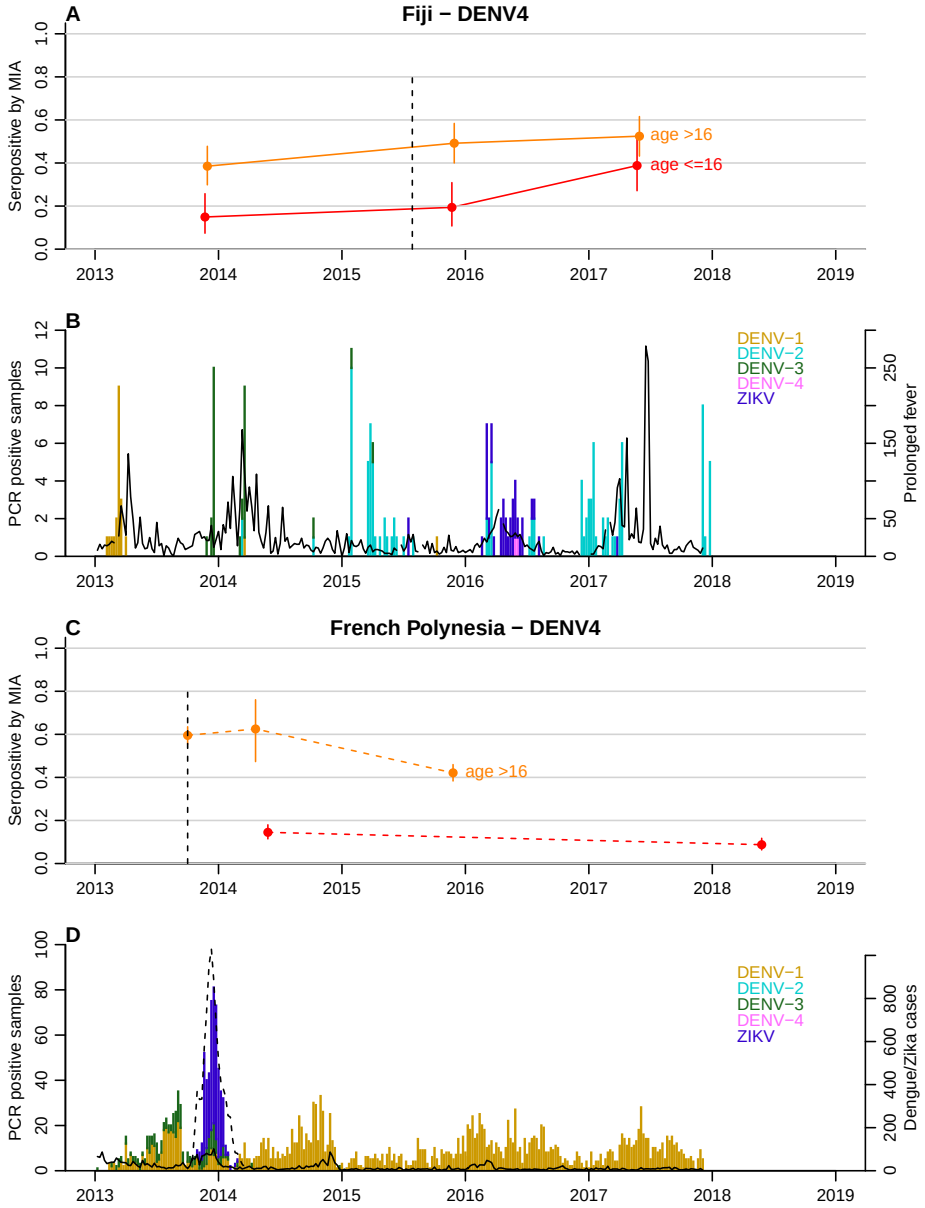

Figure 4.9: *Seroprevalence against DENV-4 in Fiji and French Polynesia, by age group. Figure colour scheme and data characteristics are same as in Figure 4.5*

Table 4.4: *Detection of IgG by MIA against ZIKV in the paired samples from participants aged under and over 16 years recruited during October-November 2015 and June 2017 in the Central division in Fiji (n = 189). Age groups are defined using age of participants when recruited to the study in 2013*

	2017					
	≥ 16 years		>16 years		Total participants	
2015	ZIKV+	ZIKV-	ZIKV+	ZIKV-	ZIKV+	ZIKV-
≥16 years						
ZIKV+	10	5	–	–	–	–
ZIKV-	4	48	–	–	–	–
> 16 years						
ZIKV+	–	–	7	23	–	–
ZIKV-	–	–	2	90	–	–
Total Participants						
ZIKV+	–	–	–	–	17	28
ZIKV-	–	–	–	–	6	138

similar to the raw values, suggesting that the decline in French Polynesia could not be explained by differences in sampling by age. However, a higher proportion of the samples in 2014 tested positive by MIA for all four DENV serotypes (Table 4.5), suggesting that the sampling included a group at higher risk for arbovirus infection than those sampled in 2015. To check that the estimated decline in ZIKV seroprevalence was not an artefact of this sampling bias, we re-estimated seroprevalence for the four DENV serotypes and ZIKV using a bootstrap sample of the 2014 responses, with replacement, weighted by the DENV exposure profile (excluding the virus of interest) in the 2015 survey so that the bootstrap sample of the 2014 responses had a similar DENV exposure profile as in the 2015 responses. For example, when generating bootstrap estimates for DENV-1 in 2014, we resampled participants based on the distribution of number of exposures to DENV-2, DENV-3, and DENV-4 in the 2015 data (Table 4.6). After adjusting for prior exposure, there was no significant decline in seroprevalence for DENV-1, DENV-3, or DENV-4, which had all circulated in the five years preceding the 2014 data collection, whereas the decline in ZIKV was still present (chi-squared test, $p = 0.0047$).

Table 4.5: *Age distribution and profile of DENV exposure history in two cross-sectional surveys conducted in the general population from the Society Islands, French Polynesia, in 2014 and 2015. While the age distribution is similar in both studies, the sample in 2014 has a higher proportion of individuals who have tested positive for infection from all four DENV serotypes by MIA*

Variable	2014 ($n = 49$)	2015 ($n = 700$)
Age distribution (median [IQR])	47 [29-56]	43 [29-57]
Number of DENV serotypes positive at time of sample collection (n [%])		
0	3 [0.061]	118 [0.17]
1	6 [0.12]	163 [0.23]
2	11 [0.22]	159 [0.23]
3	11 [0.22]	154 [0.22]
4	18 [0.37]	106 [0.15]

To explore dynamics of antibody waning at the individual level, we performed neutralisation assays (NT) on a subset of 45 participants from Fiji for whom sufficient sera were available to test against ZIKV from all three collection periods. We found that in the 31 individuals who were ZIKV seronegative (i.e. log titre < 2) in 2013 and had a rise in log titre ≥ 2 against ZIKV between 2013 and 2015, anti-ZIKV antibody responses waned significantly in 2017, with an average decline in log titre of -1.94 (t-test, $p < 0.0001$) (Figure 4.10A and Table 4.7). In total, four participants seroreverted between 2015 and 2017; all had a log titre of 4 against ZIKV in 2015. We observed a similar effect when we analysed all participants who had a rise in log titre of at least 2 between 2013–15, regardless of serostatus in 2013 (Figure 4.11).

To test whether the dynamics of anti-ZIKV antibody waning were different from the responses to DENV infection, we compared results for ZIKV to the neutralisation response following a DENV-3 infection in the same cohort from Fiji. There was a large DENV-3 epidemic during 2013–14 in Fiji (*Osuna et al.*, 2016), which meant most seroconversions to DENV-3 occurred between the collection of samples in 2013 and 2015. In those individuals that seroconverted to DENV-3 ($n = 19$) or ZIKV ($n = 31$) between 2013 and 2015, the initial rise in NT log titres against ZIKV was larger than for DENV-3, with a mean change of 5.0 and 3.37 respectively (4.10B and Table 4.7). All

Table 4.6: *Bootstrap estimated seroprevalence for each of the four DENV serotypes and ZIKV adjusted for sampling bias in two cross-sectional surveys conducted in the general population from the Society Islands, French Polynesia, in 2014 and 2015. Results from the cross-sectional surveys in the Society Islands, French Polynesia, in 2014 and 2015 show a decline in seroprevalence by MIA against all 4 DENV serotypes and ZIKV. However, the 2014 sample included more individuals that tested positive for >1 DENV serotype and are assumed to be a higher risk group. We used a bootstrap method with 10,000 iterations which estimated seroprevalence from a sample of the 2014 data set, taken with replacement, weighted by the exposure distribution to other DENV viruses in the 2015 survey. After adjusting for the sample bias, there was no evidence of a decline in seroprevalence for DENV-1, DENV-3, or DENV-4, which had circulated in the years preceding the 2014 sample collection (World Health Organisation, 2018), but there remained strong evidence that ZIKV seroprevalence declined between 2014-15*

Virus	2014 seroprevalence (95% CI) ($n = 49$)	2014 bootstrap estimates of seroprevalence (95% CI)	2015 seroprevalence (95% CI) ($n = 700$)	p-value*
DENV1	86 (73-94)	74 (61-86)	80 (77-83)	0.36
DENV2	47 (33-62)	38 (24-53)	18 (15-21)	0.0008
DENV3	76 (61-87)	64 (51-78)	55 (51-59)	0.21
DENV4	63 (48-77)	50 (37-65)	42 (38-46)	0.42
ZIKV	37 (23-52)	42 (29-55)	22 (19-25)	0.0047

* chi-squared test comparing 2014 bootstrap estimates with 2015 results

individuals who had seroconverted to DENV-3 remained seropositive to the virus in 2017, while four individuals who had seroconverted to ZIKV were seronegative in 2017. Although the NT log titres increased by a mean of 0.89 for DENV-3 between 2015 and 2017 (two-sided t-test, $p = 0.04$), log titres against ZIKV declined by a mean of 1.94 over the same period (two-sided t-test, $p < 0.001$) (Figure 4.10A and Table 4.7).

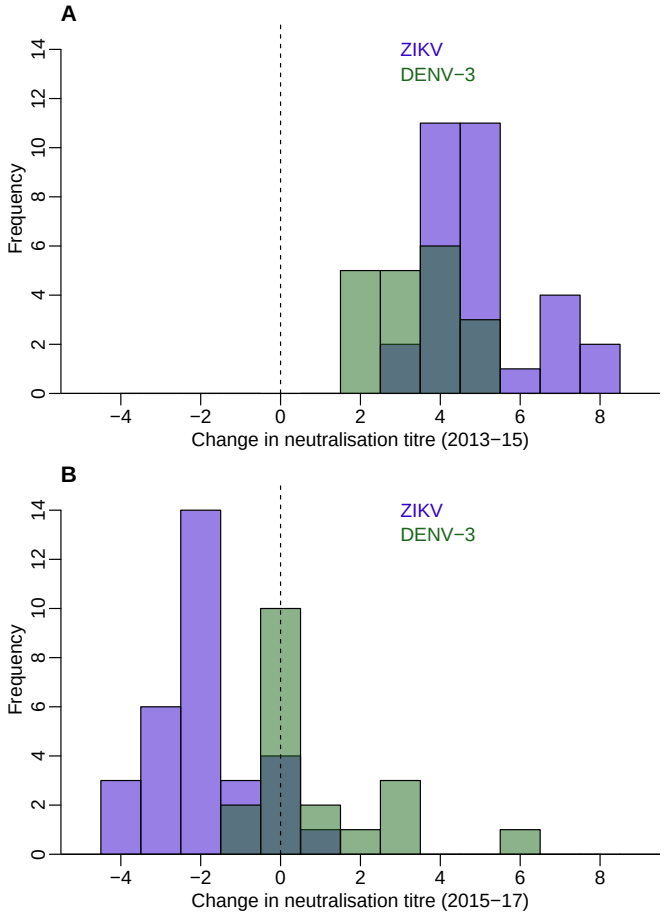

Figure 4.10: *Waning of neutralising antibody responses against ZIKV and DENV-3 in Fiji for participants who were seronegative in 2013 and seroconverted in 2015. A) Histogram of change in neutralisation assay log titre against DENV-3 (n =19) and ZIKV (n=31) between 2013–2015 for individuals who seroconverted to these respective viruses between 2013–2015 (i.e. log titre $<$ 2 in 2013 and log titre $\geq$ 2 in 2015). B) Histogram of change in log titre against DENV-3 and ZIKV between 2015-2017 for these individuals*

In Fiji, there was a delay of around 18 months between the end of the 2013–14 DENV-3 epidemic and collection of samples in 2015. As DENV titres can wane following infection, particularly in individuals with a prior DENV exposure (*Clapham et al.*,

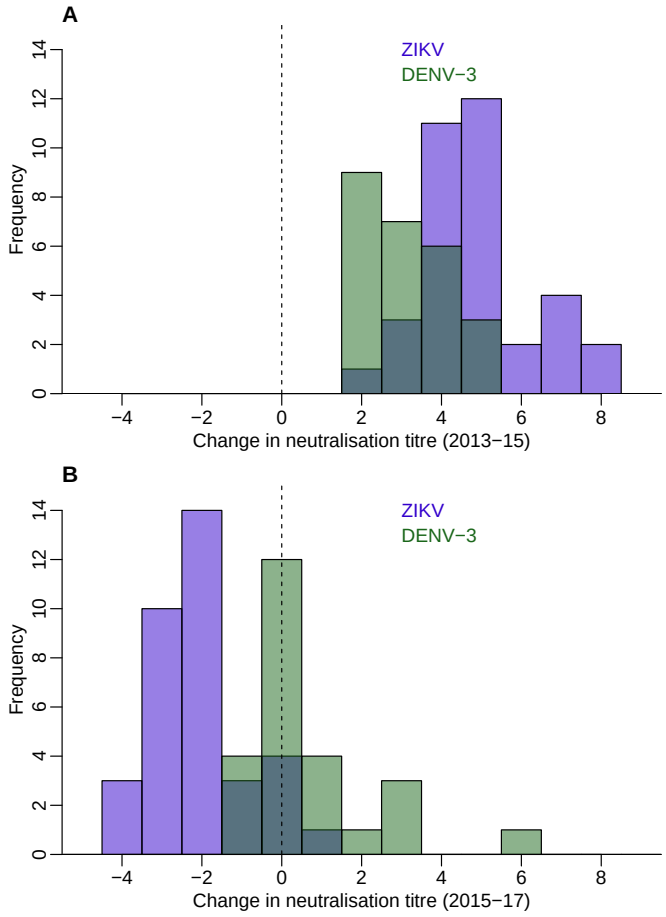

Figure 4.11: *Waning of neutralising antibody responses against ZIKV and DENV-3 in Fiji for participants who had a four-fold rise between 2013 and 2015. Histogram of change in neutralisation assay log titre against DENV-3 (n =25) and ZIKV (n =35) between 2013–2015 for individuals who had a rise in log titre of at least 2 to these respective viruses between 2013–2015. B) Histogram of change in log titre between 2015–2017 against DENV-3 and ZIKV for individuals who had a rise of at least 2 during this period*

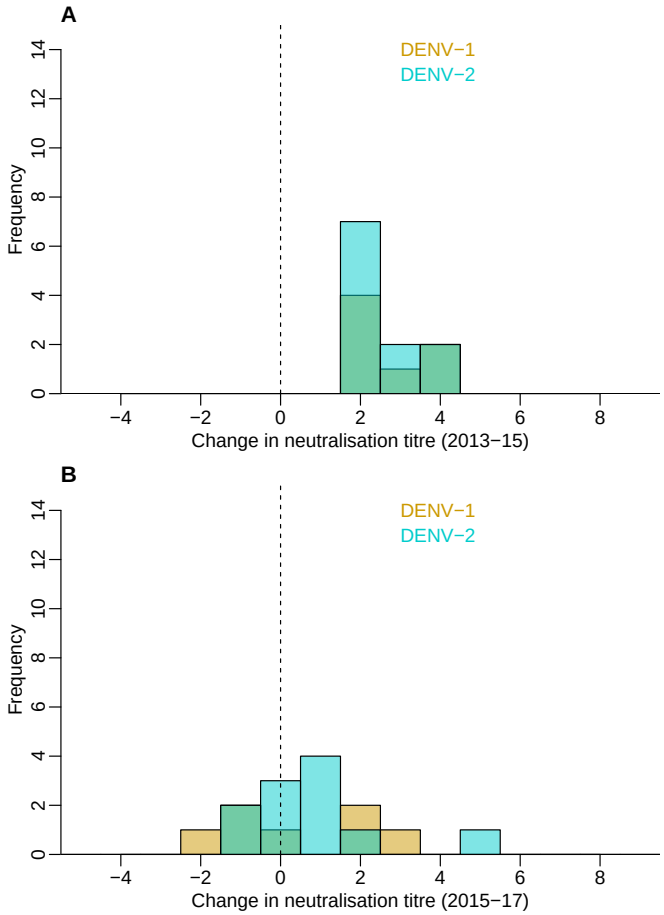

Figure 4.12: *Waning of neutralising antibody responses against DENV-1 and DENV-2 in Fiji for participants who were seronegative in 2013 and seroconverted in 2015. Histogram of change in neutralisation assay log titre against DENV-1 (n=26) and ZIKV (n=18) between 2013–2015 for individuals who seroconverted between 2013–2015 (i.e. log titre $<$ 2 in 2013 and log titre $\geq$ 2 in 2015). B) Histogram of change in log titre against DENV-1 and DENV-2 between 2015-2017 for these individuals*

Table 4.7: *Change in neutralisation titre between 2013-2017 in a cohort of 45 study participants in Fiji. ZIKV and DENV-3 both circulated between the collection of samples in 2013 and 2015 with ZIKV first reported in July 2015 and DENV-3 circulating between October 2013 and January 2015. neutralisation titre levels rose significantly over this period. Between 2015 and 2017, DENV-3 titre levels still increased with a mean change in tire of 0.89. By contrast, the mean change in ZIKV titre over this period decreased (-1.9)*

Virus	2013-2015 change, Mean [95% CI]	*p*-value*	2015-2017 change, Mean [95% CI]	*p*-value*
ZIKV ($n = 31$)	5 [4.5, 5.5]	<0.0001	-1.9 [-2.4, -1.5]	<0.0001
DENV3 ($n = 19$)	3.4 [2.9, 3.9]		0.89 [0.046, 1.7]	

* t-test comparing change in neutralisation titre for ZIKV and DENV-3 between 2013-2015, and 2015-2017

2016), titres against DENV-3 in Fiji may therefore have had more time to wane and reach a stable persistent level than titres against ZIKV, which may have circulated later than DENV-3. We therefore analysed changes in titre for participants who were initially seronegative to DENV-1 and DENV-2, which were circulating at low levels in Fiji between the two serological surveys in 2013 and 2015 (Figure 4.5). As with DENV-3, we found no evidence of a subsequent overall decline during 2015–17 for those participants who seroconverted to DENV-1 or DENV-2 during 2013–15 (Figure 4.12).

Of the 45 participants tested by neutralisation assay, 9 were initially seropositive to ZIKV by NT in 2013. Fitting a generalised additive model to these data, we found that higher baseline mean NT log titres against DENV were associated with an increased probability of seropositivity to ZIKV (Figure 4.13A). In contrast, higher baseline mean DENV titres were not associated with increased seropositivity by MIA in 2013. There was little difference between the assay results in the 2015 samples (Figure 4.13B), but we did find evidence of a difference in the 2017 results, with 15/45 participants positive by MIA and 31/45 positive by NT. This difference was associated with participants' 2013 DENV titres: those with intermediate DENV titres in 2013 had a significantly lower probability of being seropositive in the MIA in 2017 compared to NT (Figure 4.13C).

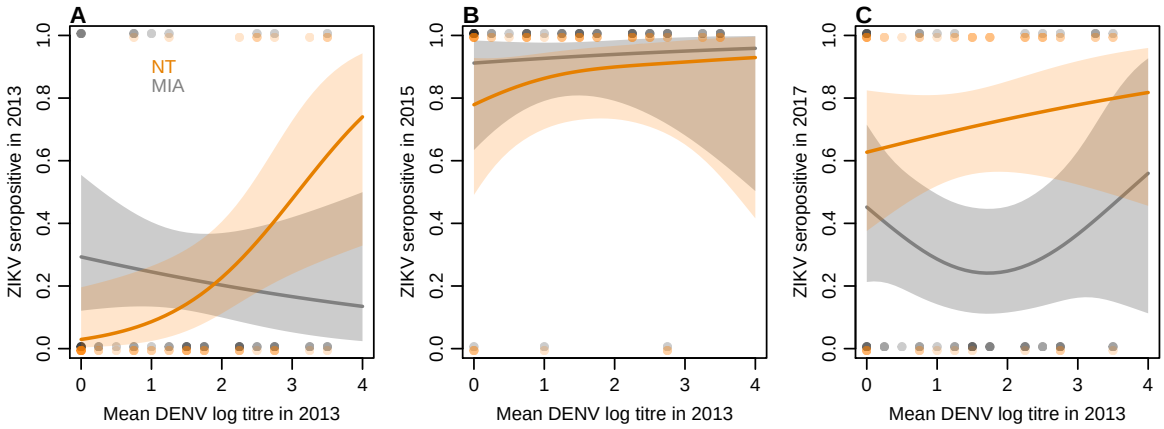

Figure 4.13: *Relationship between mean DENV log neutralisation titre across the four serotypes in 2013 and ZIKV seroprevalence using different assays. (A) Seroprevalence by MIA, shown in grey, and neutralisation test (NT), shown in orange, for sera collected in 2013. Line shows prediction from GAM fitted to each data set, with shaded region showing 95% CI, and points show raw data. (B) Seroprevalence for sera collected from the same participants in 2015. (C) Seroprevalence for sera collected from the same participants in 2017*

4.5 Discussion

Analysing data from serological surveys conducted in French Polynesia and Fiji at different time points after the first reported autochthonous ZIKV transmission, we found evidence of a decline in ZIKV seroprevalence. The high number of participants from the Fijian cohort that seroreverted between 2015 and 2017 suggested that anti-ZIKV antibody levels waned in these individuals to the point that they were no longer detectable by MIA. Using a neutralisation assay to test longitudinal sera collected in Fiji, we found that the mean change in neutralising antibody titres against ZIKV also decreased significantly between 2015 and 2017, showing that individual-level antibody titres against ZIKV as well as overall seroprevalence decreased over time. In contrast, over the same period, neutralising antibody titres against DENV-3, a closely related flavivirus which caused a large epidemic in Fiji in 2013-2014 (*Kucharski et al.*, 2018a), remained stable.

In both countries we found seroprevalence against ZIKV in individuals aged over 16 declined over the two-year period following an outbreak, while the overall level of sero-

prevalence persisted in children. This pattern was unique to ZIKV compared to DENV in both countries. It is possible that this is related to the DENV immunological profile of individuals, given that the older population is likely to have experienced more DENV infections over their lifetime. If an individual has experienced prior DENV infections, high numbers of weakly neutralising cross-reactive B cells may outcompete naïve B cells for ZIKV antigen (*Midgley et al.*, 2011), leading to a short-term boost in antibody response against ZIKV following ZIKV infection (*Robbiani et al.*, 2017) but not a persistent specific response; a similar phenomenon has been observed for other antigenically variable viruses like influenza (*Kucharski et al.*, 2018b). In the 2017 samples, more participants remained seropositive in the neutralisation assay – which measures the overall ability of sera to neutralize ZIKV – than in the MIA, which tests for IgG antibodies against domain III of the envelope glycoprotein. This difference was greatest for participants who had intermediate baseline titres to DENV in 2013 (Figure 4.13C), which would support the hypothesis that prior DENV exposure may result in a detectable short-term specific response against ZIKV following ZIKV infection (as measured by MIA), but not a persistent specific response.

To our knowledge, the only other study to date that has investigated the long-term persistence of neutralising antibodies against ZIKV was conducted in 62 residents of Miami (Florida, USA), who had a confirmed ZIKV infection in 2016 (*Griffin et al.*, 2019). This cross-sectional study found that all participants had neutralising antibodies against ZIKV 12–19 months after infection. This study also found that at least 37% of the participants had no evidence of past DENV infection, which is consistent with the hypothesis that anti-ZIKV immune responses may persist longer in populations that have had less exposure to DENV. More data are therefore needed to test hypotheses about the potential impact of pre-existing anti-DENV immune response on anti-ZIKV antibody waning.

Although we found evidence of a decline in seroprevalence for antibodies against domain III of the envelope glycoprotein, as well as waning neutralising antibody responses following two ZIKV outbreaks, the implications for susceptibility to future ZIKV infection remain unclear. Given the antigenic similarity of DENV and ZIKV (*Priyamvada et al.*,

2016), it is commonly assumed that the immune response to ZIKV infection will be similar to that following DENV infection. High levels of neutralising antibodies to DENV have been shown to correlate with protection from symptomatic infection (*Katzelnick et al.*, 2016). Moreover, infection with a single DENV serotype can confer lifelong immunity to the infecting serotype as well as a transient period of cross-neutralisation against heterologous serotypes (*Wahala and de Silva*, 2011). However, it is unclear in the context of ZIKV what the relationship is between a specific titre value and susceptibility to further infection. A key aim for future work will be establish how waning antibody levels as measured by MIA and neutralisation assays may impact protective immunity, and hence susceptibility to reinfection in populations that have already experienced transmission of ZIKV.

There are some additional limitations to our analysis. First, we did not have reverse transcription polymerase chain reaction (RT-PCR) confirmation of ZIKV infection in individuals sampled in this study. We have presented analysis of representative serological surveys in two locations with known, RT-PCR-confirmed ZIKV outbreaks (*Mallet Anne-Laure Musso, Didier and de veille Sanitaire*, 2016). However, RT-PCR confirmation for ZIKV at the individual level remains difficult to obtain, in particular from blood samples, and there have been relatively few confirmations globally compared to the number of suspected cases (*Ferguson et al.*, 2016), let alone analysis of long-term antibody dynamics in RT-PCR confirmed patients. In French Polynesia, there were approximately 32,000 reported clinical cases of ZIKV infection, but only 297 documented RT-PCR-confirmed cases (*Mallet Anne-Laure Musso, Didier and de veille Sanitaire*, 2016). As a result, antibody responses in RT-PCR-confirmed cases may not necessarily be representative of immune responses against ZIKV in the wider population, particularly following asymptomatic infection. Although MIA seropositivity in our study was defined using control sera collected over a year after RT-PCR-confirmed infection, our results suggest that this threshold may not detect long-term waning responses in individuals who had unreported, and likely less severe, infections.

Our analysis was also limited by study design. In French Polynesia, surveys were cross-sectional, so we were unable to examine temporal antibody dynamics at the individual

level. However, both cross-sectional studies of the general population were conducted using population representative cluster sampling (*Aubry et al.*, 2017) in the same remote island locations with stable population composition, which enabled robust comparisons of overall seroprevalence. We did identify one potential source of sampling bias with different DENV exposure profiles in the two surveys, but our conclusions of declining seroprevalence for ZIKV persisted once we adjusted for this bias. We also used a different serological testing method between the studies in French Polynesia in 2014 and 2015. However, both used the same recombinant antigens and it has been shown that there was good agreement between ELISA and MIA in the 2014 samples (see Materials & Methods). In Fiji, a strength of our study was the collection of longitudinal samples from the same individuals at three time points. However, our sample size was limited given the logistical challenge of recontacting participants twice over a four-year period. These data provided strong evidence that ZIKV seroprevalence declined over the two-year period following first reports of circulation, but our sample size was insufficient to fully explore the potential effect of anti-DENV pre-existing immunity on anti-ZIKV antibody waning once we stratified individuals by previous DENV exposure. Although the outbreaks of DENV-3 in Fiji and ZIKV in French Polynesia were well-documented and occurred over a relatively brief period of time (Figure 4.5), it was harder to identify the likely time of infection for other viruses – such as ZIKV in Fiji or DENV in French Polynesia – in our study populations. Several participants in Fiji were seropositive to ZIKV by neutralisation assay (NT) in 2013, but this result may be influenced by cross-reaction; participants who had high pre-existing titres to DENV in 2013 were more likely to be seropositive by NT (Figure 4.13A). In our main analysis of titre dynamics, we therefore focused on the subset of participants who were seronegative by NT in 2013 (Figure 4.10). However, we obtained the same conclusion when participants who were initially seropositive were also considered (Figure 4.11).

The global ZIKV epidemic began in the Pacific islands in 2013 before spreading in Central and South America from 2015. Seroprevalence studies following ZIKV epidemics in Latin America have been reported but data have either been non-representative (*Netto et al.*, 2017) or not enough time had elapsed since the outbreak to observe long-term dynamics (*Rodriguez-Barraquer et al.*, 2019; *Zambrana et al.*, 2018). To our knowl-

edge, these are the first studies of community seroprevalence over a long-term period following a ZIKV outbreak. Therefore, patterns observed in Pacific islands may be an early indication of what might happen to seroprevalence in Latin America where ZIKV outbreaks began two to three years after the French Polynesia epidemic (*Bogoch et al.*, 2016; *Cao-Lormeau et al.*, 2014a).

In the short-term, our findings have implications for the design of follow up studies of ZIKV. Our results provide evidence that levels of seroprevalence one to two years following ZIKV circulation may be lower than previously expected and study designs may need to be adapted to reflect this, particularly in settings that exhibit long-term low level circulation of ZIKV as opposed to large sporadic outbreaks (*Ruchusatsawat et al.*, 2019). For example, estimates of microcephaly risk may be inflated if derived from long-term seroprevalence data that underestimate the true extent of infection within the population, and results of clinical trials could also be biased if post-outbreak seroprevalence is used an indicator of infection within a population (*Cohen*, 2018). In the longer-term, our results demonstrate the value of longitudinal serological studies of flaviviruses, and analysis using multiple serological tests, including neutralisation assays (*Clapham et al.*, 2016). Such studies will be essential to understand different aspects of the short and long-term immune antibody response against ZIKV, and how prior exposures to DENV may influence these responses.

4.6 Technical Appendix

4.6.1 Baseline neutralisation titres

In this chapter I have presented an analysis of changes in neutralisation titre between 2013 and 2017. For completeness, the baseline neutralisation titre is presented here for DENV-3 and ZIKV in those that seroconverted to these viruses between 2013 and 2015 (as in Figure 4.10), and for DENV-1 and DENV-2 in those that seroconverted between 2013 and 2015 (as in Figure 4.12).

4.6.2 Comparison tests

I used a Chi-squared test for association for comparison of cross-sectional seroprevalence estimates in French Polynesia, and McNemar's test for seroprevalence estimates from longitudinal sera from Fiji. Details of both tests are presented in this section.

In the French Polynesia data I wanted to compare the proportion seropositive in 2014 and 2015. I used the χ^2 goodness of fit test with the null hypothesis that the proportion positive in 2014 π_{2014} and in 2015 π_{2015} are independent.

Table 4.8 shows an example 2x2 contingency table for the French Polynesia data.

Table 4.8: *2x2 contingency table with observed counts for serostatus in French Polynesia*

	2014	2015	Total
Seronegative	O_{00}	O_{10}	$O_{0.}$
Seropositive	O_{01}	O_{11}	$O_{.1}$
Total	$O_{.0}$	$O_{.1}$	$O_{..}$

Provided the entries in the contingency table are reasonably large the null hypothesis of no association can be tested with a χ^2 test, with test statistic (with continuity

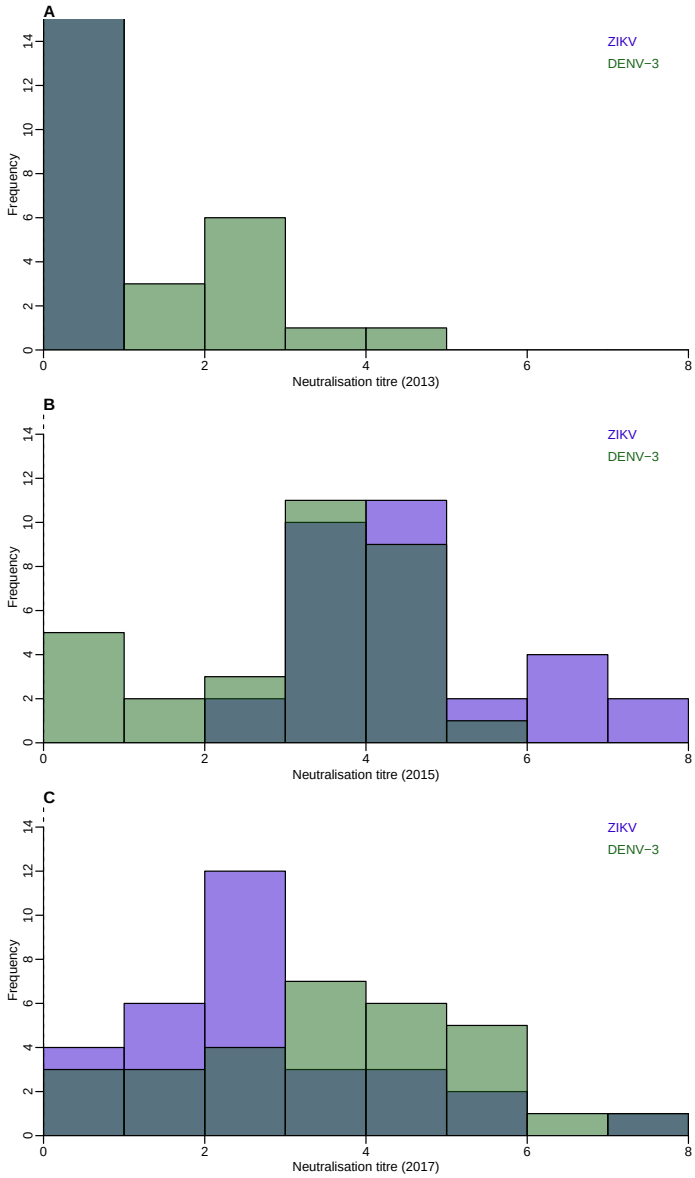

Figure 4.14: *Neutralisation titres for ZIKV and DENV-3 between 2013 and 2017 in those that were seronegative in 2013 and seroconverted in 2015 for DENV-3 (n=19) and ZIKV (n=31)*

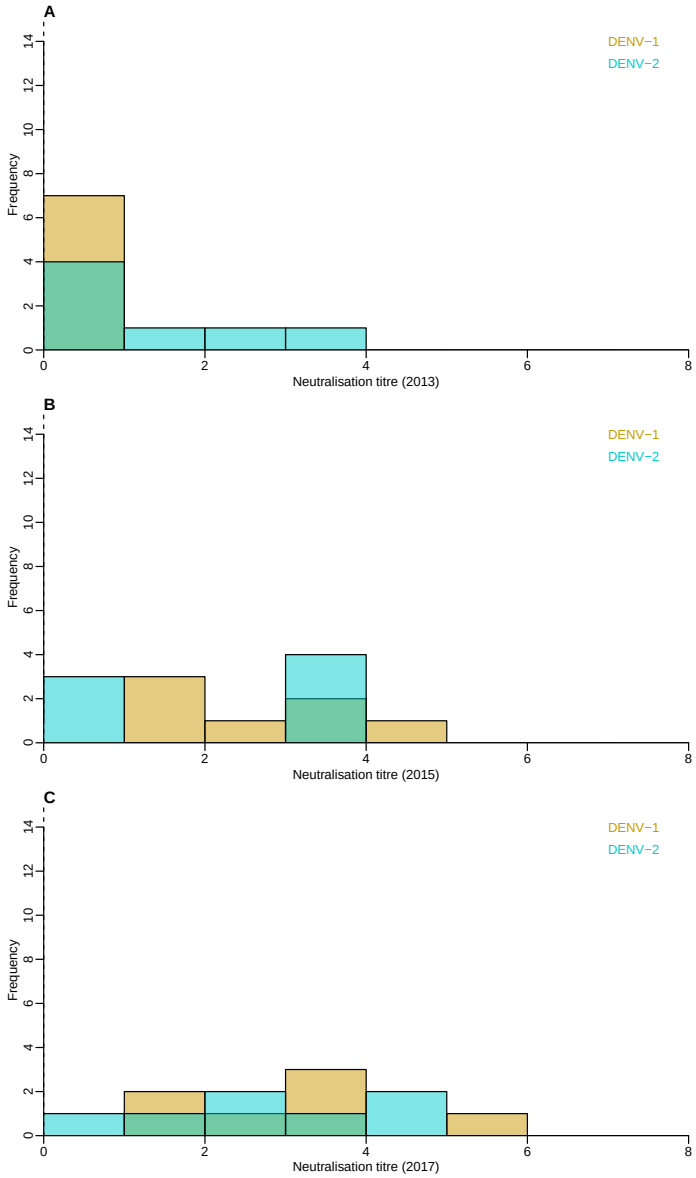

Figure 4.15: *Neutralisation titres for DENV-1 and DENV-2 between 2013 and 2017 in those that were seronegative in 2013 and seroconverted in 2015 for DENV-1 (n=26) and DENV-2 (n=18).*

correction):

$$\chi^2 = \sum_i \sum_j \left(\frac{(|O_{ij} - E_{ij}| - 0.5)^2}{E_{ij}} \right) \text{ where } E_{ij} = \frac{O_{i.} O_{.j}}{O_{..}} \tag{4.1}$$

Under $H_0 : \chi^2 \sim \chi^2_1$ so p-values are calculated comparing the test statistic to a χ^2_1 distribution using the `chisq.test` function in `R`.

The data for Fiji was matched by design so individuals could act as their own controls. This affords us greater statistical power to detect differences between samples as some unobserved variation is accounted for by samples being taken from the same individual. Table 4.9 shows an example arrangement of data from a matched study for observed serostatus in the Fijian data.

Table 4.9: *Arrangement of data for McNemar's test of serostatus in Fiji*

		2015		
		Seronegative	Seropositive	
2017	Seronegative	q	r	$q + r$
	Seropositive	s	t	$s + t$
		$q + s$	$r + t$	n

In 2015, the proportion of seropositive individuals is $p_1 = {}^{q+s}/_{n}$ and in 2017 the proportion seropositive is $p_1 = {}^{q+r}/_{n}$. I want to test whether these two proportions differ. Under H_0 the two proportions are equal and the number of discordant pairs should be balanced, i.e. the number of seroreversions and seroconversions should be equal, or $r = s$ in Table 4.9. Therefore, if H_0 is true then:

$$r \sim \text{Binomial}(r + s, 0.5) \tag{4.2}$$

I can then test this using an exact binomial test with the function `mcnemar.test` in `R`.

4.6.3 Age adjusted seroprevalence

We compared seroprevalence estimates in French Polynesia from two independent cross-sectional surveys conducted in 2014 and 2015. To remove, as far as possible, the effect of differences in age when comparing these two populations we calculated age-adjusted seroprevalence Table 4.3.

We used weights for 10-year age bands calculated as the estimated proportion in each age band in population data collected in 2017 (Table 4.1). We removed age bands with no samples for that survey, and the proportion in each age-group made a set of weights for each survey, which represented the age-specific standard population.

Using the `ageadjust.direct` function from the `epitools` package (*Aragon*, 2020) in `R`, we calculated age-adjusted seroprevalence for each survey using the direct method. The function calculates the age-specific seroprevalence rate p_j in age-group j as the number of events X_j divided by the population in that age-group N_j. The age-adjusted seroprevalence is then the rate in age-group j multiplied by the weight for that age-group ω_j. Confidence intervals are calculated according to *Fay and Feuer* (1997) which have been shown to be conservative even in cases where the two populations differ non-proportionally.

4.6.4 Bootstrap adjustment

The two seroprevalence surveys conducted in French Polynesia in 2014 and 2015 had different sample sizes. 49 samples were collected in 2014 and 672 in 2015. We wanted to check for possible biases in the two samples that could have explained the decrease in estimated ZIKV seroprevalence between the two surveys. One potential confounder was age but the two age distributions were similar and age-adjusted seroprevalence (Table 4.3) also showed a decrease in ZIKV seroprevalence.

Further investigation showed that the samples in 2014 included a higher proportion of individuals that had evidence of multiple past DENV infections (Table 4.5). To remove,

as far as possible, the effect of differences in risk of arbovirus infection in the two samples we adjusted the estimates of 2014 seroprevalence to match the DENV-exposure profile of the 2015 survey.

I estimated DENV-exposure adjusted seroprevalence in 2014. For ZIKV, I obtained the sampling frequency from the 2015 survey of individuals who were seropositive to $(0, 1, 2, 3, 4)$ DENV serotypes. For each of the DENV serotypes I excluded the serotype being adjusted. A bootstrap sample was then selected from the 2014 survey with probability equal to the sampling frequency weights, with replacement, and estimated the seroprevalence using this bootstrap sample. This process was repeated for 1,000 bootstrap samples and the DENV-exposure adjusted seroprevalence for each virus was calculated as the mean of these samples. These results are shown in Table 4.6.

I chose this method because I assumed that the 2015 sample was a better representation of the underlying arbovirus exposure profile in French Polynesia. I therefore attempted to match the 2014 results to the 2015 sample. This process could have been performed in reverse, using the 2014 exposure distribution as the weights to take bootstrap samples from the 2015 survey (Table 4.10). Using this alternative method produced consistent findings as in the main analysis (Table 4.6) for DENV-1, DENV-3, and DENV-4 which showed no evidence of a decline. All three of these viruses had circulated in the years preceding 2014 sample collection (*World Health Organisation*, 2018). However, with this alternative method there was a non-significant decline in ZIKV seroprevalence from 37% (95% CI: 23-52%) to 28% (95% CI: 25-31%) (p=0.25) between 2014 and the 2015 bootstrap estimates.

This sensitivity analysis demonstrates that the evidence for declining ZIKV seroprevalence from the two cross-sectional surveys in French Polynesia is less robust than the results from three longitudinal seroepidemiological surveys in Fiji. The high seroprevalence estimate for ZIKV in 2014 in French Polynesia could be a result of a higher proportion of high infection risk individuals in the sample. The evidence from French Polynesia alone would have been insufficient to conclude that ZIKV seroprevalence wanes within two years of an outbreak. This is likely because of the small and non-representative sampling in 2014. However, the findings from this study were consistent

with seroprevalence estimates from Fiji over a similar time frame which also demonstrated waning neutralising antibody dynamics.

Table 4.10: *Sensitivity of selected distribution to generate bootstrap estimated seroprevalence for each of the four DENV serotypes and ZIKV in two cross-sectional surveys. Result are as in Table 4.6 except 2015 sample were bootstrap sampled, with replacement, weighted by the exposure distribution to other arboviruses in the 2014 survey (i.e. in reverse to Table 4.6)*

Virus	2014 seroprevalence (95% CI) ($n = 49$)	2015 seroprevalence (95% CI) ($n = 700$)	2015 bootstrap estimates of seroprevalence (95% CI)	p-value*
DENV1	0.86 (0.73-0.94)	0.8 (0.77-0.83)	0.92 (0.89-0.94)	0.26
DENV2	0.47 (0.33-0.62)	0.18 (0.15-0.21)	0.25 (0.22-0.28)	<0.01
DENV3	0.76 (0.61-0.87)	0.55 (0.51-0.59)	0.7 (0.67-0.74)	0.55
DENV4	0.63 (0.48-0.77)	0.42 (0.38-0.46)	0.61 (0.57-0.64)	0.89
ZIKV	0.37 (0.23-0.52)	0.22 (0.19-0.25)	0.28 (0.25-0.31)	0.25

* chi-squared test comparing 2014 results with 2015 bootstrap estimates

4.6.5 GAM modelling

Generalised additive models (GAMs) have previously been introduced in chapter 3. In this chapter we used GAMs to analyse the relationship between seroprevalence as defined by MIA or PRNT and mean DENV log titre (from the PRNT) in 2013. I wanted to analyse whether the observed decline in ZIKV seroprevalence in older age groups was because of the history of DENV-exposure in adults compared to children. The original published version of this analysis includes one such model (Figure 4.13) (*Henderson et al.*, 2020). In this appendix I compare this GAM with other possible models to capture 'previous DENV exposure' and analyse the relationship with ZIKV and DENV-3 seroprevalence in 2017.

I defined our explanatory variable X as either mean log titre in 2013, age in 2013, or number of seropositive DENV serotypes in 2013 as measured by MIA. I defined serostatus in the 2017 serological survey for each assay α, Y_α. The model was as

follows:

$$g\left(E\left(Y_{\alpha}\right)\right) = \beta + s_1\left(X\right) \tag{4.3}$$

Where $E(Y)$ denotes the expected value, and $g(Y)$ denotes the link function, in this case the *logit* function because of the binary outcome. The term $s_1(X)$ defines a non-parametric function to model the non-linear relationship between X and Y_{α}, and β is the intercept. I have used GAMs to flexibly capture the relationship between the explanatory variable and the binary outcome, instead of determining the parametric form of this association *a priori* as in a generalised linear model. I used the `mgcv` package in `R` so smooth functions were represented using penalised regression splines (*Wood*, 2019).

Using GAMs to explore ZIKV seroprevalence in 2017

In the original publication we included a GAM comparing ZIKV seroprevalence from both assays by previous DENV exposure, as measured by mean DENV PRNT titre in 2013 (Figure 4.13) (*Henderson et al.*, 2020). From this analysis it appeared that those with higher levels of DENV neutralising antibodies (NAbs) in 2013 were less likely to have ZIKV specific antibodies (i.e. test positive by MIA) in 2017. However, the probability of seropositivity as measured by PRNT was similar regardless of the level of DENV NAbs in 2013.

Here I investigate this association further with supplementary GAMs. The model presented in the original publication used a subset of our study participants, those with PRNT titre values at all three time points: 2013, 2015, and 2017. This left only 45 participants to be used in the analysis. The panel for 2017 ZIKV seroprevalence has been recreated here for comparison (Figure 4.16A). If we change the variable used to quantify the level of prior DENV exposure, then we can include more data. For example, we can use age as a proxy for previous DENV exposure and include all of the MIA data, since age and previous DENV exposure are positively correlated. In a second

model I used age (in 2013) as the explanatory X variable for ZIKV seroprevalence in 2017 using different assays (Figure 4.16B). Similar to the original model, seroprevalence by PRNT shows a slight positive association with age but seroprevalence by MIA does not increase with age in this larger data set (n=320).

Age, however, is not directly related to prior DENV exposure so I used another variable that would reflect DENV infection history but keep all 320 participants in the model. I defined previous DENV exposure (the X variable in Equation 4.3) as the number of positive DENV serotypes by MIA in 2013. This set five levels of prior DENV exposure: 0, 1, 2, 3, or 4. This does not measure strength of DENV immunity as well as the original model, which used mean DENV PRNT log titre in 2013, but it is available for all 320 participants. In theory, more DENV infections prior to 2013 suggests that a participant is particularly prone to arbovirus infections and we would expect them to be more likely to have anti-ZIKV antibodies following the ZIKV outbreak. However, in Figure 4.16C we see the opposite is true for seroprevalence by MIA. As with the restricted data set in the original model (Figure 4.16A) people with more DENV exposure previously are slightly more likely to be seropositive by PRNT but are less likely to be seropositive by MIA (Figure 4.16C). The advantage of using all 320 participants in this model is clear when comparing panels A and C, where confidence intervals are more precise in the new model. The disadvantage of this explanatory variable is that it does not directly capture the level of anti-DENV antibodies in a serum sample since it relies on binary cut-offs for seroprevalence. However, despite differences in these two models, the conclusions drawn from their output are similar.

There is evidence in DENV research that during a secondary DENV infection, the presence of antibodies from a previous DENV infection are capable of responding more rapidly during the secondary infection. This altered immune response is referred to as 'original antigenic sin' (*Halstead et al.*, 1983; *Rothman*, 2011). Given the antigenic similarity between DENV and ZIKV (*Priyamvada et al.*, 2016) it is possible that previous DENV infection could also affect the immune response to a subsequent ZIKV infection. A schematic of this process is shown in Figure 4.17 comparing two hypothetical individuals, one born several decades ago and has had multiple DENV infections before

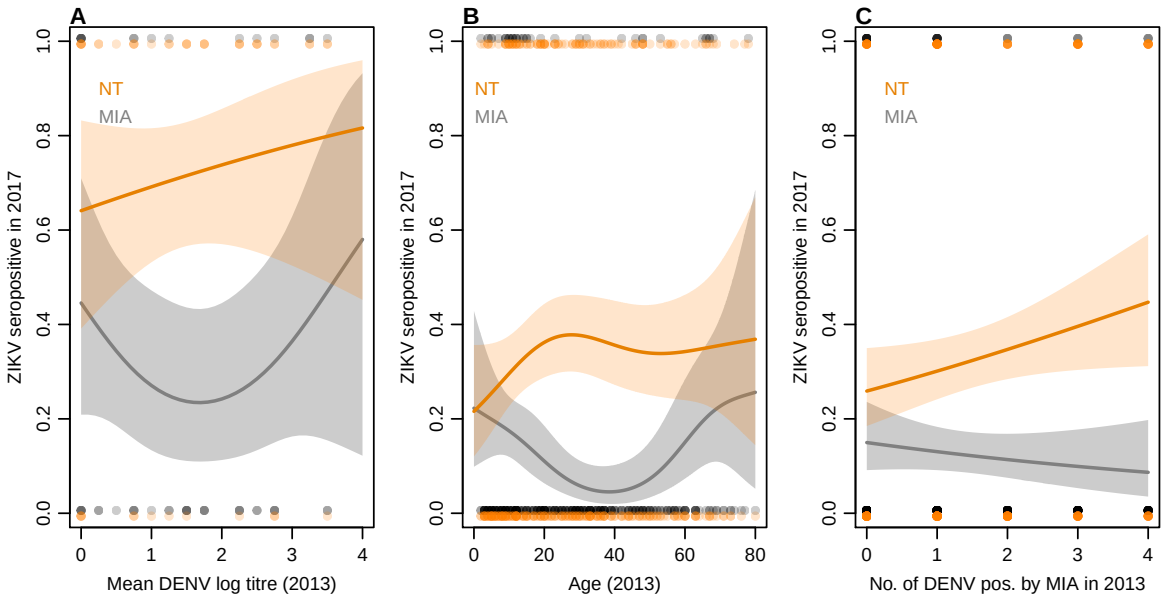

Figure 4.16: *Relationship between different exposures to DENV before 2013 and ZIKV or DENV-3 seroprevalence in 2017 using different assays. (A) The original model (Figure 4.13C) showing 2017 seroprevalence in a subset (n=45) of participants with PRNT measurements from all 3 surveys. Seroprevalence by MIA, shown in grey, and neutralisation test (PRNT), shown in orange, for sera collected in 2017. Line shows prediction from GAM fitted to each data set, with shaded region showing 95% CI, and points show raw data. (B) Seroprevalence for sera collected from all participants in 2017 (n =320) by age (in 2013). (C) Seroprevalence for sera collected from all participants in 2017 (n =320) by the number of positive DENV serotypes by MIA (in 2013)*

infection with ZIKV (A). The other individual is only infected with ZIKV without any DENV infections (B). In individual A there is a short-term boost to the specific in antibody response to ZIKV following ZIKV infection but it is lower than the immune response to earlier DENV infections. Whereas for individual B the antibody response to ZIKV is higher and persistent.

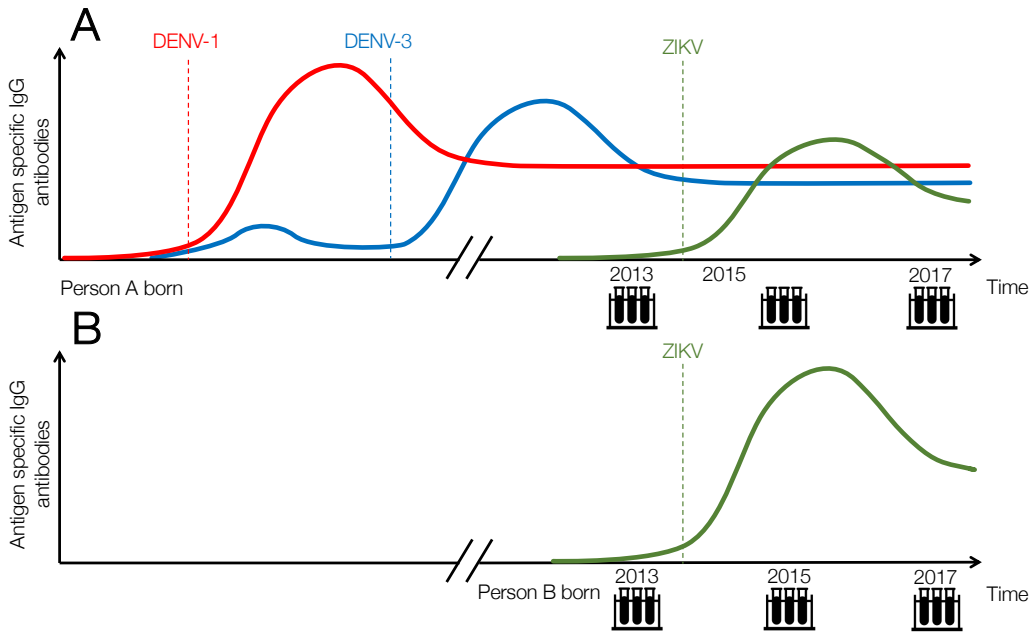

Figure 4.17: *Schematic of immune response against sequential arbovirus infections. A, an older individual with two previous DENV infections before infection with ZIKV. B, a younger individual who is naïve to DENV and ZIKV until a ZIKV infection at the same time as individual A. In this simple illustration, individual A develops high levels of antigen-specific long-term IgG antibodies to DENV-1, the first infection of their life. This infection generates a cross-reactive response in related viruses. When infected with DENV-3 the virus population is neutralised with DENV-3 specific antibodies and cross-neutralising antibodies from the previous infection. The level of DENV-3 specific antibodies therefore does not reach the antigen-specific response from the primary infection, which produces the 'antigenic seniority' effect. This effect repeats when infected with ZIKV such that the ZIKV-specific response declines in individual A. In individual B, the ZIKV-specific response is larger and persistent because it is their first infection*

Our results in Figure 4.16 show some evidence that previous DENV exposure alters the immune response to ZIKV infection. I used three different variables as a proxy for pre-

vious DENV exposure. In each model (Figure 4.16A-C) there was a trend that DENV naïve individuals were more likely to be seropositive by MIA, i.e. have ZIKV specific antibodies. This could have implications if people with previous DENV exposure do not have a persistent specific response against ZIKV following infection. Cross-reactive antibodies from previous infections can still be strong neutralisers, however they may not have optimal avidity for the infecting virus (*Midgley et al.*, 2011; *Rothman*, 2011).

The strength of these GAMs is the ability to flexibly model non-linear relationships between explanatory and dependent variables without specifying the parametric form of this association. From these models, we found some evidence that those with higher levels of DENV exposure prior to the emergence of ZIKV in Fiji (pre-2013) were less likely to be seropositive by MIA for ZIKV in 2017, but were equally or more likely to be seropositive by PRNT. Our study results suggest that previous DENV exposure has an effect on the long-term immune response to a subsequent ZIKV infection.

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Chapter 5

Interactions between timing and transmissibility explain diverse flavivirus dynamics in Fiji

Abstract

Since 2007, Zika virus (ZIKV) has caused several large, brief outbreaks in isolated populations. However, recent evidence suggests ZIKV can also persist at low levels over multiple years. The reasons for these diverse transmission dynamics remain poorly understood. In Fiji, which has experienced multiple large single-season dengue epidemics, there was evidence of multi-year low level transmission of ZIKV between 2013 and 2017. To identify factors that could explain these differences in dynamics between closely related mosquito-borne flaviviruses, we jointly fitted a transmission dynamic model to surveillance, serological and molecular data collected during this period. We estimated that the observed dynamics of ZIKV were the result of two key factors: strong seasonal effects, which created an ecologically optimal time of year for outbreaks; and introduction of ZIKV after this optimal time, which allowed ZIKV transmission to persist over multiple seasons before a combination of immunity and seasonal forcing ended transmission in 2017. We found the basic reproduction number was slightly lower in Fiji for ZIKV than for a concurrent dengue outbreak but that the main determinant of the different outbreak dynamics was the timing and amplitude of the introduction of the virus. With our model, we were also able to identify a period of high epidemic risk in Fiji. The ability to jointly fitted to multiple data sources could help identify a similar range of possible outbreak dynamics in other settings.

5.1 Background

5.1.1 Flavivirus dynamics are poorly understood

Zika virus (ZIKV) and dengue virus (DENV) are two closely related flaviviruses that share the same primary vector of transmission, the *Aedes* genus of mosquitoes (*Musso and Gubler*, 2016). Due to these similarities, both viruses often create similar outbreak dynamics in the same population with large, brief outbreaks, particularly in isolated island populations (*Craig et al.*, 2018; *Duffy et al.*, 2009; *Kucharski et al.*, 2016). However, alongside examples of clearly-defined outbreaks of ZIKV with a high attack rate during 2014-16 (*Cao-Lormeau et al.*, 2014; *Rodríguez-Barraquer et al.*, 2016; *Zambrana et al.*, 2018), there is evidence of low-level, multi-year circulation (*Ruchusatsawat et al.*, 2019). ZIKV epidemics have occurred in humans since 2007 (*Musso et al.*, 2019) and understanding the factors that caused this plurality of outbreak dynamics during the global ZIKV epidemic remains unclear.

Explanations of different outbreak dynamics are often attributed to local factors. The climate, population, people movement and mosquito populations differ greatly between locations and these all have an effect on arbovirus transmission. We do, however, expect these two viruses to behave similarly in the same location. *Funk et al.* (2016), studied DENV and ZIKV outbreaks in the islands of the Federated States of Micronesia and found greater similarity between viruses on the same island than the same virus between different islands. We might therefore expect similar outbreak dynamics from DENV and ZIKV in the same location.

Studying flavivirus outbreaks is complicated by the difficulties in collecting data on infections with these viruses. Primarily this is because of the large proportion of asymptomatic infections. A systematic review of the prevalence of asymptomatic ZIKV infection estimated that these account for 61.8% (95% CI: 33-87.1%) of all ZIKV infections (*Haby et al.*, 2018). Collecting data during an emerging outbreak is challenging, even for well-established surveillance systems and this is especially true in the case of a novel virus like ZIKV which circulated in the Pacific before there was global attention once

outbreaks began in the Americas (*Musso et al.*, 2019). The challenges of collecting data during an emerging outbreak mean that the reasons for these diverse flavivirus dynamics are currently not well understood.

5.1.2 Recent DENV and ZIKV in Fiji

To investigate which factors shape the invasion dynamics of DENV and ZIKV outbreaks we studied recent outbreaks in Fiji in the South Pacific. We combined surveillance, serological and molecular data to analyse the emergence of ZIKV and re-emergence of dengue virus serotype 3 (DENV-3) in the same population in Fiji, which resulted in very different outbreak dynamics.

In 2013-14, a large number of DENV-3 cases were reported October 2013 and May 2014. During this period, 12,413 suspected cases were reported in Central Division (*Kucharski et al.*, 2018). Of the 8,734 laboratory tested cases from Central Division that were notified to the Fiji National Centre for Communicable Disease Control, 3,633 (41.6%) were reactive for DENV nonstructural protein 1 (NS1) and/or anti-DENV immunoglobulin class M antibodies (IgM) (*Kucharski et al.*, 2018). This was a large and short outbreak that likely ended due to a combination of increased herd immunity, seasonal forcing and an additional reduction in transmission possibly from interventions on the vector population (*Kucharski et al.*, 2018). This outbreak was similar in dynamics to typical flavivirus outbreaks in Fiji (*Kiedrzynski et al.*, 1996; *Roth et al.*, 2014; *Singh et al.*, 2005).

In contrast, there is evidence that ZIKV was locally transmitted in Fiji but there were only 16 PCR-confirmed cases between July 2015 and February 2017 in Central Division. *Kama et al.* (2019), studied data from a longitudinal community serological survey and found an increase from 7.8% seroprevalence to ZIKV in November 2013 to 21.9% in November 2015, with evidence of low-level circulation over multiple seasons. This suggests that ZIKV had been circulating during this period despite only 2 confirmed cases being reported. This outbreak was very different to typical flavivirus outbreaks in Fiji. Firstly, the serological data suggests that transmission mostly occurred unobserved, be-

fore any ZIKV cases were detected. Secondly, there was confirmed transmission of ZIKV over several years when typical DENV outbreaks occur over a single high temperature season in Fiji (*Kiedrzynski et al.*, 1996; *Kucharski et al.*, 2018).

Further, the estimated time to most recent common ancestor (tMRCA) estimated in phylogenetic analysis of available ZIKV gene sequences – including from 3 ZIKV cases from Central Division, Fiji – suggested that ZIKV may have been introduced into Fiji in late 2013 or 2014 (*Kama et al.*, 2019) (Figure 5.1). This raises the possibility that DENV-3 and ZIKV were circulating at the same time. This could be a potential factor that determined unusual flavivirus dynamics since DENV outbreaks in Fiji have typically been of a single serotype and co-circulation has not been common (*Kiedrzynski et al.*, 1996; *Singh et al.*, 2005).

Studying the data on ZIKV transmission raises several questions about this outbreak. What was the overall burden of infection? When did the virus emerge and start spreading in Fiji? Where might it have arrived from? We wanted to analyse this ZIKV outbreak to understand why it looked so different to the DENV-3 outbreak of 2013-14.

5.1.3 Available flavivirus data in Fiji

A unique aspect of this study is the combination of three different data sources with a mathematical model of transmission. By working closely with partners and collaborators in Fiji and international researchers, we acquired surveillance, serological and molecular data on flavivirus transmission between 2013 and 2017. A summary of available data is shown in Figure 5.1.

Surveillance data was provided by the Fijian Ministry of Health and includes information on the timing and burden of confirmed cases of ZIKV and DENV-3. Cases of ZIKV were confirmed using reverse transcription polymerase chain reaction (RT-PCR), cases of DENV-3 were suspected DENV with a proportion reactive for DENV NS1 and/or anti-DENV IgM in laboratory tests (*Kucharski et al.*, 2018). There were 16 cases of ZIKV confirmed in Central Division between 2015 and 2017 but only 2 of these were

reported in 2015 and one in 2017. Between October 2013 and May 2014 there were 12,413 suspected cases of DENV-3 in Central Division (*Kucharski et al.*, 2018).

Data are available from a longitudinal serological study in Fiji with data collected in 2013, 2015 and 2017. Colleagues at Institut Lois Malardé in French Polynesia tested sera to test if they were reactive for immunoglobulin class G (IgG) antibodies for the four DENV serotypes and for ZIKV (*Henderson et al.*, 2020; *Kama et al.*, 2019). DENV-3 seroprevalence increased from 33.1% (95% CI: 27.4-39.1%) in November 2013 to 53.2% (95% CI: 47-59.4%) in November 2015, after the outbreak (*Kucharski et al.*, 2018). ZIKV seroprevalence in participants that were sampled three times in 2013, 2015 and 2017 was low in 2013 at 6.3% (95% CI: 3.3-11%) and increased to 24% (95% CI: 19-31%) in November 2015 before decreasing to 12% (95% CI: 7.9-18%) in 2017 (*Henderson et al.*, 2020).

Finally, molecular data was collected by colleagues for a separate study on ZIKV transmission in Fiji and they helpfully shared the raw data for this analysis. Envelope (E) gene sequences were isolated from five ZIKV patients in Fiji and three of these were from individuals in the Central Division. A previous phylogenetic analysis of these sequences combined with other Pacific and global sequences estimated a tMRCA of November 2013 (95% HPD: March 2013-July 2015) (*Kama et al.*, 2019).

5.1.4 Modelling ZIKV transmission in Fiji

Both ZIKV and DENV can cause asymptomatic or subclinical infections (*Haby et al.*, 2018; *Mitchell et al.*, 2019), which means many infections will not appear in routine surveillance data. We therefore wanted to combine the information available in these three separate data sources to estimate transmission dynamics of DENV-3 and ZIKV.

We chose a simple deterministic model structure for arbovirus transmission that was appropriate for modelling DENV or ZIKV transmission. We used a Susceptible-Exposed-Infectious-Recovered framework for the human population and a Susceptible-Exposed-Infectious framework for the mosquito population. We jointly fitted our model to

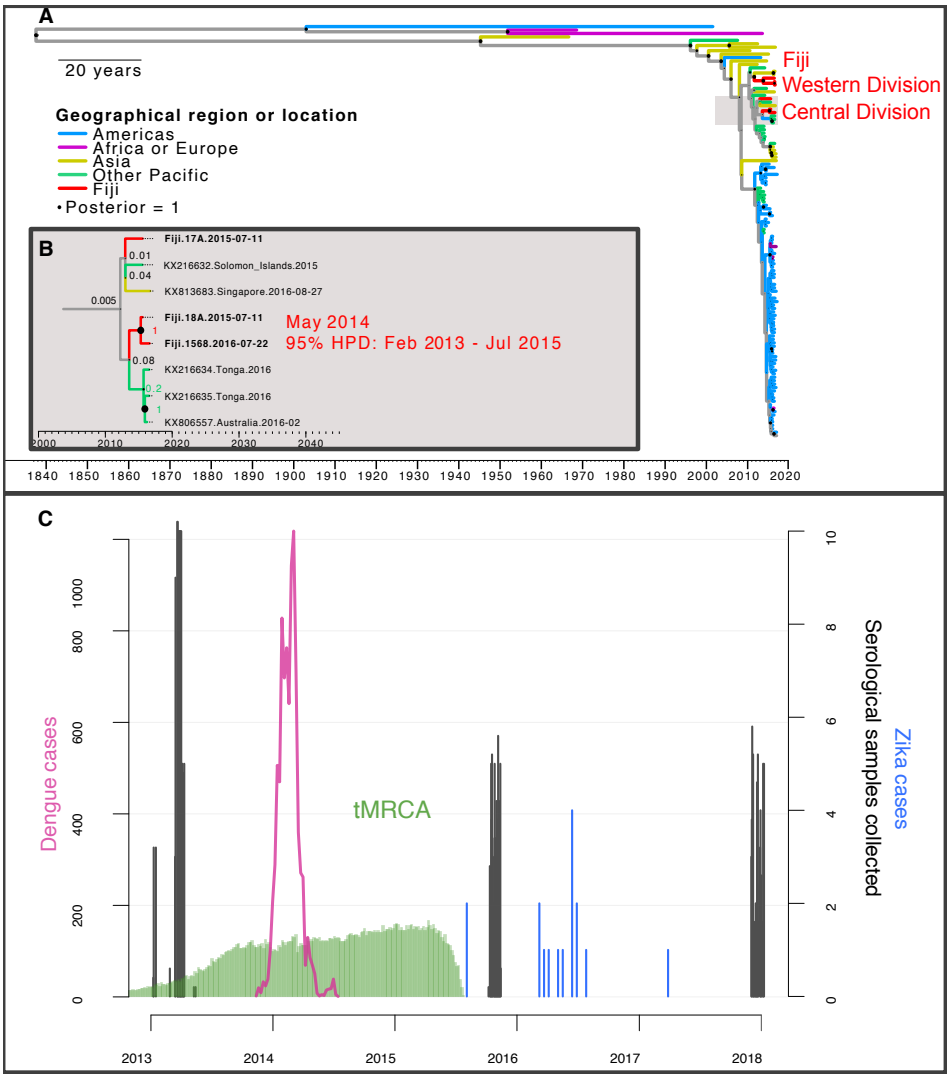

Figure 5.1: *Available data on ZIKV transmission in Fiji. (A) Dated Bayesian phylogeny of three sequences recovered from Central Division, Fiji, and other locations in the Pacific and Americas. Nodes with a posterior probability of 1.00 are indicated. Branch lengths correspond to time in calendar years. (inset B) Detailed phylogeny of the Central Division cluster. The estimated time to most recent common ancestor (tMRCA) for the two closely related Central Division sequences, selected based on previous analysis (Kama et al., 2019), is shown. (C) Green region, density of estimated tMRCA from phylogenetic analysis. This distribution was used as a prior for ZIKV introduction time in the main transmission model fitting. Pink line, cases of DENV3. Blue bars, cases of ZIKV. Grey bars, serological samples collected in Central Division.*

surveillance data, three longitudinal serological surveys and virus sequences to estimate unobserved ZIKV infection dynamics in Fiji during 2013-18. We used this model to identify factors that could explain why the dynamics of ZIKV and DENV-3 were so different.

There are several possible explanations for differences in flavivirus dynamics in the same population. We designed a mathematical model that would be flexible enough to compare four possible explanations for the diverse outbreak dynamics. Firstly, that ZIKV was less transmissible than DENV in Fiji: analysis of flavivirus outbreaks on other Pacific islands found that ZIKV can have a slightly lower basic reproduction number, R_0, than DENV in the same location (*Champagne et al.*, 2016; *Funk et al.*, 2016). Another factor is seasonality: because mosquito populations are influenced by environmental factors like temperature and rainfall (*Lourenço et al.*, 2017; *Mordecai et al.*, 2017) there is a strong temporal component to flavivirus transmission in Fiji (*Kucharski et al.*, 2018); the time of year the virus is introduced therefore could influence the dynamics of the resulting outbreak. Additionally, flavivirus outbreak dynamics will depend on prior immunity within the population, as well as immunity that accumulates during an outbreak (*Funk et al.*, 2016; *Kucharski et al.*, 2018; *Netto et al.*, 2017), or wanes following infection (*Henderson et al.*, 2020). Finally, the tMRCA of ZIKV in Fiji spans the duration of a large DENV-3 outbreak (Figure 5.1C), so it is possible that infections during the DENV-3 outbreak also conferred a degree of transient cross-protection against other flaviviruses (*Gordon et al.*, 2019; *Montoya et al.*, 2018; *Rodriguez-Barraquer et al.*, 2019).

5.2 Materials & Methods

5.2.1 Ethics statement

Each serosurvey had ethical approval from both the Fiji National Research Ethics Review Committee (2013-03, 2015.111.C.D and 2017.20.MC) and the London School of Hygiene & Tropical Medicine Observational Research Ethics Committee (6344, 10207 and 12037). All participants in follow-up studies in 2015 and 2017 had agreed to be recontacted for further health research and an updated informed consent was obtained. To respect local customs and ensure research activities were culturally accepted, the head of the household or village was visited with local bilingual field teams. The study was explained in English or iTaukei at the preference of the potential participant. Parental/guardian consent was obtained for children under 18.

5.2.2 Data

Surveillance data

Between June 2015 and August 2017 there were 16 RT-PCR confirmed cases of ZIKV through laboratory surveillance in Central Division, Fiji. The collection of surveillance data in Fiji has been previously described (Chapter 1) (*Kucharski et al.*, 2018). Over the period 27th October 2013 to 31st August 2014, there were 12,413 DENV-3 suspected cases reported in Central Division. This data set has been previously published by *Kucharski et al.* (2018).

Serological data

I used serological data collected from a longitudinal seroepidemiological survey over the period 2013-2017 with three visits to the same participants in Central Division, Fiji. Samples were tested for detection of IgG antibodies against ZIKV using a recombinant antigen-based microsphere immunoassay (MIA). Full details of the data

collection method and serological tests used has been previously described (Chapters 2, 4) (*Aubry et al.*, 2017; *Cao-Lormeau et al.*, 2016; *Henderson et al.*, 2020; *Kama et al.*, 2019; *Kucharski et al.*, 2018).

Molecular data

A previous study details the recovery of the envelope (E) gene of ZIKV strains from Fiji and the original phylogenetic analysis that informed this study (*Kama et al.*, 2019). The sequences from Central Division were recovered from two saliva samples collected in 2015 and a serum sample collected in 2016. The retrieval of sequences from GenBank has been detailed previously (*Kama et al.*, 2019). In brief, sequences were retrieved from GenBank and selected using nucleotide BLAST searches (*Altschul et al.*, 1990). I retained all sequences with a reported date of sampling and country of origin sharing more than 99% genetic identity to the Fiji sequences. I removed duplicates as done in the original study, but I retained all sequences including those from Europe and Africa. In total, the ZIKV alignments contained 120 sequences including five from Fiji, three of which were from Central Division.

Climate data

I collated daily maximum and minimum temperature from the Fiji Meteorological Service which covered the study period up to June 2017. I calculated the daily average temperature as the mean of the maximum and minimum temperature recorded on that day. Data on diurnal variability were not available for this study. I was also limited by a lack of rainfall data for the study period. However, previous work has found that temperature, not rainfall, was the key determinant of seasonal fluctuations of ZIKV transmission (*Lourenço et al.*, 2017).

5.2.3 Modelling

Data on ZIKV transmission in Fiji was sparse and apparently contradictory. Serological data showed evidence that approximately 17% of the population developed ZIKV-specific antibodies between November 2013 and November 2015, however only 2 cases were confirmed in surveillance data over that period. To model the underlying transmission dynamics, I collected data from other sources that could inform ZIKV transmission dynamics in Fiji between 2013 and 2017. A summary of available data and how it was included in our final ZIKV transmission model is shown in Figure 5.2.

Each step in this analysis is outlined in detail later in this section but is briefly summarised here. I initially estimated the dynamics of seasonal forcing on transmission from temperature data in Fiji and used these values in a model of the 2013-14 DENV-3 epidemic. This DENV-3 model was fitted to DENV-3 surveillance and serology using a model similar to a previous study in Fiji (*Kucharski et al.*, 2018). Prior distributions were specified for the full ZIKV transmission model for parameters determining the seasonal forcing of transmission (β_{amp}, β_{mid}) and the effect of a mosquito clean-up campaign in March 2014 on arbovirus transmission (β_{base}) by using *a posteriori* estimates from this DENV-3 model.

At the same time, I performed a phylogenetic analysis of three ZIKV sequences from Central Division, Fiji, aligned with 117 other global ZIKV (Asian lineage) sequences. From this analysis I obtained an estimate of the distribution of the tMRCA for the Central Division cluster and used this as a prior for the midpoint of the introduction of ZIKV to Central Division (ψ_{m}).

Finally, these informative priors were used in the full ZIKV transmission model which was fitted to both surveillance and serological data using a Markov Chain Monte Carlo (MCMC) framework.

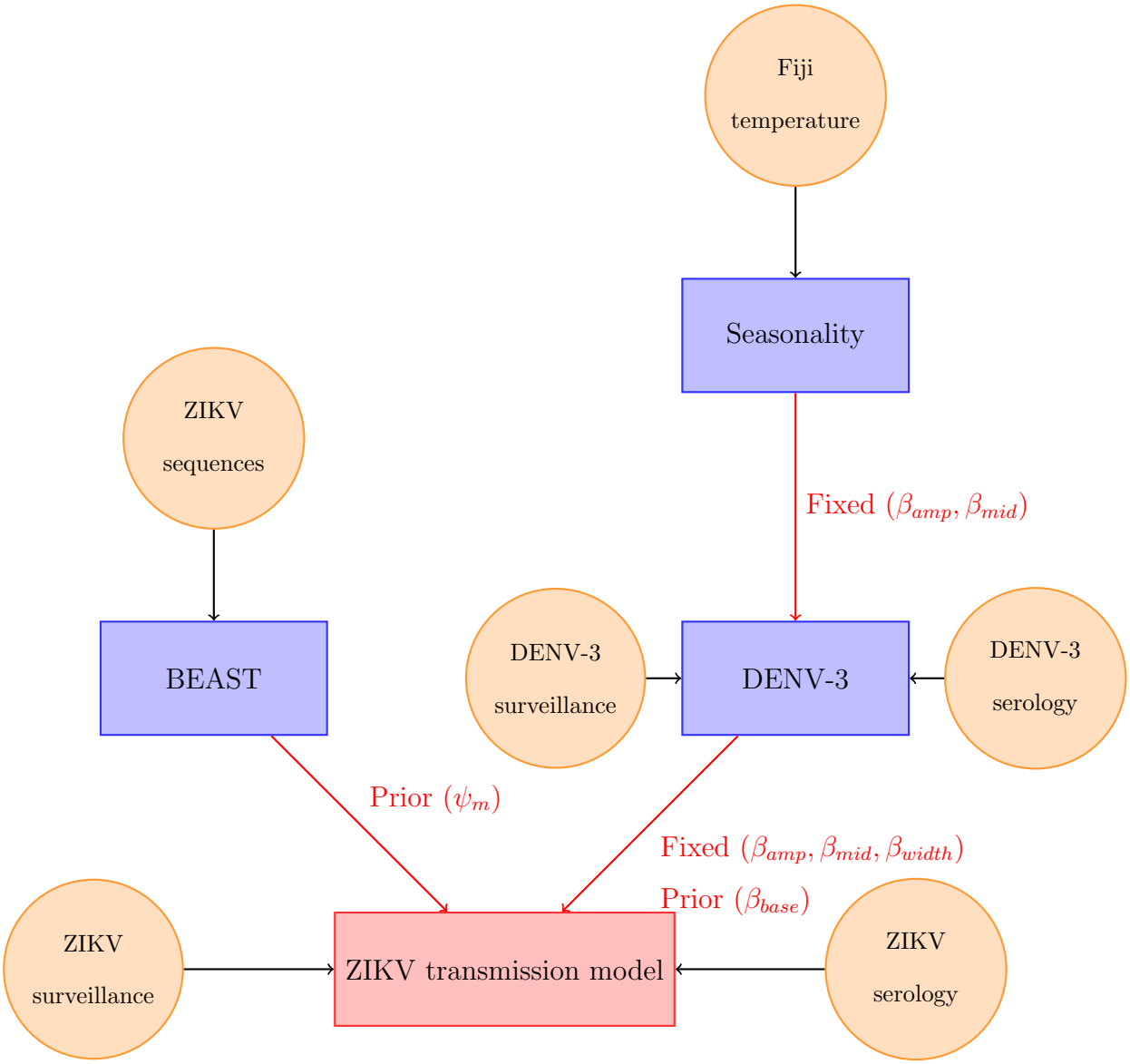

Figure 5.2: *Schematic of modelling approach and available data. The final ZIKV transmission model (red rectangle) used direct data inputs on ZIKV surveillance and serology. Data (orange circles) were used to fit models (blue rectangles) and estimate certain parameters. Information from these model fitting processes was incorporated into this final transmission model through specification of informative priors and fixed values (red arrows)*

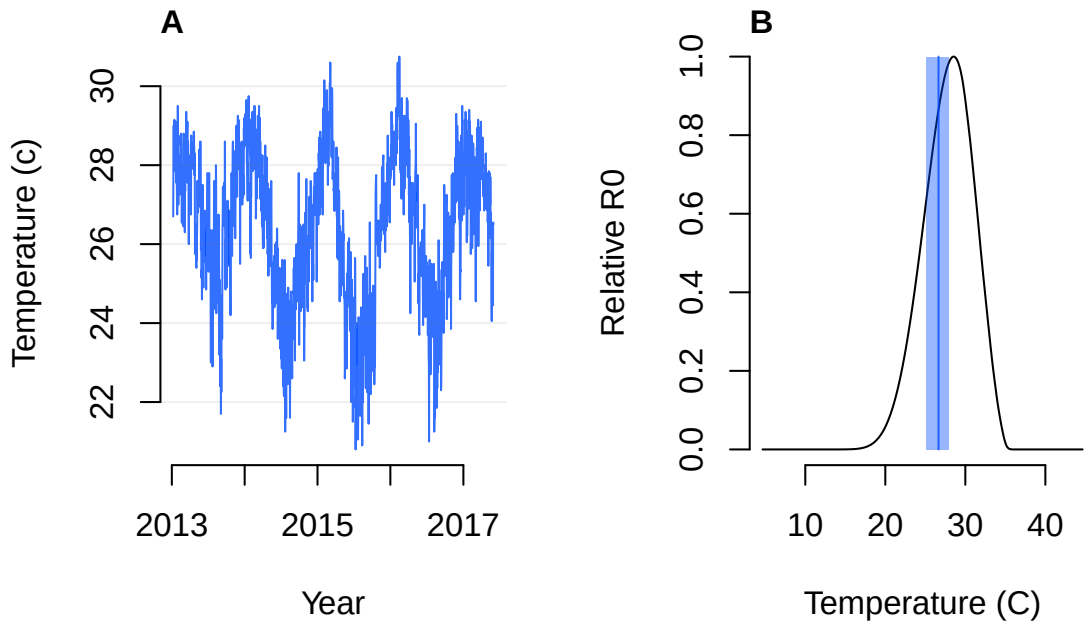

Figure 5.3: *A, daily temperature (°C) in Fiji. B, relationship between average daily temperature and R_0 in Suva, Central Division, Fiji. Black line, mean posterior estimate of the relationship between temperature and relative R_0 for arboviruses transmitted by Aedes aegypti mosquitoes (Mordecai et al., 2017). Blue line and region, median and interquartile range of average daily temperature in Fiji between November 2013 and June 2017*

Modelling seasonal forcing using temperature data

I assumed a linear relationship between temperature in Fiji and the relative transmission of ZIKV based on research from *Mordecai et al.* (2017). The authors of this study integrated data from several laboratory experiments into a mathematical model of temperature-dependent transmission. The study defined the relationship between temperature and relative R_0 of ZIKV transmitted by *Aedes aegypti* mosquitoes and found maximal transmission occurring in a range from 26-29°C. Data from this study were publicly available and are shown in Figure 5.3 along with the median and interquartile range of the average daily temperature from Fiji over our study period. Although the relationship between temperature and R_0 is non-linear, it is mostly linear in the range of temperatures observed in Fiji across our study period.

Seasonal variation in temperature is known to affect the population and ability of the primary vector of ZIKV, *Aedes aegypti* mosquitoes, to transmit viruses (*Descloux et al.*, 2012; *Gubler*, 1998; *Mordecai et al.*, 2017). Annual temperature in Fiji follows a wave-like pattern so to capture variation in transmission over time I defined a sine function with parameters that determine the amplitude (β_{amp}) and midpoint (β_{mid}) of the sine wave (Equation 5.1). I assumed that transmission of both DENV and ZIKV would vary seasonally. Under the assumption of a linear relationship, the rate of transmission from mosquitoes to humans (β_{Z}) and humans to mosquitoes (β_{M}) both vary with time t. The transmission rate at time t is defined by the seasonality function:

$$seasonal_{\mathrm{i}}(t) = 1 + \beta_{\mathrm{amp}} \sin\left(2\pi\left(t + \beta_{\mathrm{mid}}\right)\right); \qquad i = \mathrm{Z}, \mathrm{M} \tag{5.1}$$

The sine function 5.1 was fitted to daily average temperature data (*Fiji Meteorological Service*, 2017) using MCMC via a Metropolis-Hastings algorithm with weakly informative priors. I assumed that the temperature data at time t was normally distributed with mean μ and standard deviation σ derived from the overall time series temperature data. Let the parameter set $\Upsilon = (\beta_{\mathrm{amp}}, \beta_{\mathrm{mid}})$, the corresponding estimated temperature data from the sin function $S = \{s_t\}_{t=1}^{T}$ and recorded temperature data $Y = \{y_t\}_{t=1}^{T}$. The overall log-likelihood was then:

$$L\left(\Upsilon|Y\right) = \sum_{t} \log P\left(y_t|s_t\right) \tag{5.2}$$

I used bootstrap samples of the *a posteriori* estimates of β_{amp} and β_{mid} to obtain samples of the sine wave defined in Equation 5.1. I defined the amplitude of seasonal forcing as the range between peak and low temperature from the sine function rather than the maximum and minimum temperature in the raw data which could have been influenced by outliers. However, this method does exclude the delay between changes in temperature and changes in transmission intensity (*Lourenço et al.*, 2017). I then used the previously defined relationship between temperature and relative R_0 (*Mordecai et al.*, 2017) to convert this temperature range into a range of relative transmission of ZIKV or DENV. This value for β_{amp} and the median estimate for β_{mid} was then

fixed when modelling DENV-3 and ZIKV transmission subsequently. This reduced the number of parameters to estimate in the main ZIKV transmission model while capturing the effect of temperature fluctuations on arbovirus transmission in Fiji.

Modelling the 2013-14 DENV-3 epidemic and the effect of vector control interventions in March 2014

There is evidence that a vector control campaign reduced transmission during the 2013-14 DENV-3 outbreak (*Kucharski et al.*, 2018). Given the overlapping geographic region of this DENV-3 epidemic and the ZIKV transmission of this study, I assumed that the effect of the clean-up campaign in March 2014 would have the same effect on ZIKV transmission if ZIKV was circulating at this time.

I adapted the control function used by *Kucharski et al.* (2018) which was a flexible sigmoid function. An example of the relative effect of this function on transmission is shown in Figure 5.4 with parameter values set at initial conditions for the full ZIKV model.

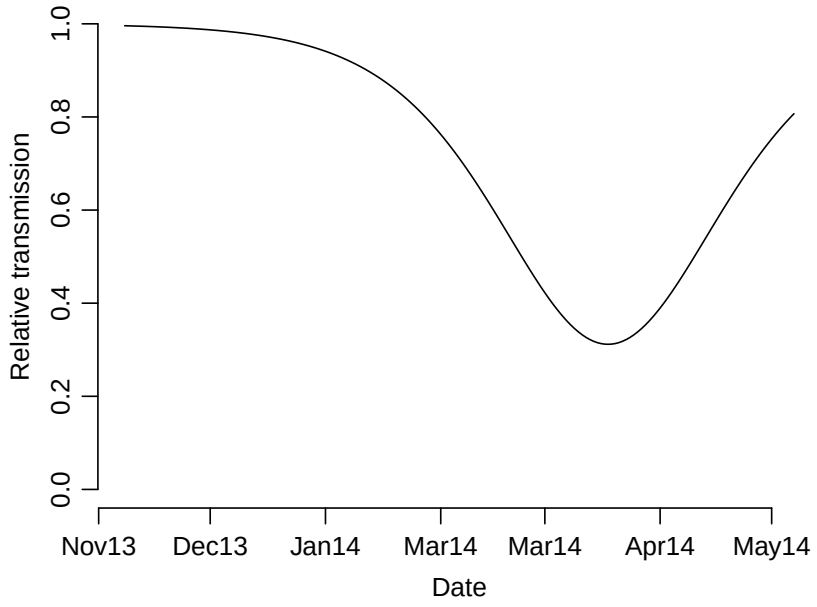

Figure 5.4: *Schematic of control function in ZIKV transmission model*

This function is defined by four parameters. According to Equation 5.3, relative trans-

mission at time t would reduce to level β_{base} with midpoint β_{centre}. In the original analysis relative transmission remained at β_{base} permanently. In this analysis of ZIKV the study period is much longer than that of the DENV-3 outbreak so I adapted the function with a quadratic denominator and a scaling factor of 4. This function then temporarily reduces transmission relative to a baseline level before returning to the original baseline level (Figure 5.4).

$$control_i(t) = 1 - 4\beta_{base}\frac{e^{(\beta_{centre} - t)/\beta_{width}}}{(1 + e^{(\beta_{centre} - t)/\beta_{width}})^2} \tag{5.3}$$

I initially used this function when fitting the 2013-14 DENV-3 epidemic. For this analysis I fixed β_{centre} so that the reduction centred around March 2014 when the vector control campaign was implemented as demonstrated in *Kucharski et al.* (2018). From fitting to DENV-3 surveillance and serological data I obtained *a posteriori* estimates for β_{base} and β_{width}.

I fitted our SEIR model to surveillance and serological data using MCMC and a negative binomial likelihood over 25,000 iterations using the priors outlined in Table 5.1 below. I then fixed the value of β_{width} and used an informative prior for the parameter β_{base} to reduce the number of parameters estimated in the final model.

Phylogenetic modelling of ZIKV sequences

I reproduced previous phylogenetic analysis by Bayesian MCMC inference (*Kama et al.*, 2019). I reconstructed phylogenies in nucleotide substitutions per sites and in unit of time ('dated' phylogenies) by Bayesian MCMC inference, using the package BEAST (v1.10.4) (*Drummond et al.*, 2012). I had a data set of 120 aligned ZIKV sequences and generated a taxon set for two of the Central Division sequences. There were three sequences isolated in Central Division, however the phylogenetic analysis gave weak branch support for a cluster of all three sequences (*Kama et al.*, 2019). There was very strong branch support for the relationship between two of the sequences – 18A, recovered in 2015; and 1568, recovered in 2016 – so I formed a monophyletic taxon

set and estimated the tMRCA for these two samples as the estimate for the tMRCA for Central Division as a whole. The analysis was performed using the General Time Reversible (GTR) model to allow flexibility in the nucleotide substitution rate matrix. I used gamma-distributed rate heterogeneity with four gamma categories (*Kama et al.*, 2019; *Lemey et al.*, 2009; *Yang*, 1995). I used a coalescent Bayesian skyline tree prior with ten groups and a piecewise-constant skyline model (*Drummond et al.*, 2005). The analysis was run using a strict clock and an uncorrelated relaxed clock with a lognormal distribution (*Drummond et al.*, 2006). The joint distributions were compared and showed improved performance from the uncorrelated relaxed clock model so this model was used. A mean substitution rate prior of 4e−4 substitutions per site per year was used. The MCMC chains were run with 20 million iterations. Convergence of the estimates was considered satisfactory when the effective sample size (ESS) calculated in Tracer v1.6.0 was >200.

Modelling ZIKV: Introduction function

The main ZIKV transmission model used a continuous flow of infectious individuals into the infectious compartment to better represent real introduction dynamics rather than a single introduction event at a single fixed point in time. This does not capture multiple separate introduction waves across multiple years. The number of introductions varied with three parameters, the peak (ψ_b), midpoint (ψ_m) and width (ψ_w) according to the introduction function (for time t):

$$\psi(t) = 4\psi_\mathrm{b}\left(\frac{e^{(\psi_\mathrm{m}-t)/\psi_\mathrm{w}}}{\left(1+e^{(\psi_\mathrm{m}-t)/\psi_\mathrm{w}}\right)^2}\right) \tag{5.4}$$

Where $\psi(t)$ is the number of infectious introductions in time t. Equation 5.4 produces a symmetric function centred around ψ_m as demonstrated in Figure 5.5. the integral of Equation 5.4 between $-\infty$ and ∞ gives the total number of ZIKV introductions and is equal to $4\psi_b\psi_w$. ψ_m had an informative prior derived from the tMRCA from a phylogenetic analysis. I took the posterior distribution of the tMRCA from the BEAST analysis and used the `fitdistr` function from the R package `MASS` to estimate

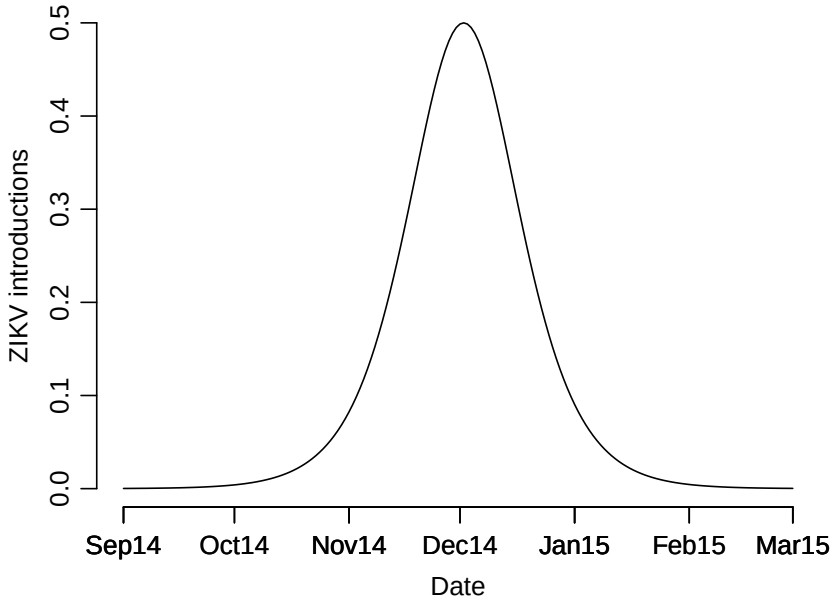

Figure 5.5: *Schematic of introduction function in ZIKV transmission model*

the empirical mean and standard deviation of this distribution. These parameters were then used to define a Gaussian distributed informative prior for ψ_{m}.

Modelling ZIKV: Transmission model

I developed a model which had flexibility to consider four possible factors that could identify the cause of diverse flavivirus dynamics: accumulation of herd immunity during the outbreak, seasonal variation in climate and the impact of virus introduction time, interaction between DENV and ZIKV resulting from cross-protection, and inherent viral transmissibility.

I modelled ZIKV transmission using a deterministic compartmental model with transitions following a susceptible-exposed-infectious-removed (SEIR) structure. The model had ten compartments in total. Upon exposure to ZIKV, humans moved from initially susceptible (S_Z) to a latent class (E_Z), then an infectious class (I_Z) and finally a recovered class (R_Z). During the 2013-14 DENV-3 outbreak, a proportion (χ) of those infected with DENV-3 but susceptible to ZIKV were temporarily removed from the S_Z compartment while clearing the DENV-3 infection. For DENV-3, the human

population similarly moved between susceptible (S_D), latent (E_D) and infectious compartments (I_D) before returning to the susceptible-to-ZIKV compartment S_Z over two transitional compartments T_{1D} and T_{2D}. Two compartments were used so that the duration to return to susceptible would follow an Erlang, not exponential, distribution (*Camacho et al.*, 2011; *Wearing et al.*, 2005). Using an exponential distribution means that there is nonzero density for the null duration in a compartment, so some people can transition artificially rapidly. Using an Erlang distribution solves this problem and is particularly valuable when the average duration of in a compartment is long as it is between T_{2D} and S_Z.

The model included seasonal forcing on the transmission rate using a sinusoidal function and a temporary reduction in transmission in March 2014 from a mosquito clean-up campaign as previously characterised (*Kucharski et al.*, 2018) and described in Equations 5.1 and 5.3. In this main transmission model, the parameters that determined the force of infection for the simultaneous DENV-3 epidemic ($S_D, E_D, I_D, T_{1D}, T_{2D}$) were fixed such that R_0 was 1.3 and a proportion (χ) of those infected with DENV-3 would become temporally immune from ZIKV. The force of infection at time t for ZIKV infection in humans $\lambda_Z(t)$ was as follows:

$$\lambda_Z(t) = \beta_Z \left(seasonal_{\mathrm{Z}}(t) \times control_{\mathrm{Z}}(t)\right) \tag{5.5}$$

$$\tag{5.6}$$

The full model was as follows. The compartment C is introduced to capture the cumulative incidence of infections in the model.

$$dS_Z/dt = \eta - S_Z(\lambda_Z(t)I_Z/N) - \chi\left(S_D\beta_D I_D/N\right) + \chi(2\omega T_{2D}) - \mu S_Z \tag{5.7}$$

$$dE_Z/dt = S_Z(\lambda_Z(t)I_Z/N) - (\mu + \alpha_Z)E_Z \tag{5.8}$$

$$dI_Z/dt = \alpha_Z E_Z - (\mu + \gamma_Z)I_Z + \psi(t) \tag{5.9}$$

$$dR_Z/dt = \gamma_Z I_Z - (\mu + \rho)R_Z \tag{5.10}$$

$$dC/dt = \alpha_Z E_Z \tag{5.11}$$

$$(5.12)$$

$$dS_D/dt = -(S_D \beta_D I_D/N) \tag{5.13}$$

$$dE_D/dt = (S_D \beta_D I_D/N) - \alpha_D E_D \tag{5.14}$$

$$dI_D/dt = \alpha_D E_D - \gamma_D I_D \tag{5.15}$$

$$dT_{1D}/dt = \gamma_D I_D - 2\omega T_{1D} \tag{5.16}$$

$$dT_{2D}/dt = 2\omega T_{1D} - 2\omega T_{2D} \tag{5.17}$$

All parameters except the force of infection $\lambda_i(t)$ and introduction of ZIKV infections $\psi(t)$ were fixed over time. I fixed values for the duration (in days) of the intrinsic incubation period $1/\alpha_Z = 1/\alpha_D = 6.1$ days (*Fourie et al.*, 2018). Likewise, the duration of the infectious period for ZIKV and DENV-3 in humans $1/\gamma_D = 1/\gamma_Z = 5$ days (*Duong et al.*, 2015). I also fixed the duration of cross-protection from DENV-3 $1/\omega = 30$ days which was a conservative estimate as ZIKV neutralising antibodies in DENV infected patients had been shown *in vitro* to not persist beyond 6 months previously (*Collins et al.*, 2017). In a sensitivity analysis I fitted the model assuming that cross-protection persisted for 6 months. I also fixed the duration of detectable ZIKV antibodies $\rho^{-1} =$ 400 days based on our serological studies in French Polynesia and Fiji which found a decrease in ZIKV-specific antibodies 18 months after outbreaks in both locations (*Henderson et al.*, 2020). This study also showed no evidence that DENV-3 antibodies did not wane over time so the parameter ρ was set to ∞ when running the model for DENV-3.

I set the initial population size N to be 342,000 as per Fiji census data from 2007 (*Fiji Bureau of Statistics*, 2018; *Kucharski et al.*, 2018). I used a death rate (μ) of the inverse of life expectancy in Fiji of 67 years (*The World Bank*, 2020a). Data from the World Bank shows that the birth rate in Fiji is approximately 2.5 times higher than the death rate (*The World Bank*, 2020b,c). I therefore set $\eta = 2.5\mu$.

The DENV outbreak for this model was fixed and did not include seasonality for parsimony. The parameters β_D, α_D and γ_D were set such that 20% of the population were infected between October 2013 and April 2014, consistent with previous modelling (*Kucharski et al.*, 2018).

As detailed above, the force of infection $\lambda_Z(t)$ was time dependent according to seasonal forcing and the effect of a clean-up campaign in March 2014. The force of infection was relative to the baseline transmission rate β_Z, which is used as a single term to incorporate number of female mosquitoes, biting rate, probability of contact and probability of transmission. Without better entomological data from Fiji I used this simpler approach with a single transmission rate.

The introductions of infected individuals $\psi(t)$ was time dependent as defined above in Equation 5.4. The introduction of DENV-3 to the model was fixed such that 160 individuals were introduced at the start of the outbreak in November 2013, consistent with previous research (*Kucharski et al.*, 2018).

The effective reproduction number, R, was defined as follows. The basic reproduction, R_0, was calculated by the same method, but assuming that both humans and vectors were fully susceptible (*Keeling and Rohani*, 2011).

$$R = \frac{S\beta_Z\alpha_Z}{(\mu+\gamma_Z)(\mu+\alpha_Z)} \tag{5.18}$$

Deterministic models can generate artificially cyclical epidemics. To better reflect reality, I included two conditions explicitly in the model. Firstly, there had to be at least one infectious individual for the virus to transmit to prohibit virus persistence at implausibly low levels over the low-transmission season. Secondly, I set the number of infectious introductions to zero if the effective reproduction number was below 1. This prevents epidemic take-off at implausible points of the year.

Full transmission model fitting

This model was used to separately fit both the DENV-3 and ZIKV epidemics to serological and surveillance data. I have described equations, compartments and parameters as ZIKV or DENV because ZIKV is the primary focus of the study. However, the same model was used to fit DENV-3 as the primary infection of interest, which was

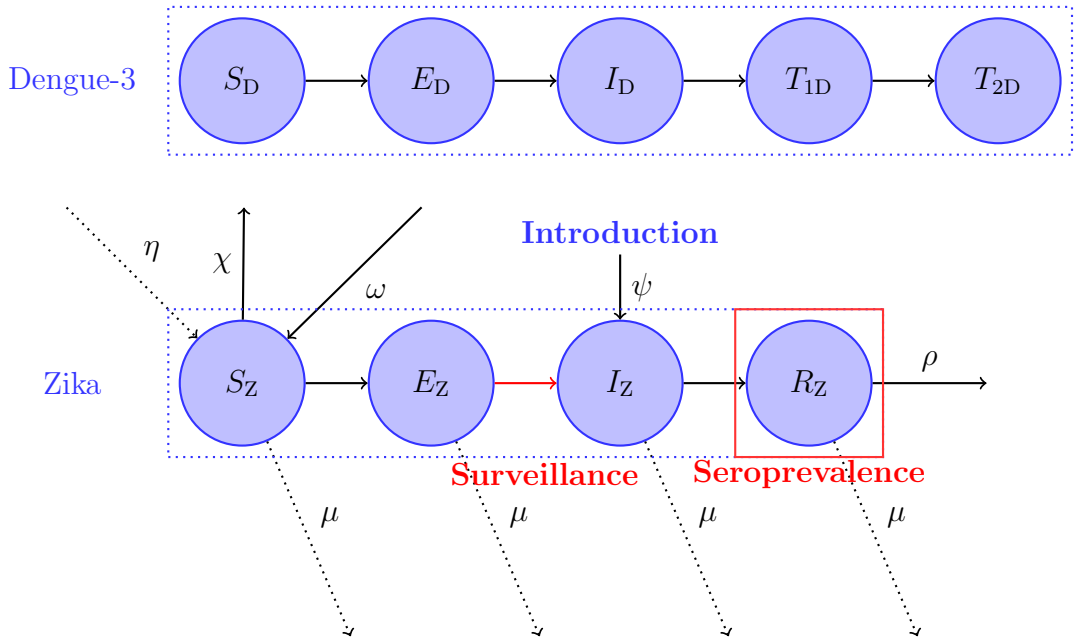

Figure 5.6: *Model schematic of ZIKV transmission model. Dotted lines show demographic transitions. People do not move from the Zika compartments to the Dengue compartments. However, a proportion (χ) of those infected with dengue are temporarily removed from the Zika compartments while they transition from S_D to T_{2D} and return to S_Z at rate ω*

done to obtain estimates for parameters controlling the seasonal forcing and reduced transmission during the clean-up campaign. The parameter χ was set to zero for this DENV-3 model run so that the $S_D, ..., T_{1D}$ compartments did not affect the dynamics in the primary infection compartments.

An informative prior was used for the introduction time for all ZIKV model runs. I fitted an empirical distribution to the posterior distribution presented in our study (Figure 5.1C) and used this as the prior for ψ_m. Since the posterior for tMRCA from the phylogenetic analysis had an imprecise estimate, the prior information in the transmission model fitting was weak. I fixed the value of ψ_w to 10 days because of mixing problems from a larger parameter set. To keep the flow of infected individuals to the model below a plausible value I used a uniform prior on the parameter ψ_b. This restricted the total number of introductions for each simulation of the model to be >1 and <800 (Table 5.1).

The full ZIKV transmission model was jointly fitted to case and serological data using

Table 5.1: *Parameter set for arbovirus model fitting*

Parameter	DENV-3 model prior	ZIKV model prior
β_Z	$U(0,1)$	$U(0,1)$
Seasonal amplitude (β_{amp})*	Fixed	Fixed
Seasonal midpoint (β_{mid})*	Fixed	Fixed
Initial immune (R^0)	$N(0.331, 0.2)$	Fixed to zero
Reporting proportion (r)	$U(0,1)$	$U(0,1)$
Test specificity ($1-\epsilon$)	Fixed	$N(0.07, 0.15)$****
Test sensitivity (ζ)	Fixed	$N(0.8, 0.15)$****
Cross protection (χ)	NA	$U(0,1)$
Waning ZIKV antibodies (ρ)	NA	$U(0,\infty)$
Relative reduction during clean-up campaign** (β_{base})	$N(0.57, 0.15)$***	$N(\mu_{DENV3}, \sigma_{DENV3})$
ZIKV introduction date (ψ_m)	Fixed	$N(\mu_{BEAST}, \sigma_{BEAST})$
ZIKV introductions peak (ψ_b)	Fixed	$U(0.25, 25)$

* Seasonal parameters are fixed from fitted values to temperature data

** Clean-up campaign in March 2014

*** (*Kucharski et al.*, 2018)

**** (*Henderson et al.*, 2020)

adaptive MCMC with a Metropolis-Hastings algorithm. I assumed that cases were distributed according to a negative binomial distribution with mean equal to the number of incident infections reported in the model $c_t = r(C_t - C_{t-1})$, where r is the reporting proportion. The dispersion parameter ϕ adjusted for the inequality between mean and variance in the case data and was fixed to improve the mixing and convergence of other parameters.

I also fitted the model to the proportion seropositive at each time point of the corresponding serosurvey in 2013, 2015 and 2017. I assumed the proportion seropositive at each survey was binomially distributed with size equal to the population size at the time of the corresponding survey and probability equal to:

$$R_j\zeta + (1 - R_j)\epsilon/N_j$$

Where R_j is the number of people in the recovered (R) compartment and N_j is the population size at time j in our model. Therefore R_j/N_j is the total proportion of true infections that could be detected by an assay. ζ is then the estimated sensitivity of the assay, and ϵ is the estimated false positive rate of the assay. I assumed that sensitivity and specificity were fixed over time which is unlikely to hold true in reality. However, I did not attempt to estimate time varying assay sensitivity and specificity because of the limited size of our data available.

Let the random variable $X_j \sim \text{Bin}(N, R_j\zeta + (1 - R_j)\epsilon/N)$. The overall log-likelihood for the transmission model with surveillance data $Y = \{y_t\}_{t=1}^{T}$ and serological data $Z = \{z_j\}_{j\in\{2013,2015,2017\}}$ is:

$$L(\theta|Y) = \sum_t \log P(y_t|c_t) + \sum_{j\in\{2013,2015,2017\}} \log P(X_j = z_j) \tag{5.19}$$

The joint posterior distribution of the parameter set θ was obtained from 1,200,000 MCMC iterations with a burn in of 480,000. I used adaptive MCMC by adjusting the covariance matrix to obtain a target acceptance rate of 0.234 (*Roberts and Rosenthal*,

2009). All models were implemented in R version 4.0.2 (*R Development Core Team*, 2011; *RStudio Team*, 2012) using the `mvtnorm` (*Genz et al.*, 2015) and `deSolve` packages (*Soetaert et al.*, 2010) and parallelised using the `doMC` library (*Revolution Analytics*, 2013). All data and code used in the analysis are available at: `https://github.com/a-henderson91/fiji-zikv-model`.

Model comparison

This full transmission model was designed to be flexible enough to test multiple explanations for the ZIKV outbreak dynamics. The model could capture reduced transmission from inherent differences in transmissibility, seasonal forcing, increased immunity and temporary cross protection during the 2013-14 DENV-3 epidemic. As a sensitivity analysis of these assumptions I ran the model with certain parameters constrained and jointly fit the transmission model using adaptive MCMC over 50,000 iterations to compare the output.

The metric to compare model performance was the Deviance Information Criterion. For a likelihood $p\left(y|\Theta\right)$, we define the deviance as:

$$D(\Theta) = -2\log p\left(y|\Theta\right) \tag{5.20}$$

Where $p\left(y|\Theta\right)$ is the likelihood of the data given Θ. The DIC can be computed as:

$$DIC = D(\bar{\Theta}) + 2p_D \tag{5.21}$$

Where $\bar{\Theta}$ is the mean of Θ with respect to the posterior distribution, and p_D is the effective number of parameters, which is approximately equal to half of the variance of the deviance with respect to the posterior distribution:

$$p_D = \frac{1}{2}\widehat{\text{Var}}(D(\Theta)) \tag{5.22}$$

A difference in DIC of >10 was considered as evidence that the model with the lower DIC was better. A difference between 5 and 10 was considered borderline evidence and any difference less than 5 was considered as no evidence that the models performed differently (*MRC Biostatistics Unit*, 2020).

5.3 Results

I fitted the full transmission model to serological and surveillance data and used prior information from an analysis of molecular data. I constrained model parameters in separate runs to compare different explanations for the observed ZIKV outbreak dynamics in Fiji and why there was not a single-year large epidemic. There were four explanations that I wanted to explicitly test. Firstly, that the reproduction number was different for ZIKV and DENV in Fiji. Secondly, that seasonal forcing impacted transmission dynamics. Thirdly, that pre-existing immunity and the immune response were different for DENV and ZIKV. Finally, that the viruses were introduced at a similar time and DENV infection conferred cross-protection against ZIKV in early 2014.

5.3.1 ZIKV arrived later than DENV and persisted for multiple years

I found the best fitting explanation for the observed ZIKV outbreak dynamics was through the following combination of factors: ZIKV was introduced into Central Division, Fiji, after the ecologically optimal time of year and transmitted at a low level over 3 years until a combination of seasonal forcing and accumulation of ZIKV immunity resulted in the end of transmission in 2017 (Figure 5.7A).

Although the first case of ZIKV was reported in July 2015, we found evidence that transmission of ZIKV likely began in early 2015 in Central Division, Fiji. Infectious individuals were introduced to our model using a continuous logistic function defined by parameters for the peak, width and midpoint of the wave of introductions. The 95% credible interval for the most likely midpoint ranges from October 2014 to February 2015 with a median of January 2015 (Figure 5.7B). By using a posterior estimate from a previous phylogenetic analysis as a prior in our model, our joint inference produced a more precise estimate than the original phylogenetic analysis alone,(*Kama et al.*, 2019) which had an inferred introduction date of May 2014 (95% HPD: Feb 2013 – Jul 2015).

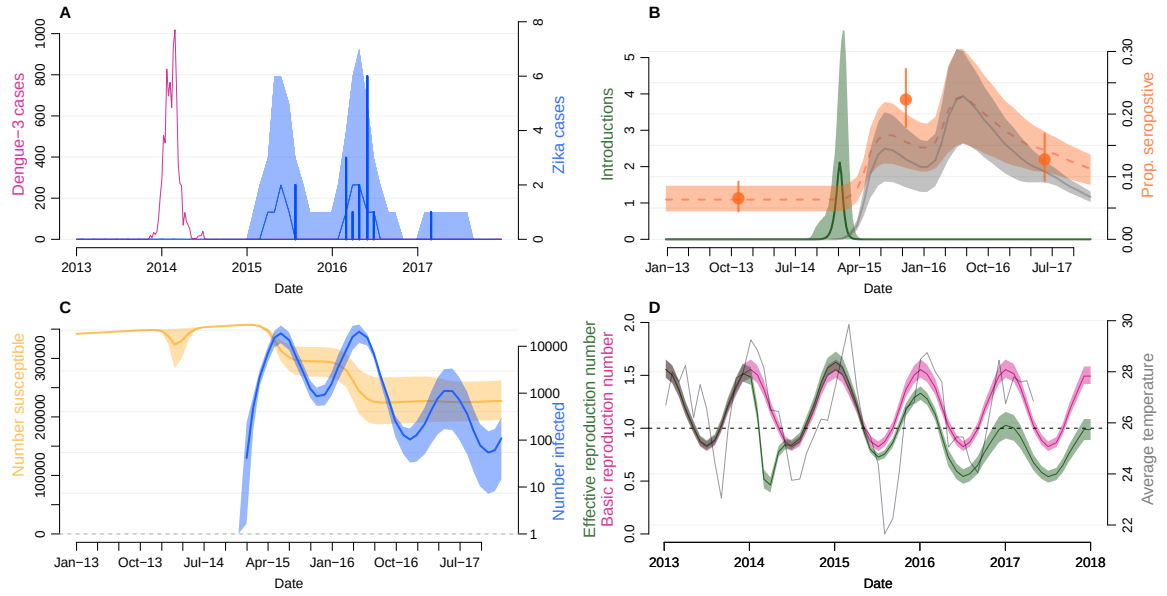

Figure 5.7: *Estimated transmission of ZIKV in Fiji using a mathematical model and multiple data sources. (A) Pink line, weekly cases of DENV-3. Blue bars, monthly cases of ZIKV. Blue dashed line and region, model estimated cases of ZIKV and 95% CrI. (B) Seroprevalence and introduction of ZIKV. Green line and region, estimated introduction of ZIKV infected individuals and 95% CrI. Grey line and region, estimated proportion of the population that had recovered from ZIKV infection (median and 95% CrI). Orange dashed line and region, estimated observed seroprevalence and 95% CrI. Seroprevalence includes an estimated 6.3% (95% CrI: 4.4–8.5%) false positive rate and 79% (95% CrI: 52–98%) assay sensitivity. Orange dots and vertical lines, observed ZIKV seroprevalence from 3 serological surveys. (C) ZIKV infection dynamics in Central Division. Yellow line and region, median and 95% CrI of the number of people susceptible to ZIKV. Blue line and region, median and 95% CrI of the number infected on the natural log scale. (D) Pink line and region, estimated basic reproduction number for ZIKV. Green line and region, effective reproduction number. This included an estimated decline in transmission coinciding with a 2014 vector clean-up campaign (Kucharski et al., 2018). Grey line, monthly temperature data from Suva, Central Division*

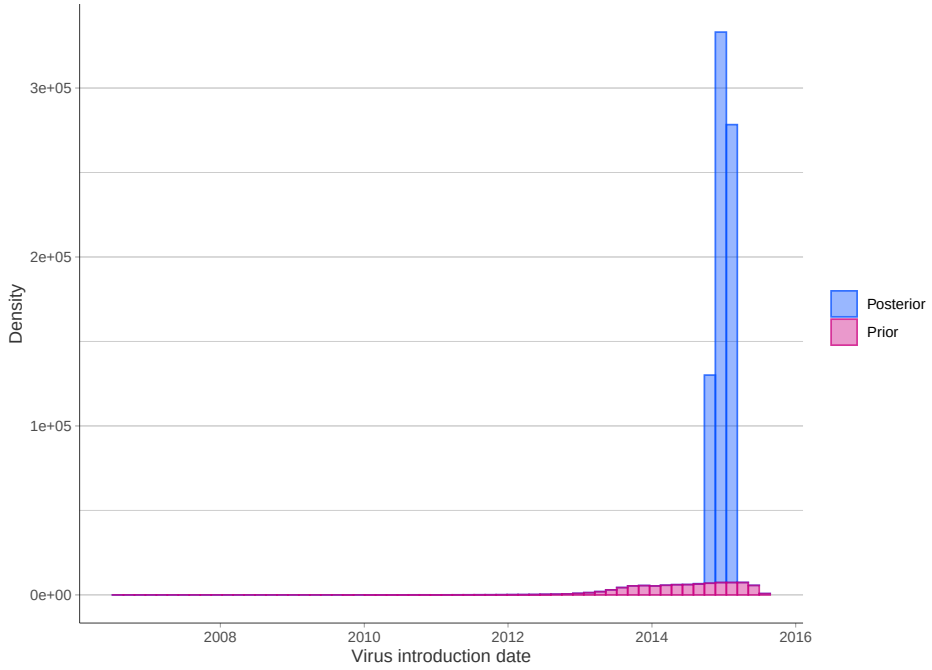

Figure 5.8: *Density of estimated introduction time of ZIKV to Central Division, Fiji. Estimate from a phylogenetic analysis of sequence data (pink) (Kama et al., 2019) used as a prior in this analysis. The estimated midpoint (ψ_{m}) from this transmission model (blue)*

5.3.2 Seasonal variation in transmission defines a period of substantially higher risk for ZIKV introduction

To estimate the role of seasonal variation in temperature on transmission, the model included sinusoidal forcing in transmission with timing and amplitude estimated from available daily temperature data (*Fiji Meteorological Service*, 2017). We then converted this into a relative transmission rate using the published data on the mechanistic relationship between temperature and basic reproduction number for transmission driven by *Aedes aegypti* mosquitoes (*Mordecai et al.*, 2017).

In Fiji, we found a strong seasonal variation in transmission which peaked in February (Figure 5.7C). The seasonality of transmission resulted in a period with an effective reproduction number (R) below 1 (Figure 5.7D). However, we estimated that this was insufficient for the epidemic to fade-out over the colder months between 2015–2016 and 2016–2017 as the prevalent number of infections was consistently above 100 (Figure

5.7C).

The seasonal pattern of transmission also created a period of heightened epidemic risk if a flavivirus was introduced during this period. Towards the end of the calendar year as temperatures, and therefore the transmission rate, increased the required number of initial cases to seed an outbreak was lower than during the colder months. We excluded the possibility of an outbreak emerging from an implausibly small introduction during the period when R was below 1.

To examine how introduction dynamics could shape subsequent ZIKV outbreaks, we simulated model trajectories using the maximum *a posteriori* estimates, then varied the midpoint of the introduction function. We found that the timing of introductions had a large effect on ensuing outbreak dynamics (Figure 5.9A-D). For example, using our model with an introduction event centred around January 2015 – slightly after peak transmission – there were three waves of infections at a low level, as in our main findings (Figure 5.9C). We found that shifting the introduction event 2 months earlier to November 2014 – slightly before transmissibility had peaked – caused a larger single season outbreak comparable to the 2013-14 DENV-3 epidemic (Figure 5.9B). An introduction centred around February 2015, generated a smaller first wave in a shorter high transmission season given the later introduction. However, this delayed the epidemic and there was a larger second wave in 2016 (Figure 5.9C). In our model, varying the timing of the introductions alone could create diverse outbreak dynamics from single large outbreaks to seasonal annual persistence for multiple years.

5.3.3 Estimated R_0 and reporting proportion for ZIKV was lower than the DENV-3 outbreak

In the model, the basic reproduction number (R_0) varied according to seasonal forcing; over the course of a year, we estimated a median ZIKV R_0 of 1.18 (95% CrI: 0.82–1.54) (Figure 5.7C). Before fitting to ZIKV data we initially fitted the same model to DENV-3 surveillance serological data from the 2013-14 epidemic (Figure 5.10). From this analysis we estimated a higher but comparable median and 95% credible interval

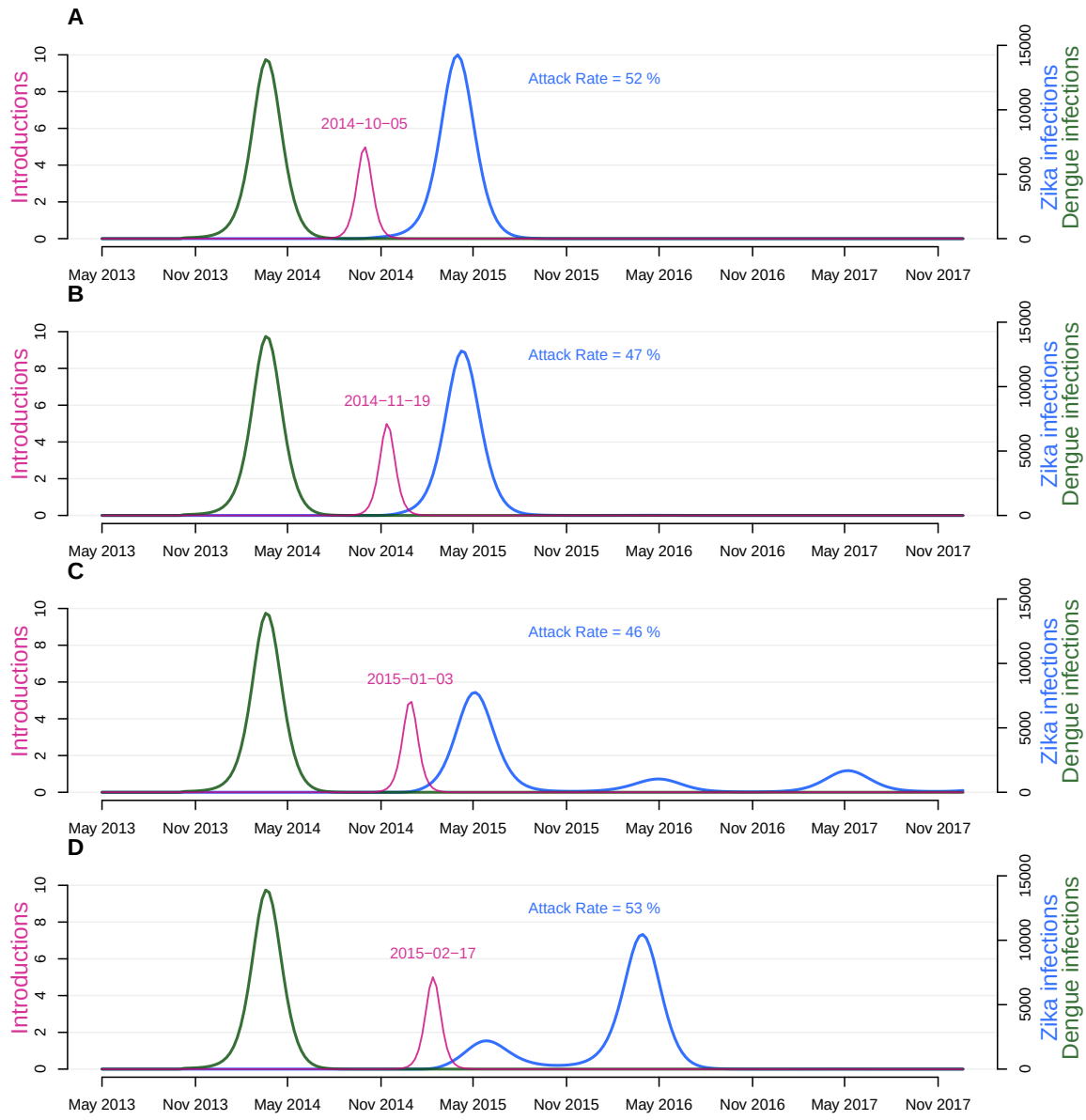

Figure 5.9: *Transmission dynamics by varying introduction time. Simulated ZIKV outbreaks using the maximum a posteriori parameter set and adjusting the midpoint of the introduction of ZIKV infectious individuals. Changing the introduction time alone can vary resulting outbreak dynamics between low level circulation over multiple years to large single-season epidemics. The modelled DENV-3 infections during the 2013-14 epidemic is reproduced here for comparison. Introduction time centred around October 2014 (A), November 2014 (B), January 2015 (C), February 2015 (D). Blue line, model simulation for the prevalence of ZIKV infections (not cases). Green line, modelled DENV-3 2013-14 infections. Pink line, introduction of infectious individuals. The date of the midpoint of the introduction function is displayed in pink. The attack rate is equal to the sum of all ZIKV infections divided by the population size at the start of the outbreak, 342,000 people.*

for R_0 for the 2013-14 DENV-3 epidemic (1.84; 95% CrI: 1.3–2.4).

Other studies have found evidence that inherent DENV transmissibility is similar or slightly higher than ZIKV in the same location (*Bowman et al.*, 2016; *Funk et al.*, 2016; *Ho et al.*, 2017; *Nishiura et al.*, 2016). A more complex modelling analysis of this 2013-14 DENV-3 epidemic estimated an R_0 of 1.12 (95% CrI: 1.02–1.25), similar to our estimate of ZIKV for the same region (*Kucharski et al.*, 2018). Our results are consistent with these findings, that ZIKV is similarly but slightly less transmissible in the same population as DENV. This likely contributed, but was insufficient, to explain the diverse outbreak dynamics between DENV-3 and ZIKV in Central Division.

I also estimated a very small reporting proportion for ZIKV from our model of 0.01% (95% CrI: 0.006–0.02%). This implies that nearly all infections were not reported as cases and were either asymptomatic, not severe enough to seek medical attention, not referred for ZIKV tests by clinicians in Fiji or undetected ZIKV in tests. This low reporting proportion is uncommon for arbovirus outbreaks in Fiji. We estimated a reporting proportion of 16% (95% CrI: 12–23%) for DENV-3 during the 2013-14 epidemic. This discrepancy is the main cause of the diverse observed outbreaks in surveillance case data. However, it is insufficient to explain why ZIKV infections transmitted at a low level for multiple years.

I initially fitted our transmission model to DENV-3 surveillance and serological data for the 2013-14 epidemic to estimate the parameter set Θ as in Table 5.1. Figure 5.10 shows the estimated transmission dynamics of DENV-3 using a mathematical model fitted to multiple data sources. Parameter estimates from this model are shown in Table 5.2.

5.3.4 Posterior parameter estimates

The estimate for the relative effect of "cross-protection" on ZIKV infection during the DENV-3 epidemic shows no evidence of an effect as the 95% credible interval extends from 0.013 (no effect) to 0.98 (total protection). This is unsurprising since most model

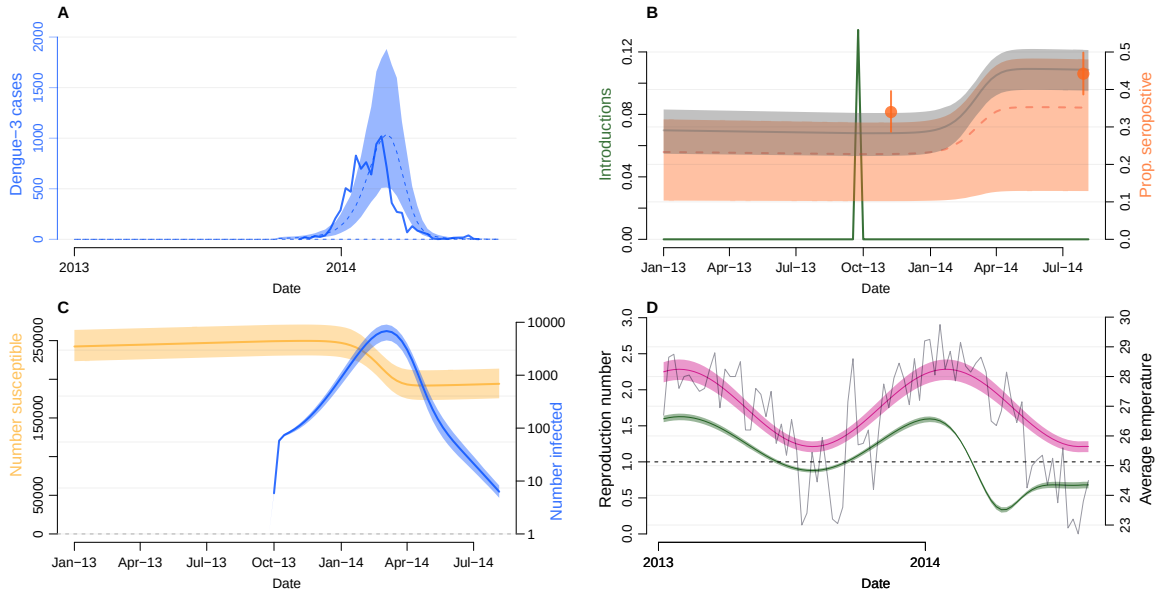

Figure 5.10: *Estimated transmission of DENV-3 in Fiji using a mathematical model and multiple data sources. (A) Blue line, weekly cases of DENV-3. Blue dashed line and region, model estimated cases of DENV-3 and 95% CrI. (B) Seroprevalence and introduction of DENV-3. Green line, fixed introduction of DENV-3 infected individuals. Grey line and region, estimated proportion of the population that had recovered from ZIKV infection (median and 95% CrI). Orange dashed line and region, estimated observed seroprevalence and 95% CrI. Seroprevalence includes an estimated 2.3% (0.35–8.2%) false positive rate and 73% (95% CrI: 23–99%) assay sensitivity. Orange dots and vertical lines, estimated ZIKV seroprevalence from 3 serological surveys. (C) DENV-3 infection dynamics in Central Division. Yellow line and region, median and 95% CrI of the number of people susceptible to DENV-3. Blue line and region, median and 95% CrI of the number infected on the natural log scale. (D) Pink line and region, estimated basic reproduction number for DENV-3. Green line and region, effective reproduction number. This included an estimated decline in transmission coinciding with a 2014 vector clean-up campaign (Kucharski et al., 2018). Grey line, monthly temperature data from Suva, Central Division*

Table 5.2: *Parameter estimates from arbovirus transmission model fitted to the DENV-3 2013-14 epidemic, and ZIKV transmission between 2013 and 2017 (with effective sample size (ESS))*

Parameter	DENV-3 estimate (95% CrI)	ZIKV estimate (95% CrI)	ESS (ZIKV)
Median R_0	1.84 (1.27-2.39)	1.18 (0.82-1.54)	-
Median R	1.08 (0.403-1.61)	0.942 (0.518-1.56)	-
β_Z	0.35 (0.33-0.38)	0.24 (0.23-0.25)	6880
Reporting proportion (%)	16 (12-23)	0.011 (0.0061-0.019)	725
Cross protection	NA	0.51 (0.013-0.98)	158
(1 minus) Test specificity (%)	2.3 (0.35-8.2)	6.3 (4.4-8.5)	1900
Test sensitivity (%)	73 (23-99)	79 (52-98)	1920
ZIKV introduction date (mid)	Oct 2014	Jan 2015 (Oct 2014-Feb 2015)	217
ZIKV introductions (n)	400	394.6 (56.51-963.7)	115
Relative reduction during clean-up campaign (Mar 2014)	0.74 (0.71-0.77)	0.68 (0.64-0.73)	22600
Initial proportion immune	0.29 (0.23-0.35)	0	–
DIC	621.6	76.3	–

simulations had ZIKV outbreaks that started after the DENV-3 outbreak had ended, so there would be no signal about cross-protection in these simulations. The effective sample size – the number of effectively independent draws from the posterior distribution – for the estimated eight parameters are above 100 and six have an ESS greater than 200. The full set of parameter estimates are shown in Table 5.2 and density plots of the eight estimated parameters are shown in Figure 5.12. Density plots of the six estimated parameters in the DENV-3 model fit are shown in Figure 5.12

5.3.5 Hypothetical ZIKV reported cases if ZIKV was reported the same as DENV-3

Figure 5.13 shows the expected observed number of ZIKV cases from our modelled outbreak if ZIKV cases were reported at the same rate as DENV-3 cases during the

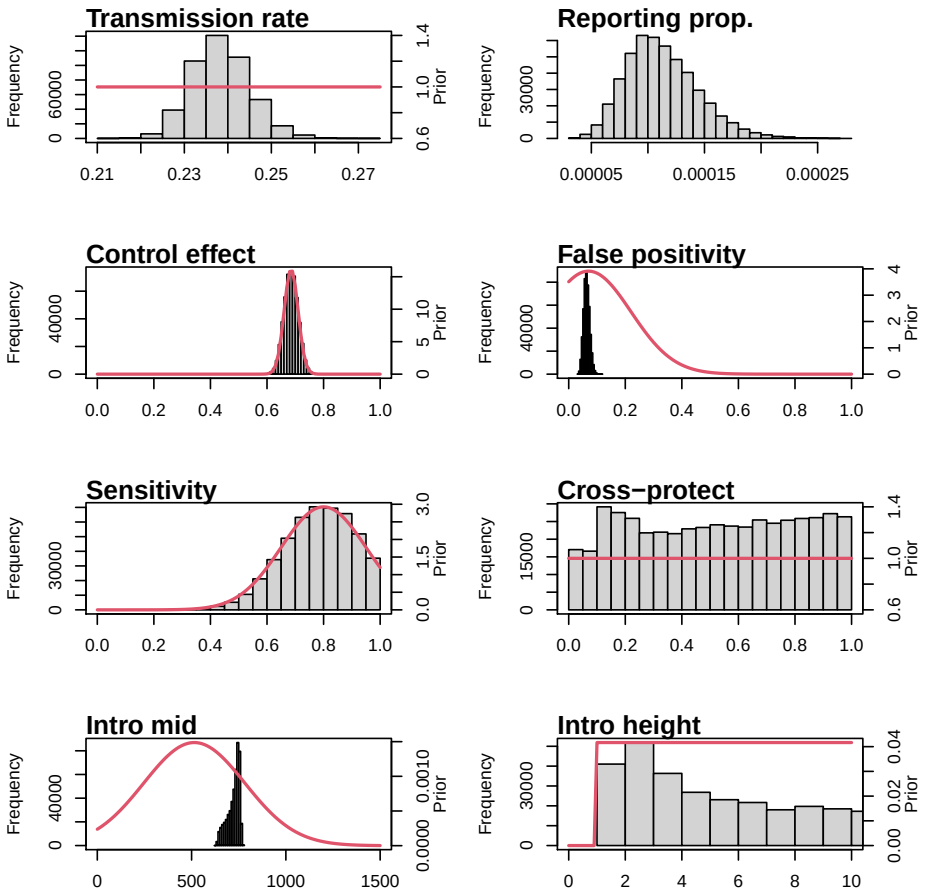

Figure 5.11: *Density plot of estimated parameters in ZIKV transmission model. Grey bars, posterior density. Red lines, prior density*

2013-14 epidemic. This demonstrates that the underlying outbreak dynamics were similar in magnitude but that the main difference in the size of outbreaks in surveillance data was the discrepancy in reporting proportions.

5.3.6 MCMC diagnostics and convergence

The trace plots for the eight estimated parameters in the main ZIKV transmission model are shown in Figure 5.14. Estimation of four of the eight parameters achieve an appropriate level of mixing in all three chains used in the MCMC fitting process. There is poor mixing for the parameter measuring cross-protection because if ZIKV transmits after the DENV-3 outbreak then there is no additional information provided

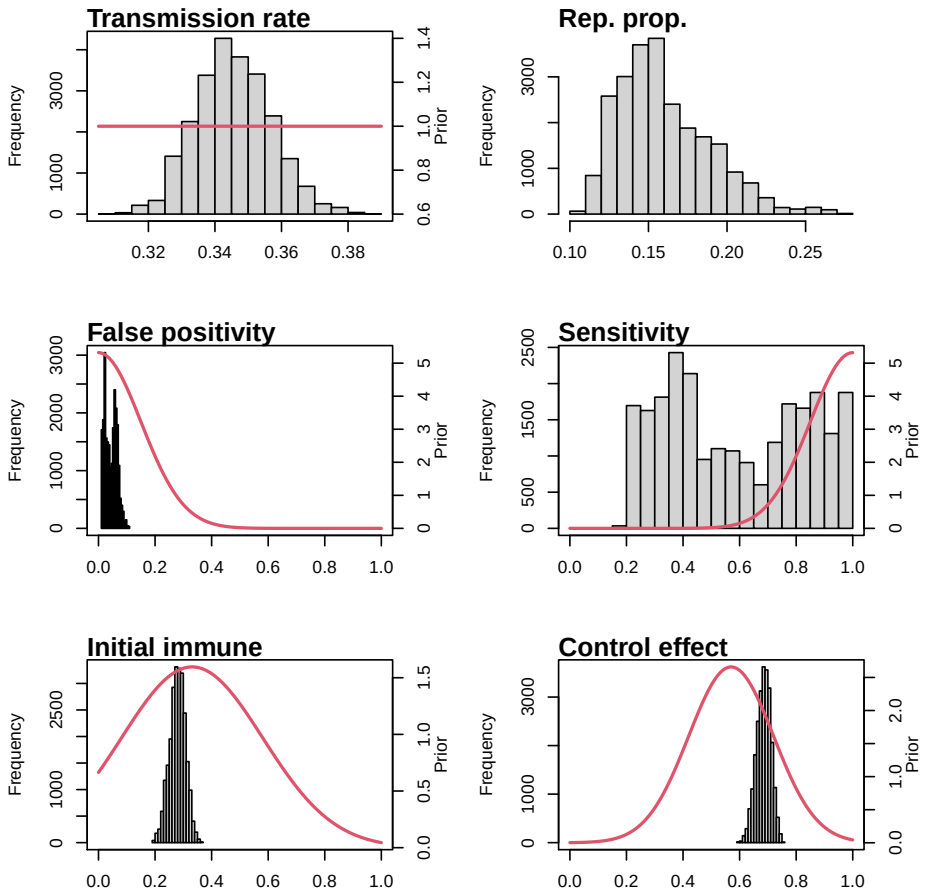

Figure 5.12: *Density plot of estimated parameters in DENV-3 transmission model. Grey bars, posterior density. Red lines, prior density*

by this parameter. The two parameters for the introduction function mix less efficiently, possibly because of their strong correlation (Figure 5.15).

The trace plots for the DENV-3 model fit are shown in Figure 5.16.

5.3.7 Sensitivity analysis of waning seroprevalence, cross-protection duration and early ZIKV introduction

I designed a transmission model that was flexible enough to test multiple explanations for the observed ZIKV transmission dynamics. There were four explanations that I wanted to explicitly test and I found evidence that three of them affected transmission

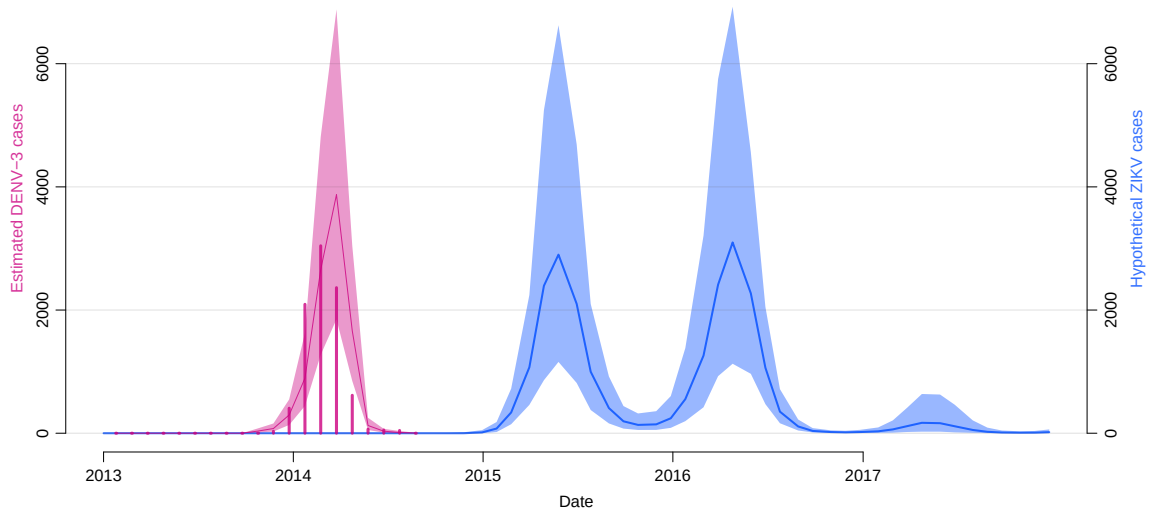

Figure 5.13: *Hypothetical ZIKV case dynamics if the reporting mechanism was equivalent to that of the DENV-3 epidemic. Pink line and region, estimated DENV-3 cases and 95% CrI. These estimates include an estimated 16% (95% CrI: 12–23%) reporting proportion. Blue line and region, hypothetical reported ZIKV cases if the reporting mechanism for ZIKV was the same as DENV-3. These estimates use the modelled infections from the ZIKV model but the reporting proportion from the DENV-3 model fit. The time scale for this plot is monthly not weekly, so the observed DENV-3 cases from surveillance data are reproduced on a monthly time scale as vertical lines.*

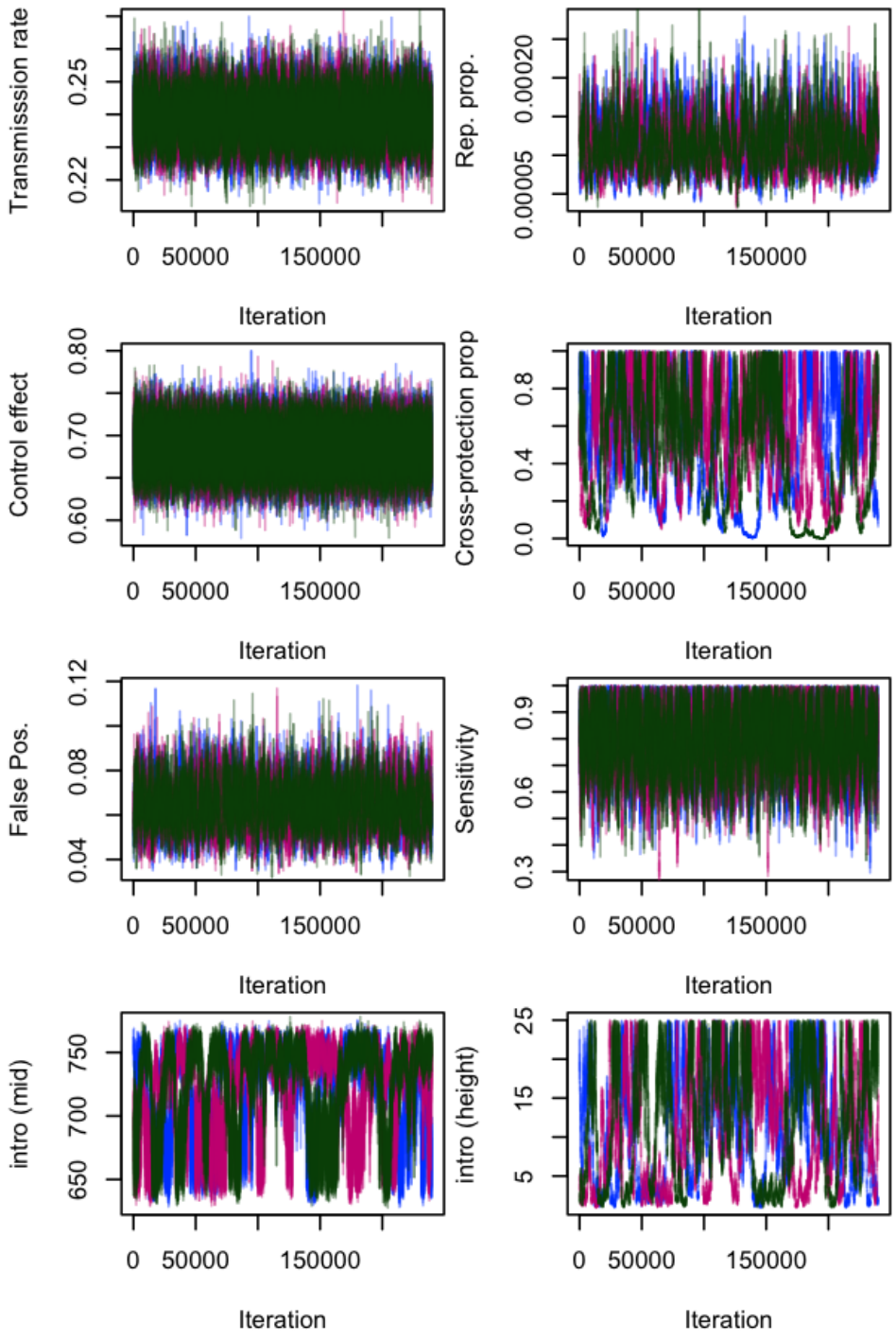

Figure 5.14: *Trace plot of MCMC convergence for full ZIKV transmission model after burn-in of 40%. The three colours represent three separate MCMC chains used in the fitting of the model to surveillance and serological data.*

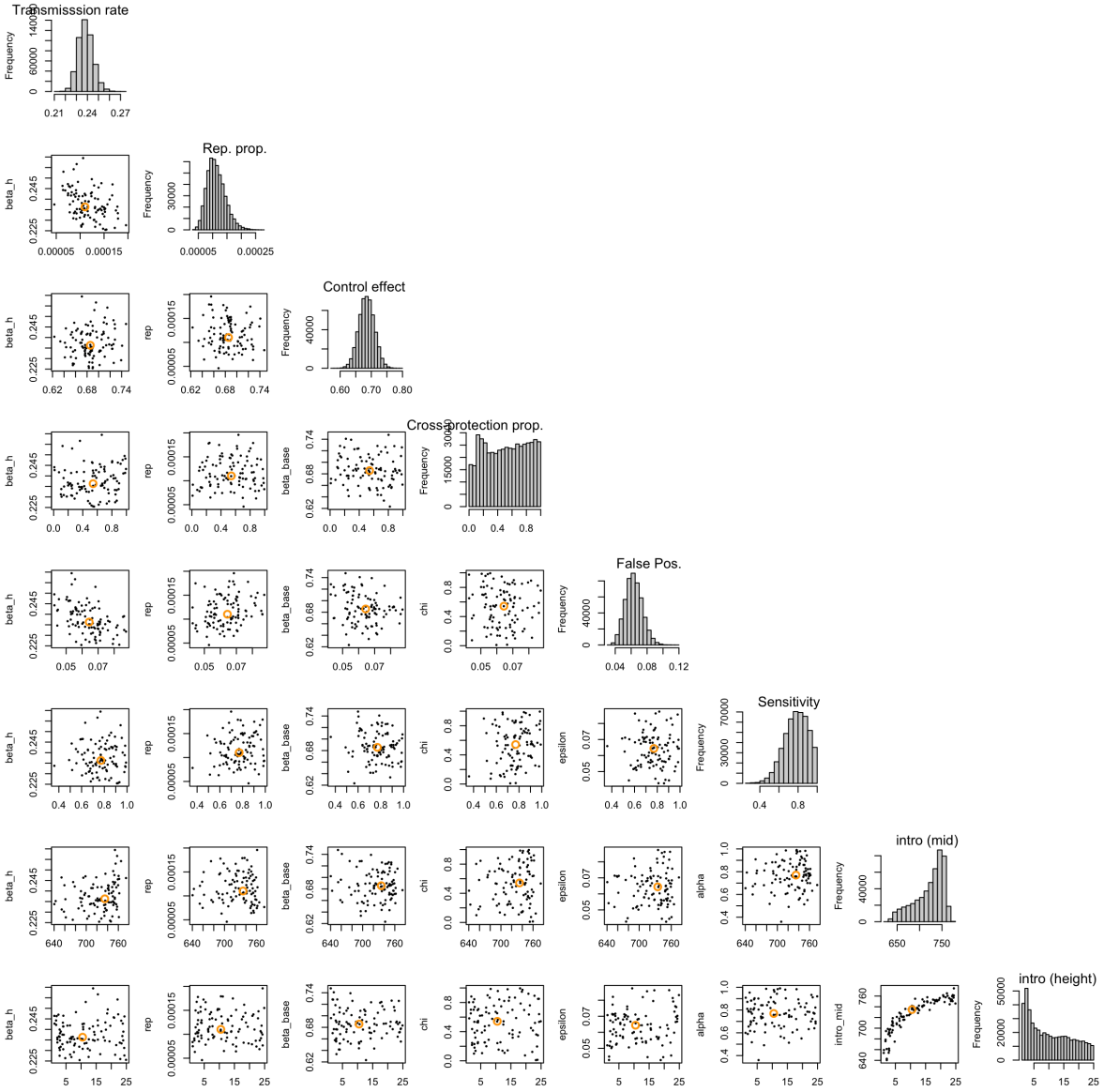

Figure 5.15: *Correlation between estimated ZIKV model parameters. The histograms show estimates of the parameter value. The scatter plot shows the relationship between estimated values of these parameters.*

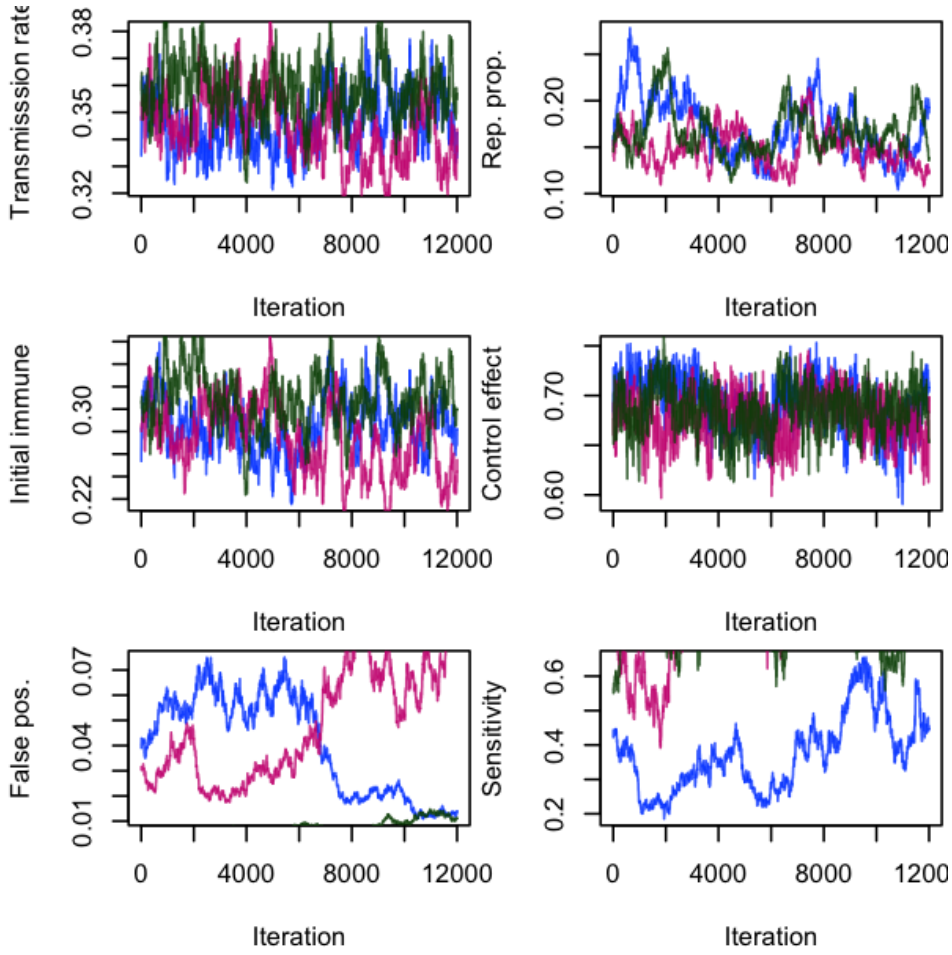

Figure 5.16: *Trace plot of MCMC convergence for full DENV-3 transmission model after a burn-in of 40%. The three colours represent three separate MCMC chains used in the fitting of the model to surveillance and serological data.*

dynamics: the reproduction number was similar but slightly lower for ZIKV than DENV in Fiji, seasonal forcing impacted transmission dynamics, and pre-existing immunity and the immune response were different for DENV and ZIKV.

The fourth explanation I considered was that ZIKV and DENV-3 were introduced to Fiji at a similar time and DENV infection conferred cross-protection against ZIKV in early 2014. It seemed plausible that ZIKV could have arrived in Fiji in 2013 given the circulation of ZIKV in the Pacific at that time (*Cao-Lormeau et al.*, 2014; *Musso and Gubler*, 2016; *Roth et al.*, 2014) and this is consistent with a previous phylogenetic analysis (*Kama et al.*, 2019). I compared the model results with an alternative model which constrained the introduction of ZIKV to Fiji to 2013. This forced DENV-3 and ZIKV to transmit at the same time in my model and therefore tested the hypothesis that DENV-3 infection provided cross-protection against ZIKV infection in early 2014.

I also tested some of the key assumptions I made when modelling ZIKV transmission. Firstly, I relaxed the assumption that seropositivity wanes in the population. Secondly, I extended the duration of the cross-protection for ZIKV following DENV infection. Finally, I forced ZIKV to start spreading in Fiji before the DENV-3 epidemic. A summary of the three models and the main ZIKV model used for this comparison are presented in Table 5.3. All four models were fitted to the same data using MCMC over 20,000 iterations.

Table 5.3: *Sensitivity analysis of key assumptions in the modelling of ZIKV transmission dynamics in Fiji. Estimated deviance information criterion (DIC) and basic reproduction number (R_0) for each model are shown*

Model		DIC	R_0 (95% CrI)
A	Main model	76.8	1.15 (0.8–1.5)
B	No reduction in seropositivity	103.1	1.06 (0.7–1.4)
C	Longer cross-protection duration	75.9	1.14 (0.8–1.5)
D	ZIKV introduction in 2013	129.8	1.24 (0.9–1.6)

Model A - full model

These are the results from the "best fitting model" but from a shorter fitting process over 20,000 iterations. To reiterate, the key assumptions that have been made are that detectable ZIKV-specific antibodies wane over time and that the model could accurately identify the reporting proportion. The estimated DIC from this main model was 76.8.

Model B - no reduction in seropositivity

I wanted to test the assumption that ZIKV-specific antibodies wane over time below a detectable threshold. There is good evidence for this assumption in serological data from Fiji (*Henderson et al.*, 2020). However this is a novel concept so I wanted to compare it to a model where antibodies do not wane over time. The results from this alternative model show that, if ZIKV seropositivity does not wane, the estimated seroprevalence from the model fitting does not recapture the observed seroprevalence data (Figure 5.17B). As a result the model fit is worse with a DIC of 103.1 compared to 76.8 from the main model.

Model C - longer period of cross-protection following DENV infection

I chose a conservative estimate for the duration of cross-protection between DENV-3 and ZIKV of 30 days. This proved uninformative in our main model findings since I estimated that ZIKV was introduced in late 2014, long after the DENV-3 epidemic. As a sensitivity analysis I set the duration of cross-protection to 6 months (*Collins et al.*, 2017) to test whether this an early introduction and long suppression of ZIKV during the DENV-3 epidemic could capture the observed ZIKV data. However the model still converged on a late 2014 introduction date (December 2014; 95% CrI: Oct 2014–Feb 2015) similar to the main model results.

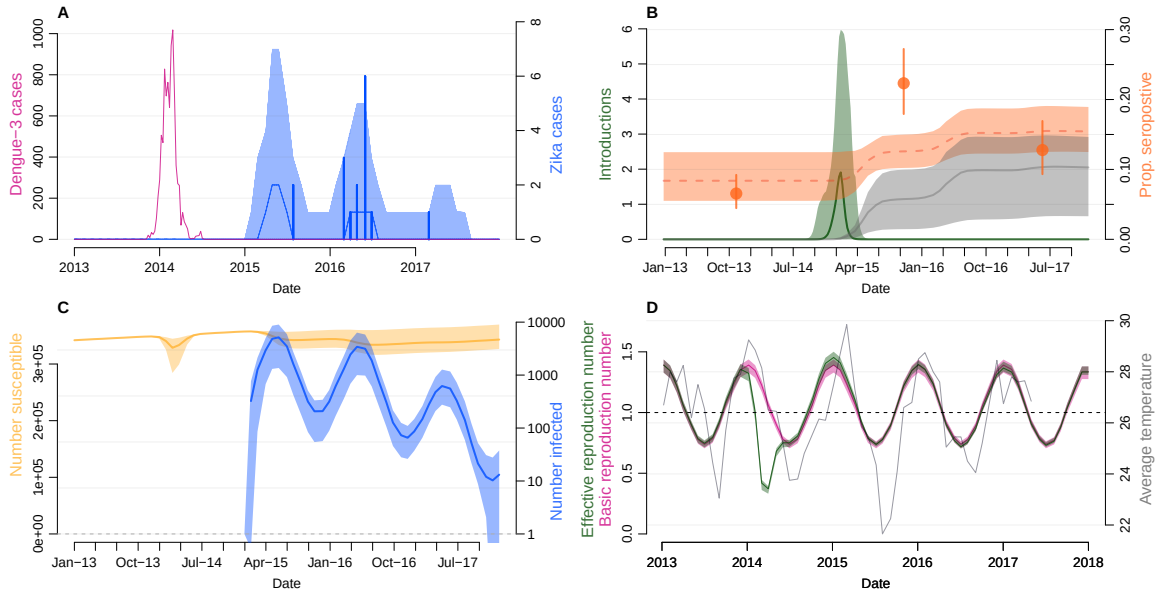

Figure 5.17: *Model B: estimated ZIKV transmission, as in Model A but seropositivity cannot decrease. (Estimated DIC: 103.1). (A) Pink line, weekly cases of DENV-3. Blue bars, monthly cases of ZIKV. Blue dashed line and region, model estimated cases of ZIKV and 95% CrI. (B) Seroprevalence and introduction of ZIKV. Green line and region, estimated introduction of ZIKV infected individuals and 95% CrI. Grey line and region, estimated proportion of the population that had recovered from ZIKV infection (median and 95% CrI). Orange dashed line and region, estimated observed seroprevalence and 95% CrI. Seroprevalence includes an estimated false positive rate and assay sensitivity. Orange dots and vertical lines, estimated ZIKV seroprevalence from 3 serological surveys. (C) ZIKV infection dynamics in Central Division. Yellow line and region, median and 95% CrI of the number of people susceptible to ZIKV. Blue line and region, median and 95% CrI of the number infected on the natural log scale. (D) Pink line and region, estimated basic reproduction number for ZIKV. Green line and region, effective reproduction number. This included an estimated decline in transmission coinciding with a 2014 vector clean-up campaign (Kucharski et al., 2018). Grey line, monthly temperature data from Suva, Central Division.*

Model D - Early ZIKV introduction and interaction with DENV was not well supported by the data

It has been proposed that infection with DENV may result in transient cross-immunity against ZIKV (*Gordon et al.*, 2019; *Montoya et al.*, 2018; *Priyamvada et al.*, 2016; *Rodriguez-Barraquer et al.*, 2019; *Zhao et al.*, 2016). I therefore examined whether the large DENV-3 epidemic in 2013/14 could have induced temporary cross-immunity against ZIKV that delayed the emergence of ZIKV until 2015. The model allowed a proportion of those infected with DENV-3 during the 2013-14 to potentially be temporarily protected from ZIKV infection. With introduction of ZIKV constrained to 2013, I found that a combination of DENV-3 cross-immunity and reduced transmission from a vector control campaign in March 2014 could have suppressed ZIKV transmission in 2014 (Figure 5.18). However, the Deviance Information Criterion (DIC) from this model was much higher than the best fitting model (Table 5.3), suggesting very little support for this alternative explanation. The reason for poor model performance is the short outbreak duration that resulted from this interaction: DENV did not just influence ZIKV in 2014 in this model: by suppressing ZIKV transmission to a large extent, subsequent multi-year outbreaks of ZIKV during 2015-2017 were not possible in the model, in contrast with the observed reported cases during this period.

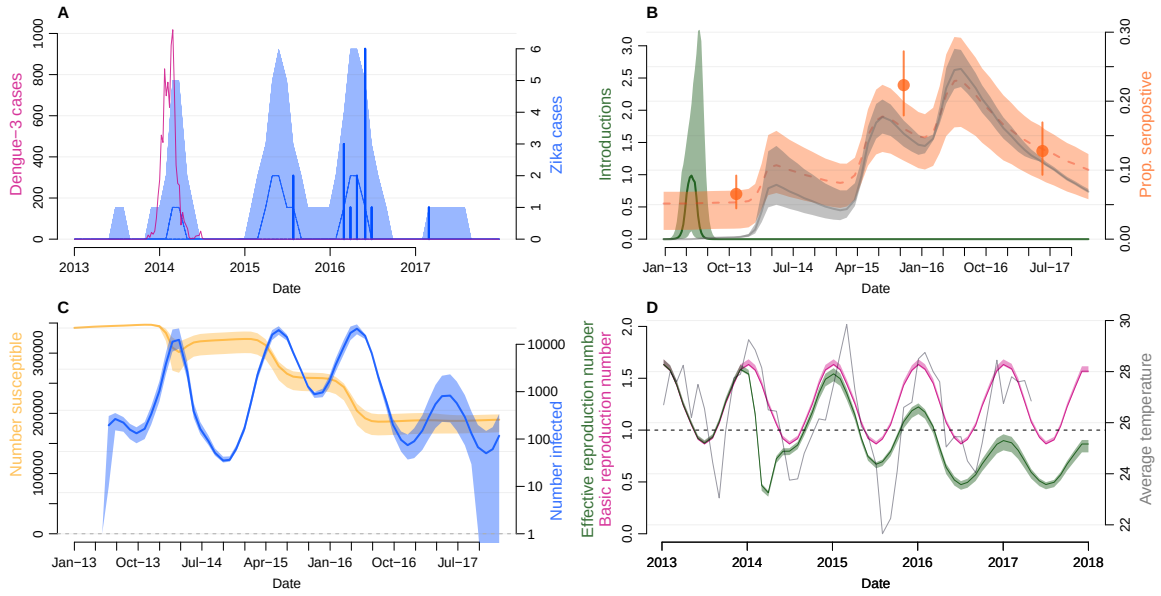

Figure 5.18: *Model D: Estimated transmission of ZIKV in Fiji in a model with ZIKV introduction constrained to 2013 (Estimated DIC: 129.8). (A) Pink line, weekly cases of DENV-3. Blue bars, monthly cases of ZIKV. Blue dashed line and region, model estimated cases of ZIKV and 95% CrI. (B) Seroprevalence and introduction of ZIKV. Green line and region, estimated introduction of ZIKV infected individuals and 95% CrI. Grey line and region, estimated proportion of the population that had recovered from ZIKV infection (median and 95% CrI). Orange dashed line and region, estimated observed seroprevalence and 95% CrI. Seroprevalence includes an estimated false positive rate and assay sensitivity. Orange dots and vertical lines, estimated ZIKV seroprevalence from 3 serological surveys. (C) ZIKV infection dynamics in Central Division. Yellow line and region, median and 95% CrI of the number of people susceptible to ZIKV. Blue line and region, median and 95% CrI of the number infected on the natural log scale. (D) Pink line and region, estimated basic reproduction number for ZIKV. Green line and region, effective reproduction number. This included an estimated decline in transmission coinciding with a 2014 vector clean-up campaign (Kucharski et al., 2018). Grey line, monthly temperature data from Suva, Central Division*

5.4 Discussion

We combined multiple data sources with a dynamic transmission model to reconstruct unobserved transmission dynamics of ZIKV in Fiji between 2013 and 2017. We found that transmission persisted over multiple years with three consecutive small annual outbreaks between 2015 and 2017, with strong seasonal forcing in transmission resulting

in a high risk period of the year for ZIKV introduction. This means there is potential for large, brief flavivirus outbreaks, as well as a period of lower risk where low-level transmission is more likely. We estimated a mean basic reproduction number of 1.18 (95% CrI: 0.82–1.54) for ZIKV, which combined with seasonality in transmission meant there was insufficient infection – and hence acquired immunity – in 2015 or 2016 to prevent re-emergence of the virus in the following year.

We found that ZIKV was slightly less transmissible than DENV in the same population and that nearly all ZIKV infections were undetected, unlike the estimated reporting proportion during the DENV-3 epidemic. We show that if the ZIKV reporting proportion was equivalent to DENV-3 then the two epidemics would appear similar in overall magnitude. However, this is insufficient to explain why ZIKV transmitted at a low level over multiple years, unlike DENV-3 which caused a large single-season epidemic. We have demonstrated that small changes to the introduction time can produce a diverse range of outbreak dynamics because of the strong seasonal forcing in ZIKV transmission in Fiji (Figure 5.9).

The estimated multi-season transmission dynamics of this ZIKV outbreak in Fiji are different to flavivirus outbreaks observed elsewhere in the Pacific, and even within Fiji, where large outbreaks typically last a single season (*Kiedrzynski et al.*, 1996; *Singh et al.*, 2005). Our ability to infer unobserved dynamics benefited from being able to simultaneously fit a transmission dynamic model to serological, surveillance and viral sequence data. Each data source provided insights into different aspects of the dynamics. The surveillance data provided information on the temporal distribution of symptomatic infections, the serological surveys provided estimates of community-level exposure at different points in time and the analysis of sequence data provided informative prior information on the potential time of ZIKV introduction to Fiji. To synthesise these complementary information sources, we used mathematical model that could generate observations representing the serological and surveillance data, then we jointly fitted the model to these data sets in a Bayesian framework, while the sequence data formed an informative prior on the time of introduction. The data available for Fiji presented a unique opportunity to compare and contrast the dynamics of ZIKV

and DENV infection; without the combination of these three data sets it would have been far more challenging to reliably infer the unobserved ZIKV outbreak dynamics.

In our study we allowed flexibility in the timing and size of the initial virus introduction, but in all scenarios we assumed that there was a single introduction wave with continuous transmission afterwards, rather than multiple separate introductions in consecutive years. We also assumed in our model and all sensitivity analyses that the virus persisted over colder months and could therefore be modelled in a deterministic framework. ZIKV outbreaks in other locations have however shown evidence of multiple introductions (*Griffin et al.*, 2019; *Grubaugh et al.*, 2019), including in island settings (*Black et al.*, 2017). Although we cannot rule out multiple early introductions that did not result in widespread transmission, a previous phylogenetic analysis of ZIKV sequences from the region identified two distinct clusters of Fiji sequences, one of which included sequences recovered from Western Division and the other from Central Division (*Kama et al.*, 2019). The small sample size and weak branch support for the Central Division cluster in both analyses means we cannot distinguish between one or more introduction events. The fact that we estimated a cluster that included all three Central Division sequences, with a close relationship between sequences from 2015 and 2016, suggests persistence rather than separate introductions. Although this clustering could also be generated by separate introductions from a similar location, we assumed a single introduction wave. Single ZIKV introduction events have also been estimated for other Pacific Islands (*Delatorre et al.*, 2018), and an introduction during 2014-15 in Fiji is further supported by context of ZIKV transmission across the Pacific. The majority of large outbreaks in the Pacific occurred in 2014 and early 2015, rather than 2016 onwards: the first large outbreak occurred in French Polynesia in late 2013; during 2014 there were ZIKV outbreaks confirmed in New Caledonia, Easter Island and the Cook Islands and in 2015 in Vanuatu and Solomon Islands (*Delatorre et al.*, 2018; *Musso and Gubler*, 2016). Moreover, the level of seroprevalence found in Fiji in 2015 suggests there was widespread transmission between 2013 and 2015, rather than a series of isolated cases (*Kama et al.*, 2019).

In our model we assumed that detectable anti-ZIKV antibodies could wane over time

and therefore seroprevalence in the population could decline over time, as has been observed in serological studies (*Henderson et al.*, 2020; *Moreira-Soto et al.*, 2020). However, as the precise relationship between a specific titre value and susceptibility to ZIKV infection is unclear, we assumed that seroreversions did not lead to loss of protection. This is consistent with other ZIKV modelling studies (*Ferguson et al.*, 2016; *O'Reilly et al.*, 2018) and the fact that many participants in the Fiji survey who were seronegative for ZIKV (as measured by MIA) still had evidence of neutralising titres (*Henderson et al.*, 2020).

In the Fiji serological survey, ZIKV seroprevalence was already 7.8% in November 2013. Given the antigenic similarity of DENV and ZIKV, we assumed that this level of ZIKV seroprevalence may be the result of cross-reactive antibody responses from prior flavivirus infections. To reflect this, we included a parameter that measured the false positive proportion (1 minus the specificity) of the assay, which was estimated as 6.3% (95% CrI: 4.4–8.5%) in the model fitting. This may explain why there was some evidence of seroprevalence before our model estimated ZIKV had arrived in Fiji. Similarly, we estimated a sensitivity of 79% (95% CrI: 52–98%). Both are consistent with the previously reported assay sensitivity and specificity for ZIKV of 79.6% and 94.9% (*Henderson et al.*, 2020). With these adjustments we found that the observed seroprevalence was broadly consistent with our expected seroprevalence from the model. However, in 2015 the observed value was at the limit of our expected seroprevalence (Figure 5.7B). It is possible the assay was more sensitive or less specific during this serological analysis. It is unlikely that there were more true infections than our model produced since this would require a higher transmission rate and therefore increase the likelihood of a single season large epidemic, which is inconsistent with the surveillance data.

The surveillance data collected during the 2013-14 DENV-3 epidemic was primarily from syndromic surveillance and did not have laboratory confirmation (*Kucharski et al.*, 2018). However, all confirmed cases attributed to ZIKV in this study had reverse transcription PCR confirmation in Fiji (*Kama et al.*, 2019). There is significant overlap in the definitions of dengue-like illness, Zika-like illness, influenza-like illness, and acute

fever and rash, so it is a challenge for doctors and nurses to classify patients into these categories and there are inherent uncertainties in the reported numbers. It is therefore possible that some of the cases defined as DENV-3 in 2013-14 were actually caused by ZIKV infection and ZIKV was introduced earlier to Fiji than we suggest here. However the probability of ZIKV arriving in Fiji in 2013 unobserved and still circulating in 2017 was not well supported by our model (Table 5.3). To estimate the proportion of reported DENV-3 cases that were actually ZIKV was not identifiable without more information on the test positive rate during the DENV-3 epidemic, and complicated by a change in reporting during the 2013-14 DENV-3 epidemic from laboratory testing to suspected cases (*Kucharski et al.*, 2018).

Despite these limitations, our results show that ZIKV does not necessarily cause large, brief outbreaks in settings where other flaviviruses have done so, and can persist over multiple seasons, mostly undetected, even in isolated locations. We found that these dynamics most likely resulted from the timing and the magnitude of the introductions of infections prior to the first reported cases. Given the strong seasonal forcing on transmission of vector-borne infections in Fiji, the timing of the introduction had a large impact on the resulting dynamics. This indicates a period of high epidemic risk in Fiji – specifically as temperatures begin to increase – during which surveillance should be particularly vigilant. It also suggests that a wide range of outbreak dynamics are possible if infections are introduced outside this period, including repeated, low-level outbreaks over varying numbers of years. By estimating this range of possible transmission dynamics with such models, it should be possible to improve forecasts about likely outbreak dynamics when new cases are identified. More broadly, with a similar joint analysis of wider data sources for virus outbreaks, there is potential to characterise the range of possible dynamics for other settings as well.

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Chapter 6

Modelling dengue virus transmission in Fiji and assessing the contribution of vector control interventions in ending DENV epidemics

Abstract

Dengue virus (DENV) outbreaks occur with increasing regularity in the Pacific islands but designing rapid responses to emerging outbreaks is limited because outbreaks are challenging to predict and there is limited evidence on the effectiveness of current intervention strategies. We combined transmission models with surveillance, serological, environmental and intervention data during an outbreak of DENV-2 in 2017 to forecast the outbreak in real-time and determine what role control measures played in the decline in transmission. We found that, by fitting models to emerging outbreak data and a historic outbreak we were able to accurately forecast the outbreak in real-time and capture the transmission dynamics before the peak of the epidemic had occurred. Additionally, using complete data after the outbreak we found that herd immunity and seasonal forcing of transmission were insufficient to recapture the transmission dynamics. We show that an additional reduction in transmission when vector control interventions were implemented helps to explain the observed outbreak dynamics. This study demonstrates the potential benefit of real-time modelling of emerging outbreaks and adds to the limited evidence base on the effectiveness of DENV control interventions in Fiji.

6.1 Background

6.1.1 History of dengue virus in Fiji

Dengue virus (DENV) outbreaks in Fiji are typically large with tens of thousands of cases reported in a country with a population of 884,887 (*Fiji Bureau of Statistics*, 2018b; *Kucharski et al.*, 2018). DENV can transmit efficiently in Fiji because there are between four and six species of *Aedes* that can transmit DENV, the most effective of which is *Aedes aegypti* (*Prakash et al.*, 2001). Since 1971 a pattern has emerged where one DENV serotype is introduced to a mostly immunologically naïve population resulting in a large outbreak. Since infection with one DENV serotype provides lifelong immunity for that serotype and temporary heterogeneous immunity against all serotypes (*Sabin*, 1952) there follows a gap of several years before a new serotype can emerge in Fiji, and over ten years before the same serotype causes another outbreak.

This arbovirus outbreak pattern is common in island populations and as a result of this they make for excellent study sites for outbreak dynamics. These locations are isolated and therefore less likely to sustain endemic transmission and have a pattern of self-contained epidemics with reintroduction after an interval period of several years (*Black*, 1966; *Keeling and Grenfell*, 1997; *Teissier et al.*, 2020). It is possible to study an outbreak in these settings for observations about the dynamics of transmission (*Kucharski et al.*, 2018), to compare different disease dynamics (*Funk et al.*, 2016), and analyse risk factors for severe disease (*Cao-Lormeau et al.*, 2016). If we are able to accurately model a previous outbreak then we may also be able to accurately model an emerging outbreak in real-time to predict outbreak dynamics.

This chapter will focus on a recent outbreak of DENV-2 in Fiji in 2017. Cases were first recorded in January 2017. Between January and September 2017 there were 755 cases recorded in Suva, Nasinu, and Nausori in Central Division, Fiji, and at the peak in May 2017 there were 61 cases recorded in one week. I studied this outbreak to analyse the contribution of control efforts to stopping the outbreak, and to assess the performance of a real-time forecast of the outbreak.

6.1.2 Summary of public health interventions for DENV in Fiji

Interventions are available in Fiji to attempt to reduce the burden of DENV cases, mostly targeted at reducing the mosquito population. The primary method used in Fiji for reducing the DENV vector population is to remove breeding sites. This is a year-round routine activity that can be intensified if an outbreak is declared but is also intended to reduce the likelihood of DENV epidemics emerging. An entomological study from 1980 found *Aedes* mosquitoes in miscellaneous containers such as tin cans and plastic food containers, as well as coconut shells, flower vases and old motor parts (*Goettel et al.*, 1980). Tyres and drums were found to be less common breeding sites for *Aedes* larvae, however they were very productive breeding sites so are responsible for the majority of adult *Aedes* produced (*Prakash et al.*, 2001). As a result, tyres and large receptacles are targeted in source reduction clean-up campaigns (*Goettel et al.*, 1980), including during the 2017 DENV-2 epidemic (Ministry of Health report).

In the case of an epidemic a more targeted response is used, which is chemical spraying to kill adult mosquitoes in areas with a high burden of reported cases. The active ingredient in the chemical spraying used is pyrethrins which is a widely used insecticide (Ministry of Health report). Previously, Malathion ULV spraying has been used (*Goettel et al.*, 1980; *Prakash et al.*, 2001). Spraying is performed with both truck ULV and hand-held portable devices (Figure 6.1). The location of chemical spraying is determined by the number of reported DENV case notification and preventative spraying in heavily populated areas. Chemical spraying takes place in the early morning and evening when *Aedes* mosquitoes are most active (*Nelson et al.*, 1978). Residents in the area are advised to open doors and windows prior to the spraying.

Intervening to reduce the DENV vector population is expensive and there is very little evidence about the effectiveness of these interventions. A study from 1980 showed evidence that environmental sanitation and, to a lesser degree, insecticide spraying were effective in reducing the adult mosquito population over the study period (*Goettel et al.*, 1980). There is no evidence about the relationship between Ministry of Health

(a)

(b)

Figure 6.1: *(A) Insecticide spraying using handheld devices at Suva grammar school (Ministry of Health report). (B) Insecticide spraying from van mounted devices at Bureta Street, Suva (Ministry of Health report)*

interventions and the burden of DENV disease in Fiji. Globally, a recent review of current vector control methods and their effectiveness on reducing DENV concluded that "almost nothing is known about how well they prevent disease" (*Achee et al.*, 2015).

My objective with this analysis was to improve understanding of how vector control interventions can reduce the burden of DENV during an outbreak in Fiji by developing a mathematical model and estimating any additional reduction in transmission during vector control campaigns.

6.1.3 Modelling of the 2013-14 DENV-3 outbreak

I developed a Ross-Macdonald model of vector-borne transmission for this DENV-2 epidemic. This class of models has previously been used in a study of the 2013-14 DENV-3 outbreak in Fiji (*Kucharski et al.*, 2018). Between November 2013 and August 2014 a large DENV-3 epidemic emerged with over 25,000 suspected cases across all of Fiji, and 12,413 suspected DENV cases in Central Division, where longitudinal serological data were available. Pre- and post-outbreak sera were collected in Central Division and tested for evidence of previous DENV infection using a multiplex microsphere im-

munoassay (MIA) and found an increase in DENV-3 seroprevalence from 33.1% (95% CI: 27.4–39.1%) in November 2013 to 53.2% (95% CI: 47–59.4%) in November 2015. One major strength of this study was that the model combined surveillance data and serological data in the likelihood when model fitting, which was an approach we carried into this DENV-2 analysis.

The 2013-14 DENV-3 outbreak could have ceased for a variety of reasons, but three likely explanations were built into the model. Firstly, the outbreak was self-limiting driven by a decline in the susceptible population. The change in climate conditions over the outbreak could have introduced seasonal forcing on transmission of DENV-3. There was also a large clean-up campaign in March 2014, at the peak of the outbreak, to remove mosquito breeding sites and this could have had a direct effect on transmission. The inclusion of serological data allowed for the estimation of the relative effect of these three factors, which was not possible using surveillance data alone. The outbreak ceased because of a combination of increased immunity, seasonal forcing and control measures.

This analysis of the 2013-14 epidemic found evidence that vector control had an effect on DENV transmission. However, the signal was too weak to analyse further and, working with the Ministry of Health, it was clear that they wanted more detail on how effective these interventions were and how to improve their effectiveness. Control measures are not implemented uniformly across the Division which presents an opportunity to compare different levels of interventions in different areas. I wanted to analyse the relationship between intervention and transmission reduction at a finer spatial scale. This was the prior objective of this study, however, while I was working on data collection in Fiji the DENV-2 epidemic was ongoing so I was able to use this model to forecast the outbreak in real-time. In this chapter I will firstly present work on forecasting the DENV-2 epidemic, then examine control measures retrospectively.

6.1.4 Real-time forecasting the 2017 DENV-2 outbreak

The use of mathematical modelling of infectious diseases to conduct real-time analyses of outbreaks and consequently forecast the likely path of an emerging epidemic is growing rapidly (*Camacho et al.*, 2015; *Finger et al.*, 2019; *Kucharski et al.*, 2020). The main advantage of this practice is that forecasting the burden of an outbreak can improve preparedness and allow public health bodies to mobilise resources appropriately. Additionally, a successful forecast can assess underlying factors that are affecting the transmission dynamics. In the case of arboviruses such as DENV this may be changing climate conditions or the role of direct interventions on the vector population. A case study of a diphtheria outbreak in a crisis setting demonstrates the value of effective real-time forecasting. *Finger et al.* (2019), fitted a compartmental transmission model to incidence data in real-time. The model was able to produce reliable forecasts three weeks before the outbreak ended which supported the operational response, such as bed need, and advocacy for control measures. Similarly, during the 2014 West Africa Ebola virus disease epidemic *Camacho et al.* (2015), used a stochastic mathematical model to forecast the course of the Ebola virus epidemic in the worst affected country, Sierra Leone. Their results suggested that the epidemic had already peaked in Sierra Leone. A subsequent assessment of the model performance during this outbreak found that the model was well calibrated – the ability of the model to correctly identify its own uncertainty in making predictions – up to 2-3 weeks ahead (*Funk et al.*, 2019).

Real-time forecasting has been proven to provide valuable information during an outbreak, however they have serious limitations. Most importantly is the quality of data that is available during an outbreak. Fiji has an established and successful health surveillance system (*Sheel et al.*, 2019) which produces reports for the Pacific Public Health Surveillance Network (PPHSN). However, during the DENV-2 epidemic, the burden on the laboratory analysis meant that only a small proportion of test results were available in real-time. In addition a mixture of diagnostic methods were used. Reverse transcription polymerase chain reaction (RT-PCR) was used to identify the circulating serotype (*Aubry et al.*, 2012), the majority of laboratory diagnosis was either through detection of the nonstructural protein 1 (NS1) or an assay testing for

evidence of anti-DENV immunoglobulin class M (IgM) antibodies. Another issue is the rapid evolution of outbreaks and the impact of changes in behaviour and direct interventions may have on transmission dynamics during the outbreak (*Bausch and Edmunds*, 2018). Finally, improving real-time modelling performance is a challenge without an agreed characterisation of what constitutes a 'good' forecast. This is being addressed in the literature (*Funk et al.*, 2019) but will also depend on the priorities during the outbreak. In the case of DENV-2 in Fiji in 2017 the priority was to learn of the possible overall magnitude of the outbreak, and therefore potential benefits for intervening and reducing the vector population. Real-time modelling remains just one tool in a public health response during an outbreak, but in the case of DENV-2 we were able to demonstrate its utility when working directly with the Ministry of Health in Fiji.

In May 2017 I was in Fiji setting up the third in a longitudinal seroepidemiological study (chapters 3 and 4). A DENV-2 outbreak had emerged with the first confirmed case in January 2017 and by the time I arrived in Fiji there had been 223 suspected and confirmed cases reported. We extended the model developed for analysis of the DENV-3 2013-14 outbreak (*Kucharski et al.*, 2018) to forecast the epidemic. Importantly, the Ministry of Health was most concerned that this could become another large epidemic with thousands of cases unless the mosquito density was reduced. After the outbreak, I obtained the full surveillance data with final results from all laboratory tests performed during the outbreak. I used these data to assess the performance of the forecast and wanted to identify how early in the outbreak we would have been able to reliably predict the epidemic dynamics.

6.1.5 Assessing the role of interventions in the end of the 2017 DENV-2 outbreak

I also performed a retrospective analysis of control measures that were introduced during the outbreak. Previous work in Fiji found evidence that DENV-3 transmission dynamics could be captured in a model with an additional reduction in transmission

intensity at the same time as a government intervention removing mosquito breeding sites (*Kucharski et al.*, 2018). Evidence on the effectiveness of mosquito control programmes to reduce DENV transmission is weak (*Bouzid et al.*, 2016; *Bowman et al.*, 2016; *Heintze et al.*, 2007; *Reiter*, 2016). As a result, I wanted to use this island epidemic of DENV-2 to analyse the impact of vector control interventions during the outbreak. I was based at Mataika House in Suva, Fiji, between May and July 2017 and I worked with the Ministry of Health and the entomological control department to obtain more information on control measures and interventions used during the outbreak. Having contributed to update and decision-making meetings during the outbreak it was clear that the vector control response was heterogeneous across Suva, some areas were more intensely targeted than others. I aimed to expand on published DENV analysis in Fiji (*Kucharski et al.*, 2018) and jointly fitted a model in different regions of Suva, the capital city of Fiji. These regions within Suva had varying case incidence, pre-outbreak population immunity and interventions during the DENV-2 outbreak. Using this model, I aimed to estimate the effectiveness of different intervention strategies in different areas.

Interventions used during the outbreak were mostly chemical spraying to reduce the adult mosquito population. Information were available from detailed reports on when and where these spraying activities were performed and the area they covered. In addition to this the entomological control department kept details on clean-up campaigns to reduce mosquito breeding sites, including location, date and the amount of waste disposed of. For serological data, because population representative sampling across Fiji was used (*Lau et al.*, 2016; *Watson et al.*, 2017), the sample included a large number of participants from Suva. There were thirteen clusters in our DENV-2 study area in Suva, each with twenty-five participants sampled either once in 2013 or followed up in 2015 and/or 2017 as well. These sera were tested using a microsphere immunoassay (MIA) so we had estimates of pre-outbreak DENV-2 seroprevalence which was collected in November 2015. The third sample collection was during the tail end of the DENV-2 outbreak, collected in June 2017. Combining these data sources with complete surveillance data I was able to fit a model to DENV-2 transmission at a more detailed spatial level.

The aim of this section of the study was to analyse the DENV-2 2017 outbreak to identify any effect of control measures on transmission intensity at a Division level. Following this, I aimed to estimate variation in effectiveness of interventions in different areas by fitting the model to seroprevalence estimates and reported cases in each region. Given the variety in vector control intervention efforts in these regions during the outbreak, I expected that it would be possible to identify differences in the effectiveness of different intervention strategies.

6.2 Materials & Methods

6.2.1 Ethics

Ethics approval was obtained from the London School of Hygiene & Tropical Medicine (reference 12037) and the local research ethics committee in Fiji. In 2018 we submitted an ethics amendment application for our project titled "Serosurvey to study Zika and related arboviruses in Central Division, Fiji" (reference 2017.20.MC). Approval for the amendment was granted on 23rd October 2018. Our original ethics application was focused on researching Zika virus (ZIKV) but included an objective to understand the "potential association with serological evidence of other arbovirus infections, such as dengue". We submitted an ethics amendment request for additional data to study the effect of vector control interventions during the 2017 outbreak by combining surveillance and serological data.

6.2.2 Data

Real-time modelling projections were made between May and June 2017 and were fitted to weekly reported case data as they became available. Cases were defined as positive if they met one of the following criteria: DENV RNA detected by RT-PCR, positive for NS1 antigen, anti-DENV IgM antibodies detected by Enzyme-linked immunosorbent assays (ELISAs). A dengue outbreak is declared in Fiji once the incidence rate exceeds a

predefined threshold of more than two standard deviations above the average incidence rate for the last five non-outbreak years (*Ministry of Health and Medical Services*, 2016). In an outbreak setting dengue surveillance can move from laboratory confirmation to clinical-based reporting, i.e. dengue-like illness. In the case of the DENV-2 epidemic, all reporting was laboratory confirmed because the incidence rate never exceeded the threshold for declaring an outbreak. This switch of reporting method was cited as a limitation when analysing the 2013-14 DENV-3 epidemic (*Kucharski et al.*, 2018). Real-time data were unreliable as a result of the logistical constraints of laboratory testing during the epidemic. The proportion of samples tested was variable week-to-week and most test results were from rapid NS1 detection tests instead of ELISAs. As a result, when performing retrospective analyses of forecast performance and analysing intervention strategies I used post-outbreak confirmed case data. This data should be more accurate as it includes ELISA data where available and will include cases that may have been confirmed later in the outbreak. After the outbreak all samples collected during the outbreak had been tested and we had NS1 or ELISA confirmation for cases in the Suva-Nausori study region between January and September 2017. I obtained seroprevalence estimates from serological surveys previously described in Chapter 2.

Study regions in Suva

The study area was divided into five regions based around a health centre that could collect and record cases during the outbreak (Figure 6.2). Colonial War Memorial hospital (CWM) is the largest health facility in Suva. The area around the hospital is the central business district and tourism hub of the capital and includes the working docks and most of the coastal region of Suva. North of CWM are two suburban districts of Suva, on the east coast is Raiwaqa and to the north is Samabula, both of which are predominantly residential areas. Moving further north the regions increase in altitude as well from 57m above sea level at CWM hospital to 124m at Nuffield Health Centre. The Nuffield region is a peri-urban area and forms the 'corridor' that connects Suva city to Nausori town. Finally, the Nasinu/Nausori area has several health facilities across the region but the largest is Makoi Health Centre in the north of the region, where

elevation dips again down to 58m above sea level (Figure 6.2).

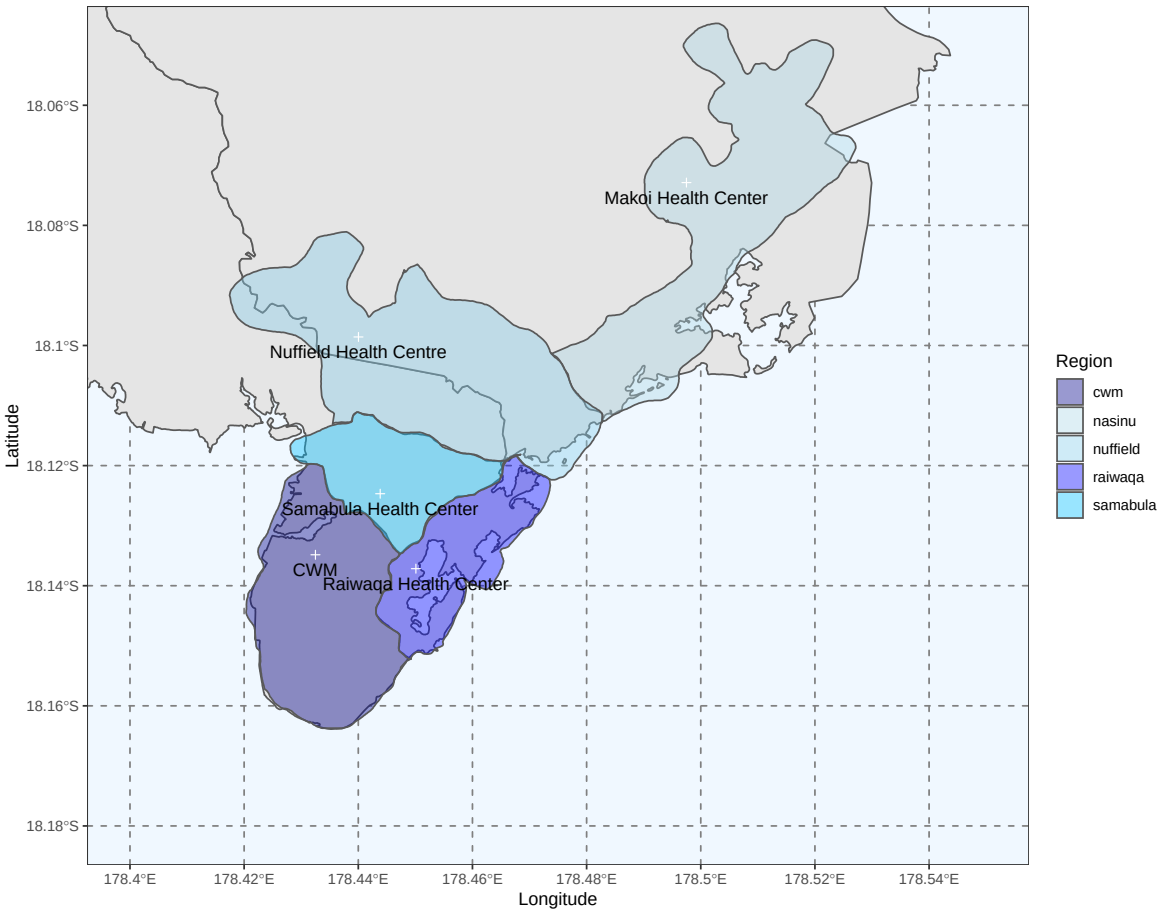

Figure 6.2: *Map of study area. Suva and Nausori in the southeast of Viti Levu, Fiji. Each region is represented by a different shade of blue. White crosses indicate the largest health care centre in each of the five regions*

Our serological sampling included several clusters in Suva, Nasinu or Nausori because this is where most of the Central Division population resides (*Fiji Bureau of Statistics*, 2018a). Figure 6.3 shows the centroid for a cluster of up to twenty-five participants recruited in the original 2013 study (*Lau et al.*, 2016; *Watson et al.*, 2017). Participants in the serological study were well distributed across our study with samples available from all five regions so it was possible to capture regional differences in pre-outbreak community immunity.

Finally, the entomological department at the Ministry of Health recorded 49 individual events of vector population reduction interventions. The vast majority, 42 (86%)

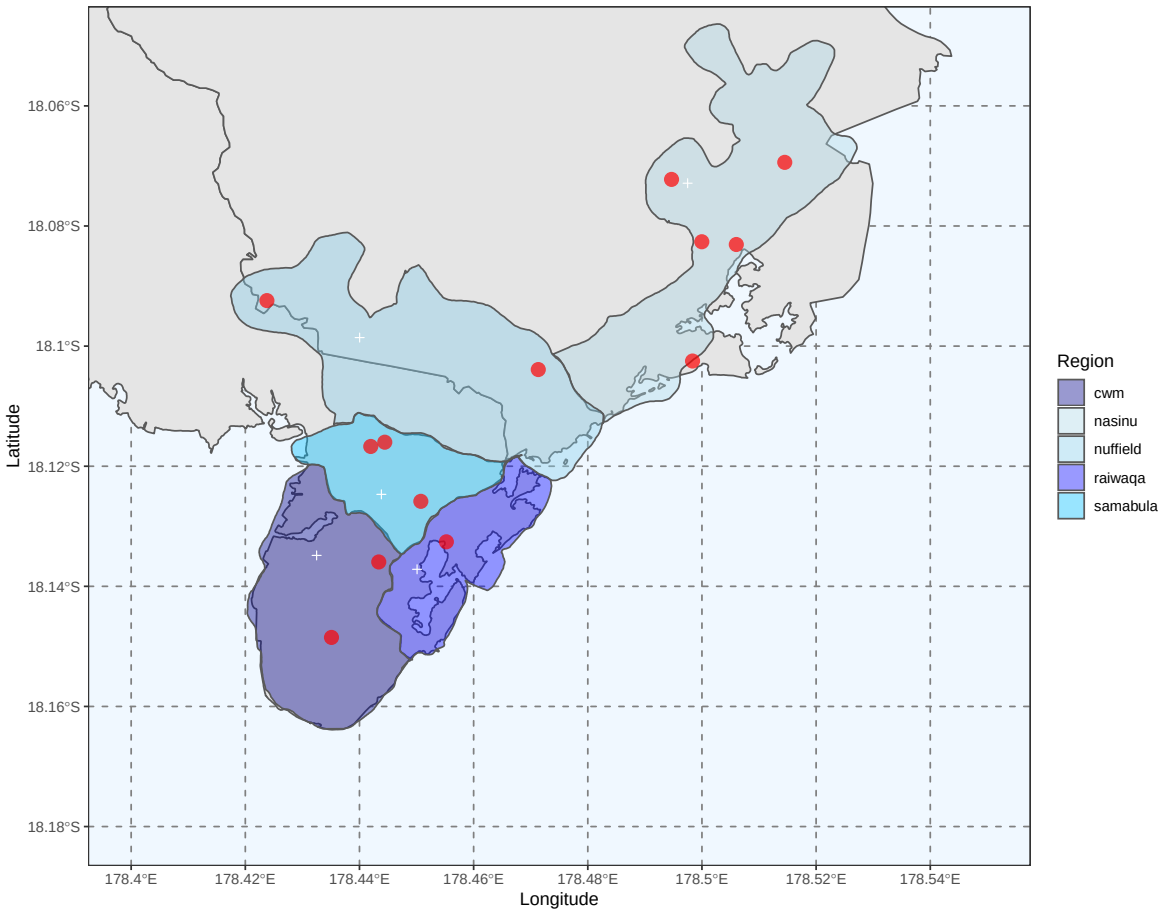

Figure 6.3: *Map of study area and location of collected serological samples. Red dots, centroid of a cluster of up to twenty-five participants in the original 2013 serological survey. A subset of participants at each of these cluster locations were sampled in 2015 and in 2017. White crosses show the largest health care centre in each of the five regions as in Figure 6.2*

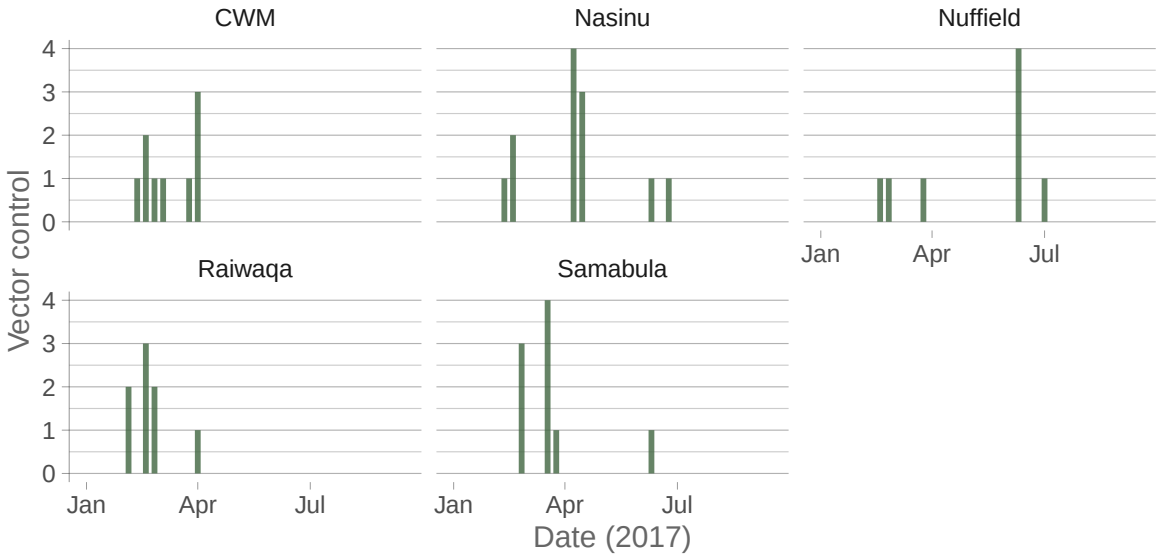

Figure 6.4: *Timing of vector control interventions during the 2017 DENV-2 outbreak. One intervention was defined as reported by the Fijian MOH during the outbreak and could correspond to an area sprayed with insecticide or targeted source reduction in a specific area*

of these were chemical spraying and the remaining 7 were removal of breeding sites. The first round of spraying was in February as a preventative measure to reduce the likelihood of a large outbreak and was followed by a second round of chemical spraying throughout March and April as cases increased quickly (Figure 6.4).

The sites of these interventions were well distributed across the study region. Chemical spraying is designed to target areas with a high case load so a lot of the activity is in Nausori, especially in the north around the Davuilevu housing estate, and in the area immediately surrounding CWM hospital (Figure 6.5).

6.2.3 Model structure

I used a compartmental deterministic model introduced in Chapter 2. The model defined the human population as either Susceptible, Exposed (pre-infectious), Infectious, or Recovered (SEIR). The mosquito population followed a similar process without a Recovered compartment because I assumed that mosquitoes did not recover from infection. The full model was as follows:

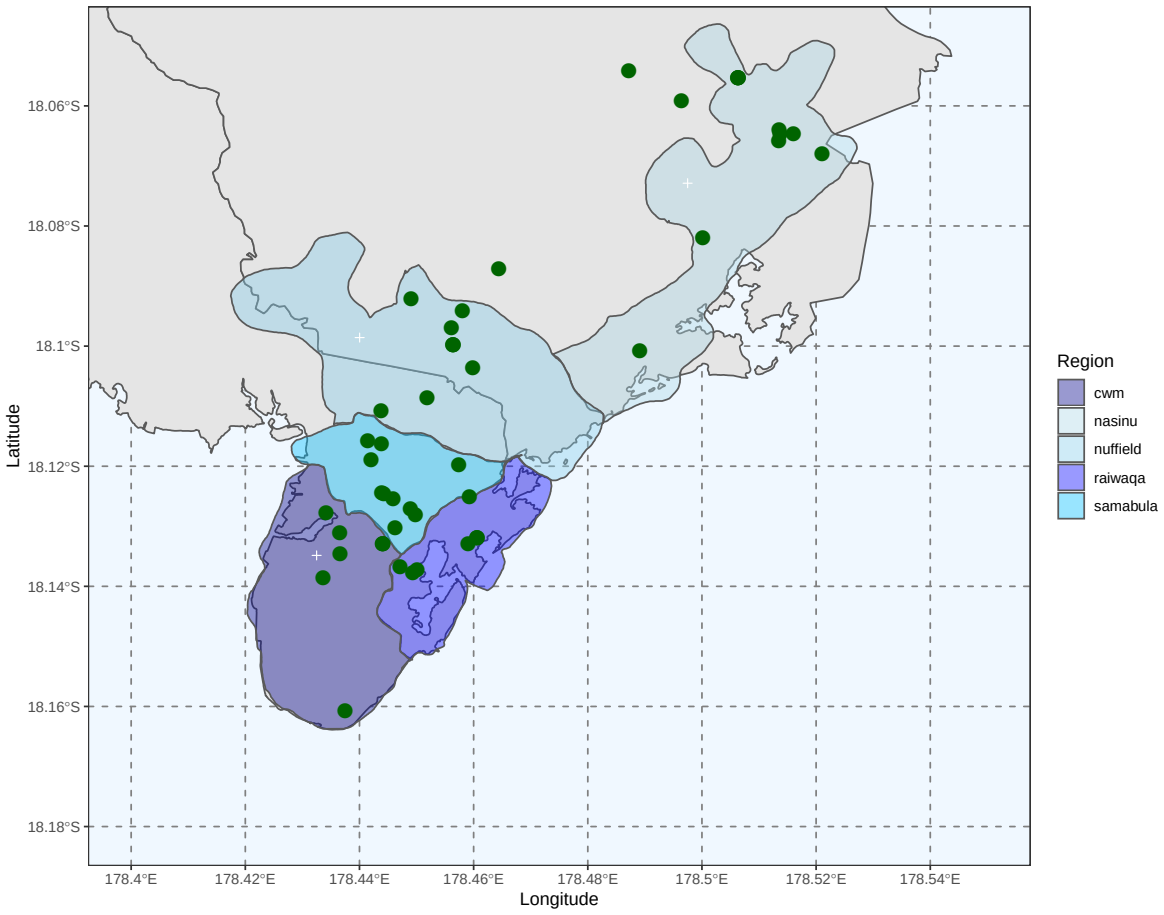

Figure 6.5: *Map of study area and location of vector control interventions during the 2017 DENV-2 outbreak. Green dots, a vector control intervention implemented, either insecticide spraying or source reduction in this area. White crosses show the largest health care centre in each of the five regions as in Figure 6.2*

$$\frac{dS_H}{dt} = -\beta_H(t) i_M S_H \tag{6.1}$$
$$\frac{dE_H}{dt} = \beta_H(t) i_M - \alpha_H E_H \tag{6.2}$$
$$\frac{dI_H}{dt} = \alpha_H E_H - \gamma I_H \tag{6.3}$$
$$\frac{dR_H}{dt} = \gamma I_H \tag{6.4}$$
$$\frac{dC_H}{dt} = \alpha E_H \tag{6.5}$$
$$\frac{ds_M}{dt} = \delta - \beta_M \frac{I_H}{N_H} s_M - \delta s_M \tag{6.6}$$
$$\frac{de_M}{dt} = \beta_M \frac{I_H}{N_H} s_M - (\delta + \alpha_M)\, e_M \tag{6.7}$$
$$\frac{di_M}{dt} = \alpha_M e_M - \delta i_M \tag{6.8}$$

Data were available on the size of the human population but not the mosquito population so the human compartments were specified in terms of numbers and the mosquito compartments in terms of proportions. The birth and death rates of mosquitoes was assumed to be equal so the population density remained constant throughout the outbreak but the proportion susceptible was variable. The human population was assumed to be constant throughout the outbreak with no deaths or births in this model.

Humans are assumed Susceptible (S_H) until exposed to infection with DENV-2 when they transition to a latent class (E_H), then an infectious class (I_H), and finally a recovered and immune class (R_H). Mosquitoes followed a similar process from susceptible (s_M), through latency (e_M) to infectious (i_M) where they remained until they died at rate δ. The model uses six parameters in total: the force of infection from mosquitoes to mosquitoes ($\beta_H(t)$) and mosquitoes to humans (β_M), the intrinsic (α_H) and extrinsic incubation period (α_M), the recovery from infection period in humans (γ), and the birth rate of mosquitoes (δ) which was equal to the death rate to keep mosquito density constant. The force of infection to humans varied over time due to seasonal variation in transmission (details below). All other parameters in the model were constant over time. The full model was defined as follows:

The next generation matrix for humans and vectors was defined as follows (*Kucharski*

et al., 2018; *Manore et al.*, 2014):

$$\begin{pmatrix} R_{HH} & R_{HM} \\ R_{MH} & R_{MM} \end{pmatrix} = \begin{pmatrix} 0 & \frac{S_h}{N}\frac{\beta_H(t)}{\delta}\frac{\alpha_M}{\delta+\alpha_M} \\ \frac{s_M\beta_M}{\gamma} & 0 \end{pmatrix} \tag{6.9}$$

The effective reproduction number, R, was equal to the dominant eigenvalue of this matrix. The basic reproduction, R_0, was calculated by the same method, but assuming that both humans and vectors were fully susceptible.

6.2.4 Climate and control effect on transmission

The effect of seasonal variation in temperature was introduced to the model directly into the transmission rate as has previously been described in Chapters 2 and 5. The transmission rate at time t was dependent on a baseline mosquito-to-human transmission rate β_H and sinusoidal forcing defined by an amplitude (β_{amp}) and a midpoint (β_{mid}) as given in Equation 6.10.

$$\beta_H(t) = \beta_H\left(1 + \beta_{amp}\sin\left(2\pi\left(t + \beta_{mid}\right)\right)\right) \tag{6.10}$$

Similarly, I modelled the 'control effect' – any additional reduction in transmission during vector control intervention campaigns – as a direct effect on the transmission rate. Unlike in Chapter 5 the transmission rate fixes at the reduced value for the duration of this outbreak because the study period is short and a single season. As in Chapter 5 the transmission rate could gradually reduce over time with the reduction centred around time β_{centre}. The total reduction in transmission rate (β_{base}) was defined as a proportion between 0 and 1. The steepness of the decline in transmission rate was defined by the parameter β_{grad}. The 'control effect' on transmission was defined as follows:

$$\beta_H(t) = \beta_H \left(1 - \frac{\beta_{base}}{1 + \exp\left(-\beta_{grad}\left(\frac{t-\beta_{centre}}{365.25}\right)\right)}\right) \tag{6.11}$$

6.2.5 Model fitting

The model fitting process I used has previously been described (Chapters 2 and 5) (*Kucharski et al.*, 2016, 2018). Briefly, the model was jointly fitted to case and serological data using adaptive MCMC with a Metropolis-Hastings algorithm. I used adaptive MCMC by adjusting the covariance matrix used to resample and obtain a target acceptance rate of 0.234 (*Roberts and Rosenthal*, 2009).

6.2.6 Assessing model forecasts performance

During the outbreak, I assessed model performance visually by comparing previous forecasts with newly available data. When I performed a retrospective analysis of model performance I used three metrics of forecast performance – calibration, sharpness and bias (*Funk et al.*, 2019). I judged our best performing forecast to be the one that maximises the sharpness of predictive distributions subject to calibration (*Gneiting*, 2008). I therefore calculated the calibration of forecasts before assessing sharpness and bias. I compared three types of prediction model to identify which component led to the greatest improvement in forecast performance. I started with our null ('naïve') model where I assumed that the entire population was susceptible to DENV-2 and fitted to surveillance data only. I then fitted to our pre-outbreak DENV-2 seroprevalence estimate as well as the surveillance data. Finally, I jointly fitted to both the 2017 DENV-2 epidemic and the 2013-14 DENV-3 epidemic.

Calibration

Calibration of forecasts is the ability of a forecast to correctly identify its own uncertainty in making predictions. I used data up to a specific fixed point in the outbreak

and then used these model fits to forecast at weekly horizons up to 12 weeks. For each forecast horizon, at time t I took bootstrap samples of the estimated weekly incidence from our *a posteriori* model fits and derived the empirical cumulative probability distribution P_t. I then calculated the calibration at time t, u_t, according to the formula:

$$u_t = P_t(k_t) + \nu \left(P_t(k_t) - P_t(k_t - 1)\right) \tag{6.12}$$

This estimates the density of the data point at time t from the empirical probability distribution defined by simulations from the forecast. Where k_t is the observed data at time t, and ν is the standard uniform distribution. I took a random draw from a uniform distribution between the probabilities at k_t and $k_t - 1$ to adjust for the discretised cumulative probability distribution when handling count data. I calculated u_t for weekly forecast horizon from 1 to 12 weeks after the data used to fit the model.

I obtained a distribution of u_t from a bootstrap sample from the posterior of model forecasts. If a model is perfectly calibrated then the observed data at each time point will look as if they came from P_t at that time point. Therefore, if P_t is the true cumulative probability distribution, then u_t is standard uniform (*Czado et al.*, 2009). I tested whether u_t followed a standard uniform distribution using the Anderson-Darling test of uniformities. I used the function `ad.test` from the package `goftest` to perform the test. The p-value from this test was used as evidence of good or poor calibration. I considered that there was no evidence to suggest a forecasting model was miscalibrated if the p-value found was greater than a threshold of $p \geq 0.1$, some evidence that it was miscalibrated if $0.01 < p < 0.1$, and good evidence that it was miscalibrated if $p \leq 0.01$ (*Funk et al.*, 2019).

Sharpness

After assessing the calibration of the models I wanted to find the model that maximised sharpness and reduced bias. Sharpness is a data-independent measure and is the ability of the model to generate predictions within a narrow range of possible outcomes. I eval-

uated the sharpness at time t, by calculating the normalised median absolute deviation about the median of y:

$$S_t(P_t) = \frac{1}{0.675}\text{median}(|y - \text{median}(y)|) \tag{6.13}$$

where y is a variable with CDF P_t. The sharpest model would focus all forecasts on one point and have $S = 0$, whereas a completely blurred forecast would have $S \to \infty$.

Bias

I evaluated bias at time t as:

$$B_t(P_t, x_t) = 1 - (P_t(x_t) + P_t(x_t - 1)) \tag{6.14}$$

Where x_t is the observed data point at time t. The least biased model would have $B_t = 0$, whereas a completely biased model would systematically over-predict ($B_t = 1$) or under-predict ($B_t = -1$) the data.

6.3 Results

6.3.1 DENV-2 transmission was low and ended by August 2017 as forecast in real-time

There were 755 cases of DENV-2 confirmed by detection of RT-PCR, NS1 or IgM antibodies in the medical subdivision of Suva and neighbouring Nausori between January and September 2017 (Figure 6.6). The population of Suva, Nasinu and Nausori is estimated as 243,795 (*Fiji Bureau of Statistics*, 2018a). The epidemic peaked in May with 61 confirmed cases in one week. 497 cases (65.8%) were positive for NS1 antigen and 258 (34.2%) were confirmed ELISA positive for anti-DENV IgM antibodies.

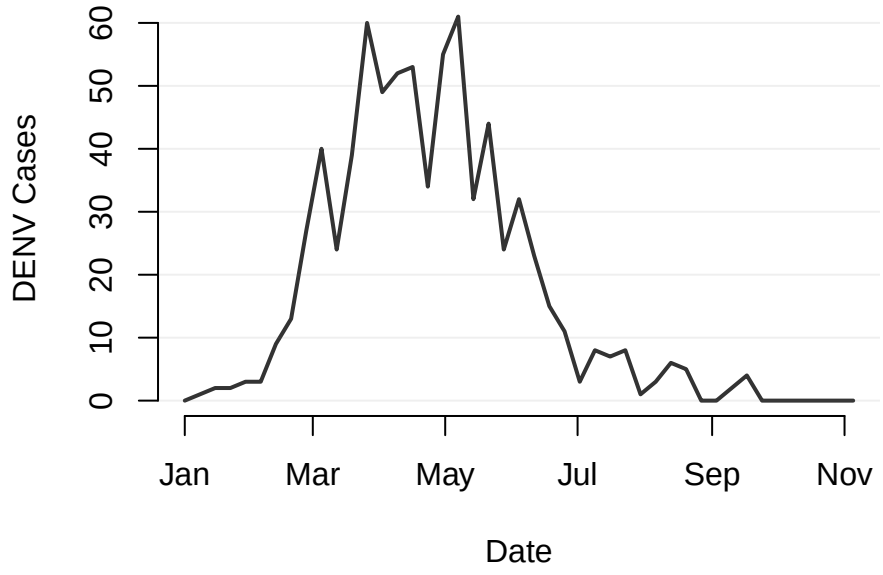

Figure 6.6: *Confirmed weekly DENV-2 cases during the 2017 epidemic*

I arrived in Fiji on 1st May 2017, by which point data were available up to the end of March. There were 223 confirmed cases (29.5% of the total outbreak) in the final data set released after the outbreak. However, the real-time data used to forecast the outbreak had a slightly higher incidence and more variable trajectory, a comparison of the real-time and post-outbreak case data is shown in Figure 6.7. A combination of testing capacity and repeated tests led to this discrepancy. Real-time data recorded more suspected cases at that point of the outbreak with 299 suspected cases between January and March 2017.

There was concern in early May 2017 that this outbreak could increase rapidly and cause a similar disease burden as the DENV-3 outbreak in 2013-14. This concern was valid especially when taking into account the serological data collected from Fiji. In November 2015, sera collected from 390 participants estimated that DENV-2 seroprevalence was 15.3% (95% CI: 12.6–19%) so there was a large proportion of the population susceptible to DENV-2. In November 2013, at the start of the DENV-3 outbreak, estimated DENV-3 seroprevalence was 45.3% (95% CI: 40-51%), so population immunity

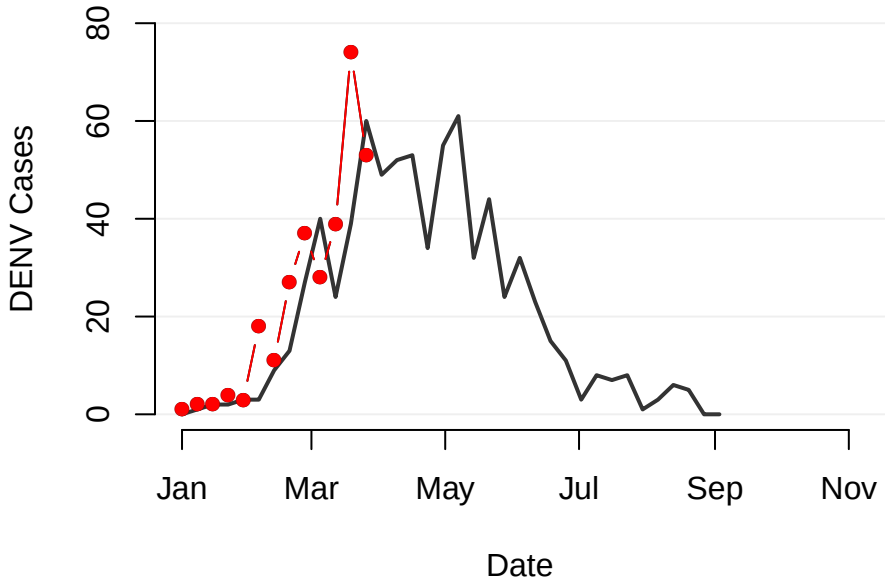

Figure 6.7: *Comparison of available DENV-2 data. Both lines show DENV-2 case data during the 2017 epidemic. Red line and dots shows the epidemic case data that was available in real-time. After the outbreak some data were revised and the final epidemic curve for confirmed cases is shown with the black line*

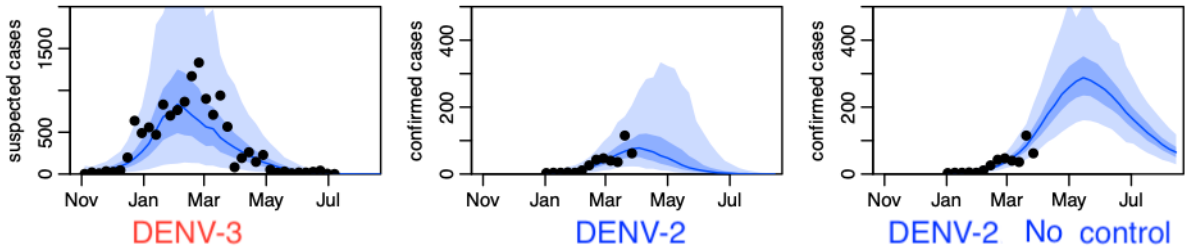

Figure 6.8: *DENV model predictions provided to Fiji MOH during the DENV-2 on 12th May 2017. Left panel: black dots, DENV-3 2013-14 case data. Blue line and region, model projections with median (blue line) 95% CrI (light region) and 50% CrI (dark region). Middle panel: black dots, real time DENV-2 2017 case data up to 8th March 2017. Blue line and region, model projection as in left panel with seasonal forcing and additional 'control effect'. Right panel: black dots, case data as in middle panel. Blue line and region, model projection as in middle panel but without any 'control effect' (assumed to be 50% at time of analysis). All model projections include seasonal forcing.*

to DENV-2 in May 2017 was lower than DENV-3 immunity before a large outbreak in November 2013.

Together with my supervisor, Dr. Adam Kucharski, who was in Fiji with me at the time to set up the serological survey, we began modelling the outbreak by jointly fitting the available data in 2017 and the whole 2013-14 DENV-3 outbreak. We assumed that some of the drivers of transmission were constant across the two outbreaks, primarily parameters controlling the effect of climate on DENV transmission. On 12$^{\text{th}}$ of May 2017 we presented projections of DENV forecasts to the Ministry of Health (Figure 6.8). We found good evidence that this outbreak was unlikely to grow rapidly, total weekly cases were not likely to exceed 200, and that the outbreak would have ceased by August. We found that, because of the decline in temperature in Fiji between April and August (Figure 6.9) and the effect this had on DENV transmission in 2013-14, continued circulation of DENV-2 beyond July was unlikely.

I also analysed the effect of control interventions in real-time on the assumption that interventions in 2017 were similarly effective to those implemented in 2013-14. I assumed that interventions reduced transmission by 50% and was able to show that the epidemic would be shorter with a lower peak (Figure 6.8 middle panel) than if no interventions

were applied (Figure 6.8 right panel). Even in the absence of interventions the 2017 outbreak was forecast to be smaller than the DENV-3 epidemic had been.

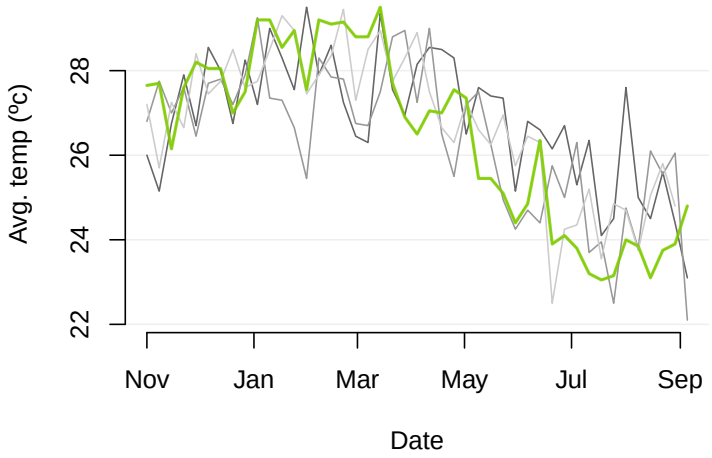

Figure 6.9: *Average daily temperature in Suva, Fiji. Green line, daily temperature between November 2013 and October 2014. Grey lines, average daily temperature in 2012-13, 2011-12 and 2010-11*

6.3.2 The forecast could have been accurate only using data up to March 2017

After the 2017 outbreak I was able to assess the performance of our forecast. Visually comparing our forecast to the final data there was good agreement between the model projections and the final epidemic (Figure 6.10). The projection made in May 2017 was broadly accurate in terms of the number and timing of peak cases and duration of the outbreak. Incident DENV-2 cases slowed rapidly in June as predicted. The epidemic did have a longer tail than expected with cases reported as late as September 2017.

I wanted to analyse which aspect of our modelling approach led to the success of the forecast. Then, using the best fitting model structure, I wanted to analyse how early in the epidemic we could have made an accurate forecast on the trajectory of the epidemic. I compared three versions of the same model for forecasting the epidemic and used data

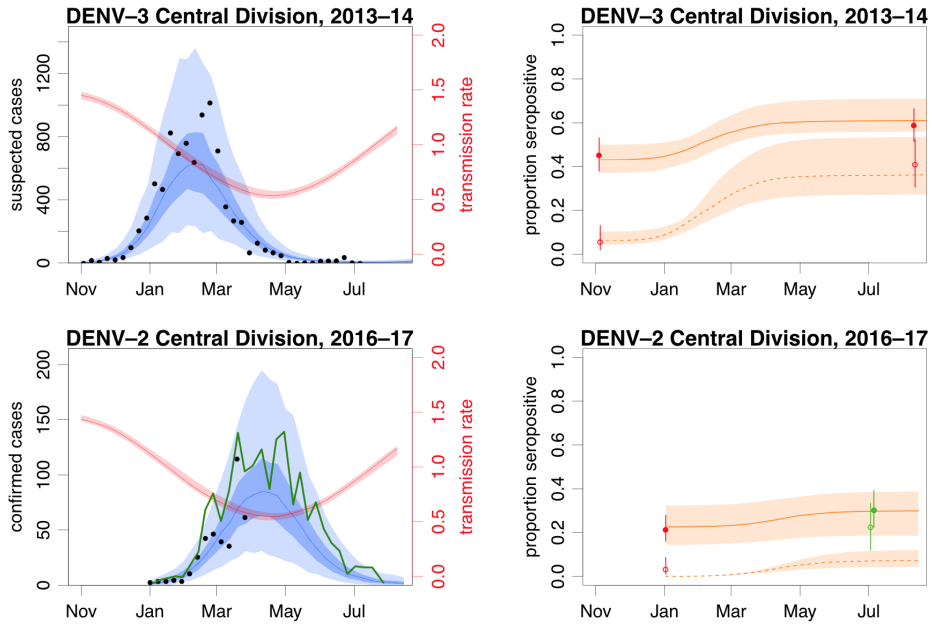

Figure 6.10: *Comparison of real-time model forecasts with final outbreak data. (Joint work with Dr. Adam Kucharski and this forecast was presented to public health officials on 12th May 2017). Top row, 2013-14 DENV-3 epidemic. Bottom row, 2017 DENV-2 epidemic. Black dots, weekly case data. Blue line, median model projected cases. Blue region, 95% CrI (light region) and 50% CrI (dark region). Red region, estimated seasonal forcing of transmission. In this model we estimated that DENV transmission intensity peaked before temperature peaked in Fiji. Red dots and lines, estimated seroprevalence and 95% CI from serological surveys conducted in November 2013, November 2015 and June 2017 included in model fitting. Green dot and lines, estimated seroprevalence and 95% CI presented for reference but not included in model fitting. Orange line, median estimated seroprevalence from the model. Orange region, 95% CrI for estimated seroprevalence from the model*

up to 8th March 2017 for all three models to forecast the epidemic. Firstly, a null (or 'naïve') model, as described in the methods section but assuming that we have no information on prior immunity and the whole population is susceptible. This first model was fitted to surveillance case data only. Secondly, the same model but fitted to both surveillance case data and the estimated pre-outbreak DENV-2 seroprevalence in Fiji. This should improve the estimate of the proportion of the population that were susceptible to DENV-2 at the start of the outbreak. Finally, I fitted the model to both 2017 DENV-2 cases and serological data and also jointly fitted this with the 2013-14 DENV-3 epidemic. For this third model I included pre- and post-outbreak

seroprevalence estimates for DENV-3 but only pre-outbreak DENV-2 seroprevalence when model fitting.

The results from the null model show a forecast of a very large outbreak with post-outbreak seroprevalence above 80% (Figure 6.11A). In the second model, even with a pre-outbreak seroprevalence estimate we would still project a large outbreak if we only included 2017 data (Figure 6.11B). In this second model a smaller proportion of the population can get infected compared to the null model because an estimated proportion are immune before the outbreak begins. The increase in suspected cases up to March 8th 2017 therefore suggests that the virus is more easily transmitted than in the null model assuming total susceptibility. This higher estimated transmission in the early stages of the outbreak then leads to an overestimate of the peak cases in April in the forecast. Neither of these first two forecasts are particularly accurate from a visual comparison to the data. In the final model however, by incorporating information from the 2013-14 DENV-3 epidemic the forecast would accurately project a small outbreak and accurately predict the peak cases, peak timing and timing of the end of the outbreak (Figure 6.11C). I used an MCMC sampler where the total likelihood for each iteration was the sum of the likelihood from the 2013-14 DENV-3 epidemic and 2017 DENV-2 epidemic. In this joint fitted model, information from both outbreaks was therefore used to estimate parameters that were constant across both outbreaks, such as the seasonality and baseline transmission rate.

Even from a visual comparison it is clear that the third model provided a more accurate forecast. However I also used three metrics to compare the forecast performance: calibration, sharpness and bias. I compared these metrics for the three models using forecast horizons up to 12 weeks. I found that the forecast which jointly fitted to both DENV outbreaks preformed much better. The naïve model and joint fitted models were both well calibrated – p-values greater than 0.1 – up to approximately a 2 month forecast horizon (Figure 6.11D). However, the joint fitted model was sharper, which measures the ability of the model to generate predictions within a narrow range of possible outcomes, with values closer to 0 across all forecast horizons (Figure 6.11E). Finally, the joint fitted was the least biased forecast with values closer to 0 whereas both

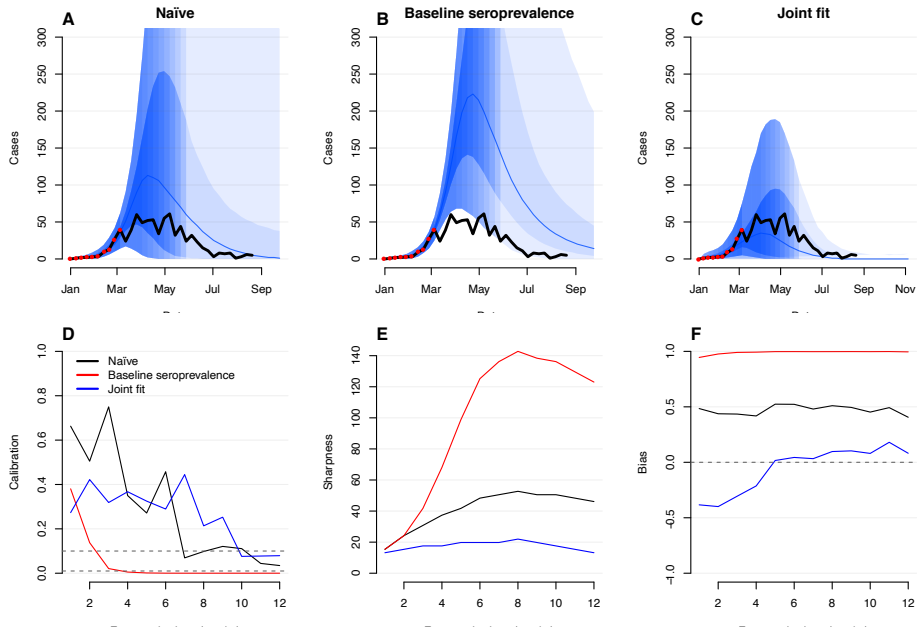

Figure 6.11: *Assessment of model forecast performance using three models. A, a null model assuming the whole population is susceptible to DENV-2 infection. B, model fitted to pre-outbreak seroprevalence as well as surveillance data. C, Model jointly fitted to the 2017 DENV-2 and 2013-14 DENV-3 epidemics including fitting to seroprevalence estimates. Red dots, DENV-2 case data used in model fitting. Black line, complete 2017 DENV-3 epidemic case data. Blue line, median model projected cases. Light region, 95% CrI. Darker region, 50% CrI. Gradient of blue regions shows weekly projections from the end of the available case data in model fitting up to the 12 week forecast horizon used in assessing model performance. D, calibration - p values from a test of uniformity of the calibration at increasing forecast horizons from 8th March (1-12 weeks). Lower p-value shows more evidence against the null hypothesis that the model is well calibrated. E, sharpness - the ability of the model to generate predictions within a narrow range of possible outcomes. Lower values are preferable. F, bias - unbiased model with have a value of 0. D, E, and F. Black lines, null model. Red line, model including pre-outbreak seroprevalence. Blue line, model jointly-fitted with DENV-3 2013-14 epidemic*

of the simpler models systematically over-predicted the case burden (Figure 6.11F)

Having found that the joint-fitting model had superior forecast performance I wanted to analyse the performance of this model with varying lead times for data availability. My objective was to evaluate how early in the outbreak we could have provided a reliable forecast of the 2017 DENV-2 outbreak. The epidemic take-off was slow in 2017.

Two months after the first confirmed case, at the end of February, there were only 68 confirmed cases of DENV-2 (Figure 6.6). This slow emergence of the epidemic meant that using data available up to February, we would have predicted that there would likely be no epidemic (Figure 6.12A). In March the epidemic started to increase and our forecast improved. Using data available up to 1st March 2017 we overestimated the total size of the epidemic but accurately predicted that cases would decline by July with good confidence (Figure 6.12B). As more data became available from April through June, the overall conclusions of the forecasts did not change substantially but the credible intervals narrowed giving a more precise estimate. (Figure 6.12).

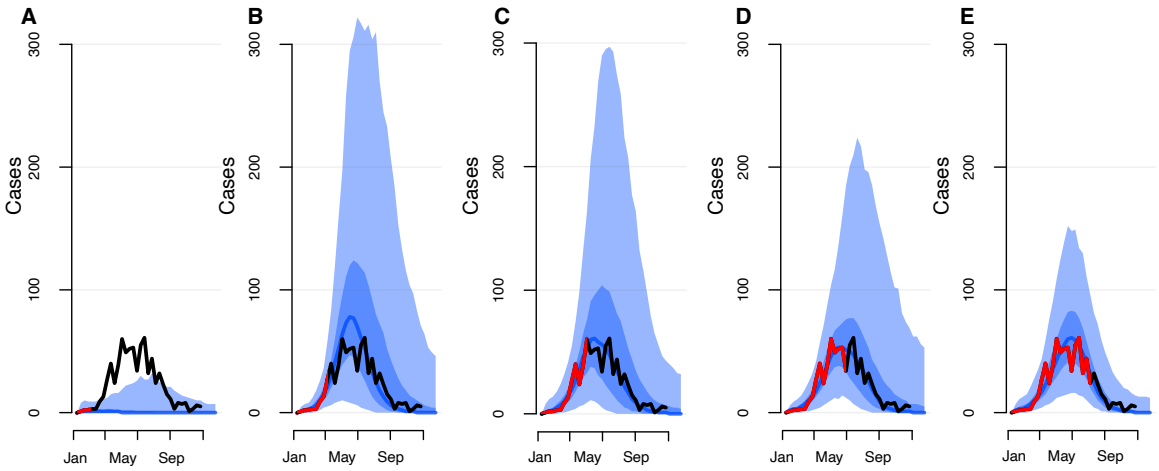

Figure 6.12: *Model forecast performance with increasing data used in model fitting. Model forecasts used data up to 1st February 2017 (A) then 1st March (B), April (C), May (D), June(E). Red line, DENV-2 case data used in model fitting. Black line, complete DENV-2 outbreak case data. Blue line, median model projection. Light region, 95% CrI. Dark region, 50% CrI*

6.3.3 Summary of data available to assess interventions

When we were modelling the outbreak in real-time we provided two forecasts, one that assumed no additional reduction in transmission due to interventions, and another that assumed a 50% reduction (Figure 6.8). In the analysis of forecast performance I removed any additional effect of interventions on transmission for simplicity. To better analyse the role of public health interventions I collected detailed data on incidence,

seroprevalence and entomological interventions during the DENV-2 outbreak from the capital of Fiji, Suva, and neighbouring regions Nasinu and Nausori. I combined surveillance and serological data with information on entomological interventions to model outbreak dynamics in each of these five regions.

In follow-up serological studies conducted in November 2015 and June 2017 we were able to recontact and recruit participants from all five regions for another sample collection. In 2015 there were 153 samples collected from Suva, Nasinu and Nausori, and in 2017 we collected 123 samples. 82 participants were sampled in both years. Samples were available at both time points from all regions. The sample size in Nasinu was the largest in our study with 128 samples across both time-points, and it also saw a large estimated increase in DENV-2 seroprevalence (as measured by MIA) between collection dates from 18% (95% CI: 11-29%) to 35% (95% CI: 22-49%). The seroprevalence pattern in CWM is hard to estimate because only 6 samples were collected from this region in 2015 and all were negative. Post-outbreak seroprevalence in CWM was comparable to the rest of Suva. Estimates were also uncertain in Raiwaqa with the smallest sample size of 31 over both time periods (Table 6.1).

Table 6.1: *Number of participants who were seropositive to DENV-2 as measured by MIA in 2015 and 2017, divided by study area regions*

	2015			2017		
Region	Positive	N	% (95% CI)	Positive	N	% (95% CI)
CWM	0	6	NA	4	17	24 (7.8–50)
Nasinu	14	76	18 (11–29)	18	52	35 (22–49)
Nuffield	3	28	11 (2.8–29)	4	15	27 (8.9–55)
Raiwaqa	4	15	27 (8.9–55)	4	16	25 (8.3–53)
Samabula	5	28	18 (6.8–38)	5	23	22 (8.3–44)

The location of cases in the data were defined according to the location of the health centre/hospital where the case was reported. Unsurprisingly, most cases reported at the largest hospital in Suva, CWM. The second largest case burden was in Nasinu/Nausori and there were very few cases in Nuffield. In both Raiwaqa and Samabula, the more

residential areas of Suva city, the case burden was small but the epidemic curve was well defined in surveillance data. Overall, we had a detailed description of the outbreak within each of the five study regions, allowing us to model the DENV-2 outbreak for the area as a whole and in each of the five regions defined in this section (Figure 6.13).

6.3.4 Climate and herd immunity were not enough to recreate epidemic dynamics

I modelled DENV transmission in Suva, Nasinu and Nausori by jointly fitting the DENV transmission model to both 2017 DENV-2 surveillance and serological data, and the 2013-14 DENV-3 outbreak. This model was able to reproduce the transmission dynamics of DENV-2 in Suva, Nasinu and Nausori in 2017, and of DENV-3 in Central Division in 2013-14 (Figure 6.14). For the 2017 DENV-2 outbreak the model captures the dynamics of the outbreak well in terms of peak cases, peak timing and duration of outbreak. The basic reproduction number (R_0) was variable across the calendar year because of the seasonal forcing in the model so I used the median R_0 over one year to compare values. Median R_0 values were similar for both outbreaks, 1.4 (95% CrI: 0.93-1.78) for DENV-2 and 1.5 (95% CrI: 0.94-1.92) for DENV-3.

To capture any potential effect of vector control interventions the model included a flexible function to capture any additional reduction in transmission separate from immunity dynamics and seasonal forcing. The effect of interventions on transmission was weaker in 2017 than it had been in 2013-2014 (Figure 6.14B and D). However, even though our estimate for relative reduction in transmission was less precise in 2017 I still found strong evidence of a non-zero effect. The estimated reduction in transmission after interventions in 2017 was 36% (95% CrI: 31-42%). For the 2013-14 DENV-3 outbreak the estimated reduction in transmission from this model was 71% (95% CrI: 68-75%). The dynamics of the 2013-14 DENV-3 and 2017 DENV-2 epidemics were different for several reasons. The DENV-3 epidemic was larger and peaked earlier in the year and had a smaller susceptible population at the start off the outbreak. This analysis suggests however that for both epidemics seasonal forcing in transmission

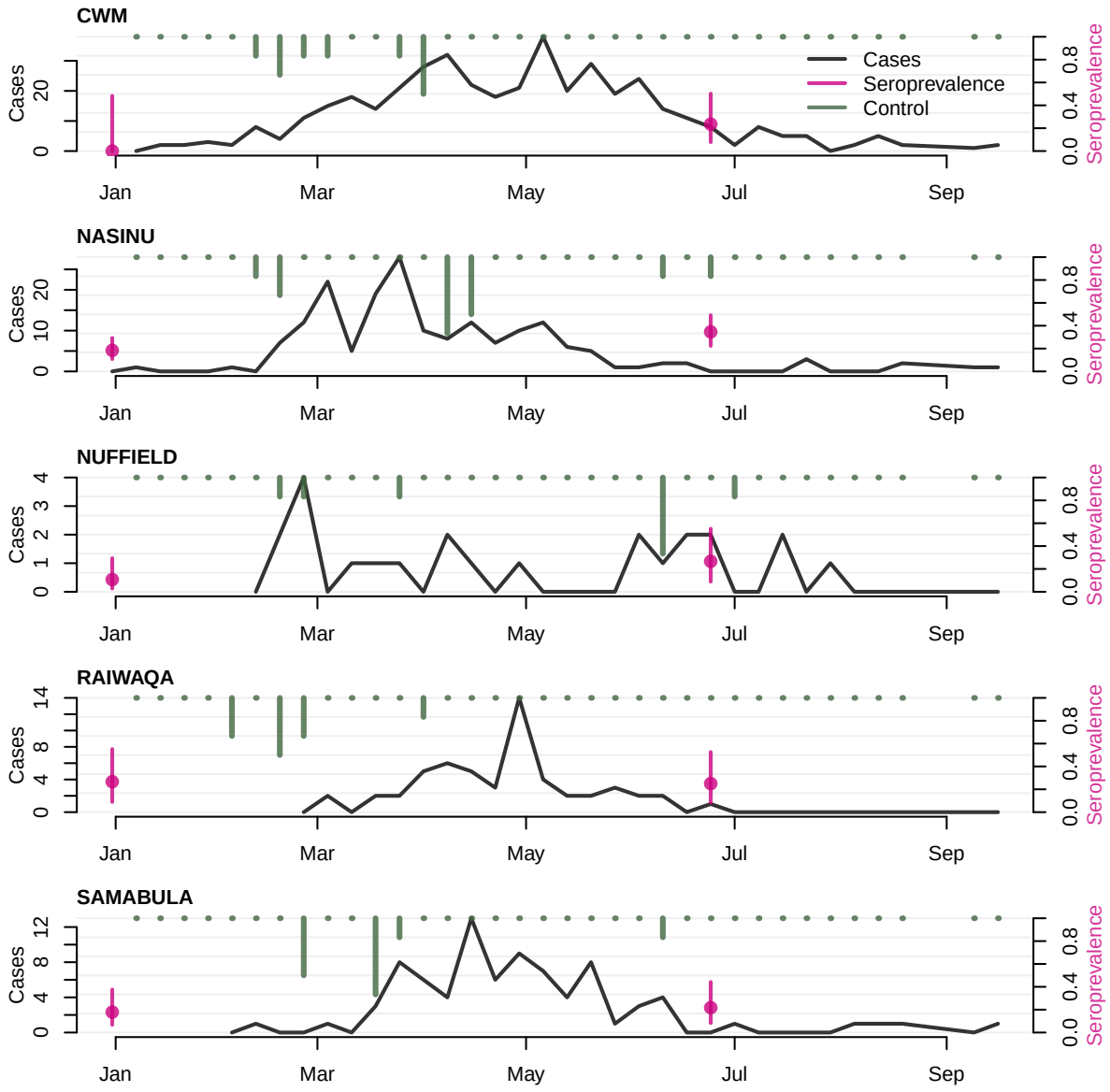

Figure 6.13: *Summary of all available data in each of the five study area regions. Black lines, weekly reported cases of DENV-2 during the 2017 outbreak. Pink dots and lines, estimated DENV-2 seroprevalence as measured by MIA in each region and 95% CI before the outbreak (samples collected in November 2015) and at the tail end of the outbreak (June 2017). Green lines indicate timing of vector control interventions in each region*

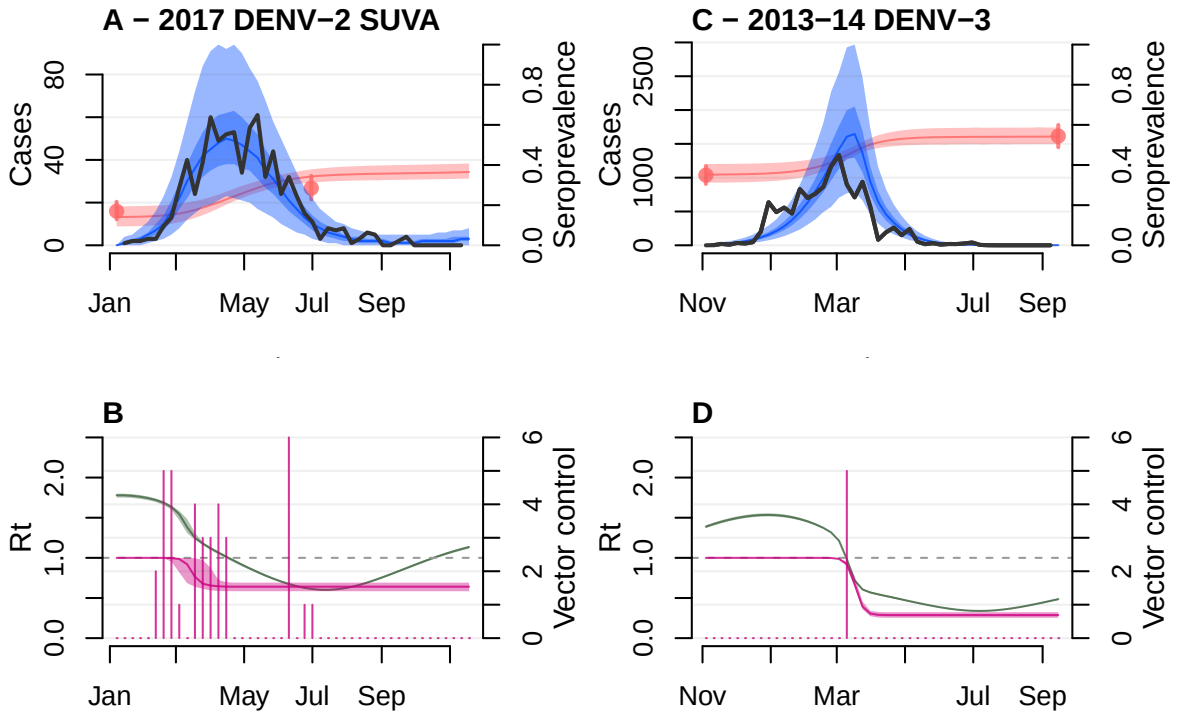

Figure 6.14: *Impact of climate and control measures on DENV transmission during the 2017 DENV-2 outbreak (A, B) and 2013-14 DENV-3 outbreak (C, D), using a model that was jointly fitted to both outbreaks. (A, C) Black lines, weekly reported DENV case totals. (A, C) Blue line and region, median estimate from the jointly fitted model, 95% CrI (light region), and 50% CrI (dark region). (B, D) Green line and region, estimates of the effective reproduction number (R_t). Pink line and region, estimated additional reduction in transmission potentially as a result of vector control interventions which are shown with vertical pink lines*

intensity and increase in immunity during the outbreak were insufficient to fully explain observed outbreak dynamics and an additional reduction in transmission better fits the observed data.

I estimated the start of the reduction in transmission for the 2017 DENV-2 epidemic. I put no constraints on this parameter because I wanted to analyse whether a reduction in transmission coincided with the actual interventions over this period. I estimated that the central point of the 'control effect' was 68 days (95% CrI: 56-87) after the start of the outbreak. This means the estimated reduction in transmission in the model is centred around early March 2017, which is between the two rounds of interventions during the outbreak (Figure 6.14B). For the 2013-14 DENV-3 outbreak I limited the start of any reduction in transmission to be after the clean-up campaign in March 2014 (Figure 6.14D).

I compared this model for Suva with another model without a 'control effect' using the Deviance Information Criteria (DIC). A difference in DIC of >10 was considered as evidence that the model with the lower DIC was better. A difference between 5 and 10 was considered borderline evidence and any difference less than 5 was considered as no evidence that the models performed differently. The model without a 'control effect' (Figure 6.15) had a higher DIC (1003) than the model with control (851). This suggests that the inclusion of an additional reduction in transmission improved the model fit. Without this additional 'control effect', the estimated peak of cases in Suva in 2017 is later than the observed reported cases and the estimated seroprevalence post-outbreak is greater than that observed in our study (Figure 6.15A). This model comparison provides further evidence that, at a regional level, the additional reduction in transmission on top of herd immunity and seasonal climate effects better captures the observed transmission of the 2017 DENV-2 outbreak.

I estimated five parameters in the better performing model with a 'control effect' (Figure 6.14). I used multiple MCMC chains and all five parameter values converged such that parameter estimates were normally distributed (Figure 6.16). From this model I estimated that 14% (95% CrI: 9-19%) of Suva were DENV-2 immune at the start of the outbreak, slightly lower than the estimate from serological data, 17% (95% CI:

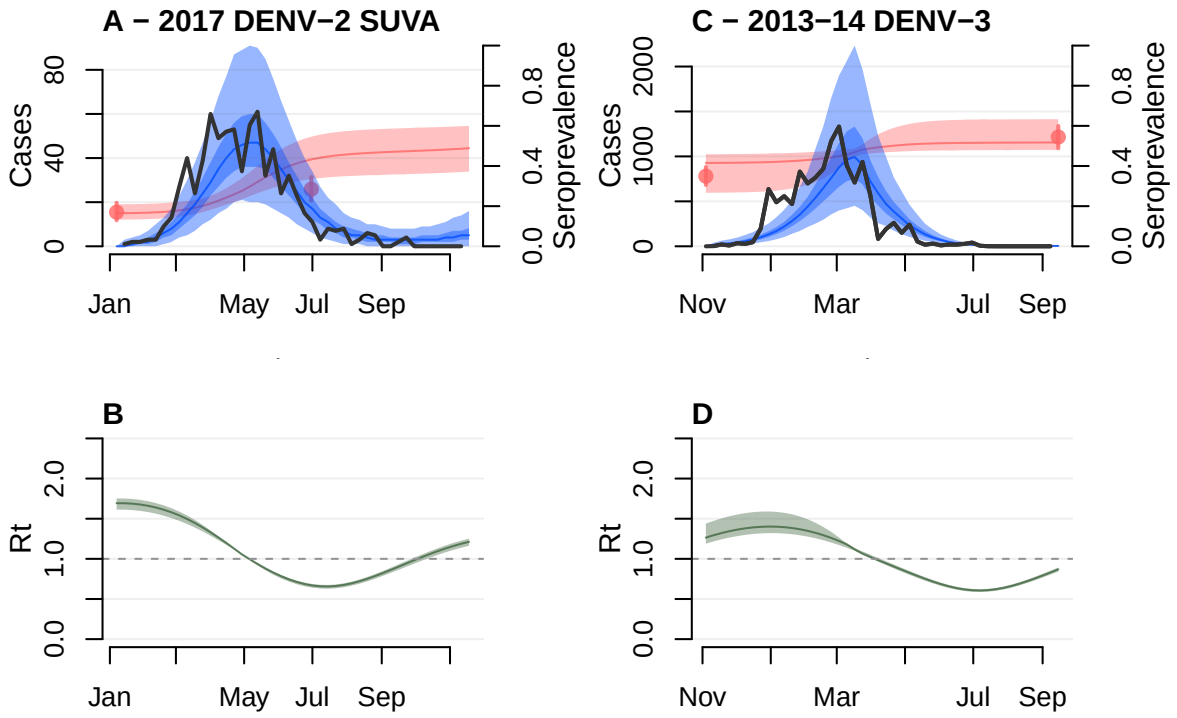

Figure 6.15: *Estimated DENV transmission dynamics without any additional reduction in transmission during the 2017 DENV-2 outbreak (A, B) and 2013-14 DENV-3 outbreak (C, D), using a model that was jointly fitted to both outbreaks. (A, C) Black lines, weekly reported DENV case totals. (A, C) Blue line and region, median estimate from the jointly fitted model, 95% CrI (light region), and 50% CrI (dark region). (B, D) Green line and region, estimates of the effective reproduction number (R_t)*

13-22%). I also estimated a lower reporting rate in 2017 of 1.8% (95% CrI: 1.2-2.5%) compared to 2013-14 DENV-3, 24% (95% CrI: 17-40%).

6.3.5 There was no evidence that more interventions led to different transmission dynamics between local regions

There was a lot of variability in the timing and intensity of vector control interventions across the five regions of our study site. All regions were covered as part of the first round of chemical spraying in February, but some were targeted more heavily than others such as Raiwaqa which had 7 different spraying events before the end of February.

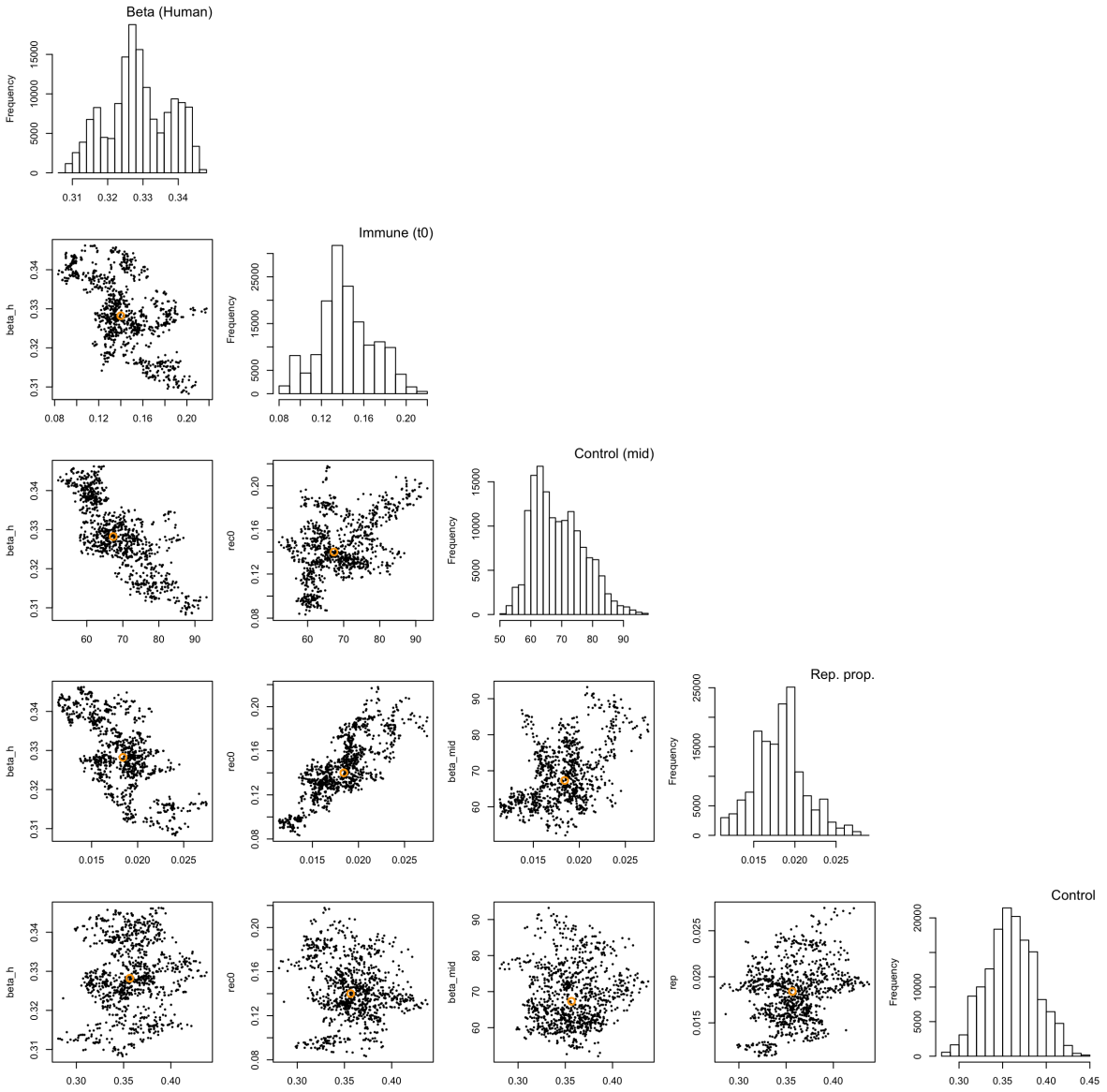

Figure 6.16: *Correlation between estimated parameters from full DENV transmission model. The transmission rate was a global parameter (the same value for the 2017 DENV-2 and 2013-14 DENV-3 epidemic). All other parameters are from the 2017 DENV-2 model only. Histograms show estimated parameter values*

By contrast, Nasinu saw more spraying in the second round in April compared to the February interventions (Figure 6.4).

Due to these heterogeneous intervention strategies, I analysed whether different intervention strategies were related to different transmission dynamics by running our model in each of the five regions. Population data for each region were estimated from Fiji census data collected in 2017. I fitted the model to outbreak data from the 2017 epidemic for each study region and the 2013-14 DENV-3 outbreak at the Division level using an MCMC sampler. The likelihood for each step of the MCMC was the sum of the likelihood for each study region and the DENV-3 outbreak.

I was able to recapture DENV transmission dynamics in all five regions in 2017 with this model, as well as the 2013-14 DENV-3 epidemic (Figure 6.17). The model was able to reproduce the observed temporal trend of cases and seroprevalence within each of the five regions for the 2017 DENV-2 outbreak (Figure 6.17A-E), however estimates were less precise than the previous model fitted to the region as a whole (Figure 6.14). Results were more precise in locations with more data available such as CWM and Nasinu. These results show that it was possible to recapture transmission dynamics in smaller areas with our model which included seasonal forcing on DENV transmission and a 'control effect' coinciding with interventions in each area.

The estimates for the effect of interventions on DENV transmission were imprecise. Even with the combination of surveillance and serological data and jointly fitting the 2013-14 DENV-3 outbreak, there was not enough data to accurately characterise the 'control effect' in each region (Figure 6.18). There was a stronger signal for reduced transmission in Nasinu (Figure 6.18B) and Raiwaqa (Figure 6.18D). In both these areas I estimated a $> 50\%$ reduction in transmission when interventions were implemented. There was no evidence of an additional reduction in transmission due to a 'control effect' in CWM, the area with the most interventions and most cases. This could be because CWM is the largest health centre in Suva and cases reported at this location were from infections in other locations, so interventions in this area will have less of an effect on reported cases in the area.

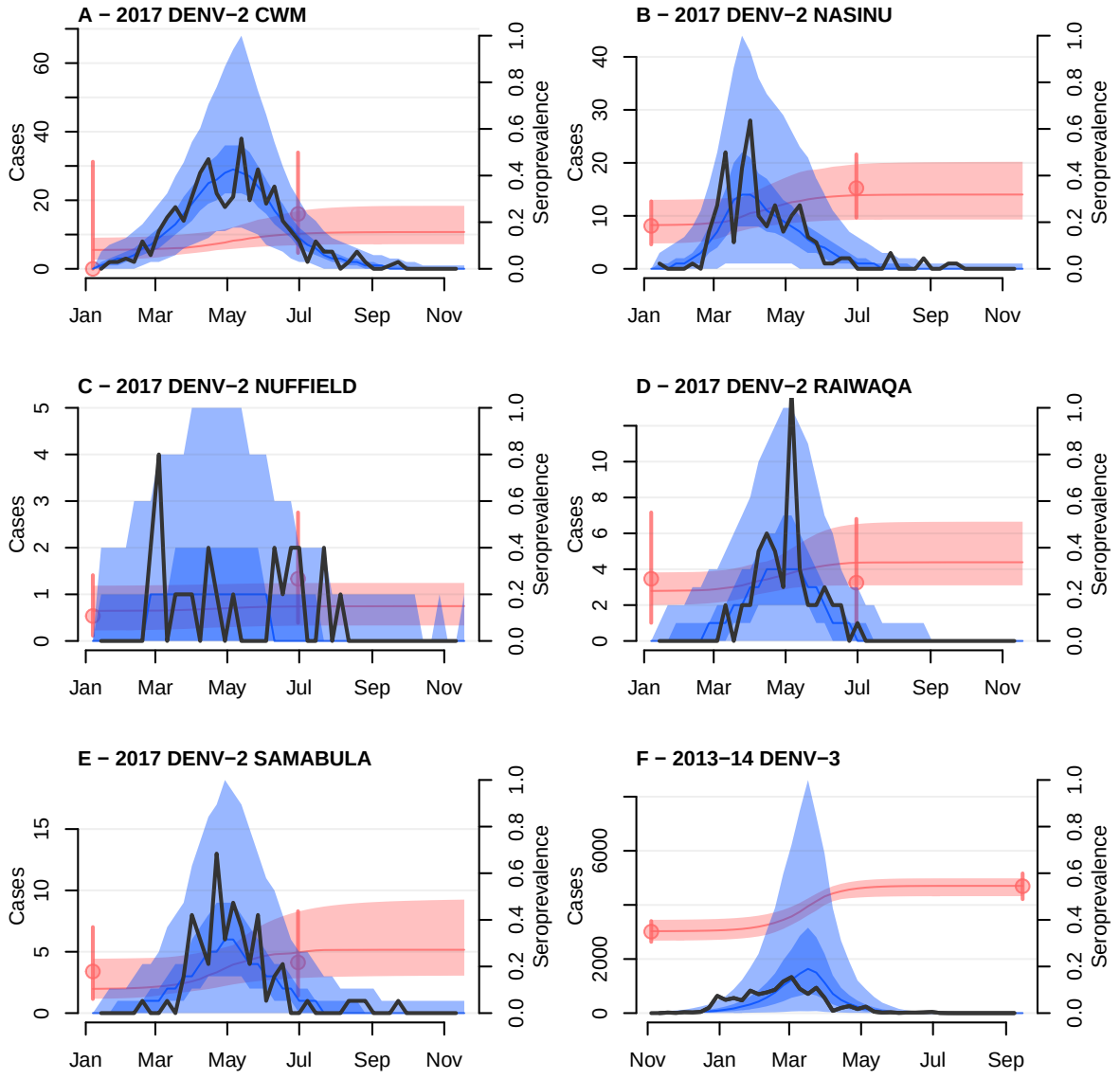

Figure 6.17: *Dynamics of DENV transmission in Central Division, Fiji. Transmission dynamics in five regions of Suva during the 2017 DENV-2 epidemic (A-E), and the whole region during the 2013-14 DENV-3 epidemic (F). Black lines, weekly reported DENV case totals. Blue line and region, median estimate of reported cases from the model, 95% CrI (light region), and 50% CrI (dark region). Red line and region, median and 95% CrI of estimated of seroprevalence from the model. Red dots and vertical lines, estimated seroprevalence from serological surveys conducted pre-outbreak and in June 2017*

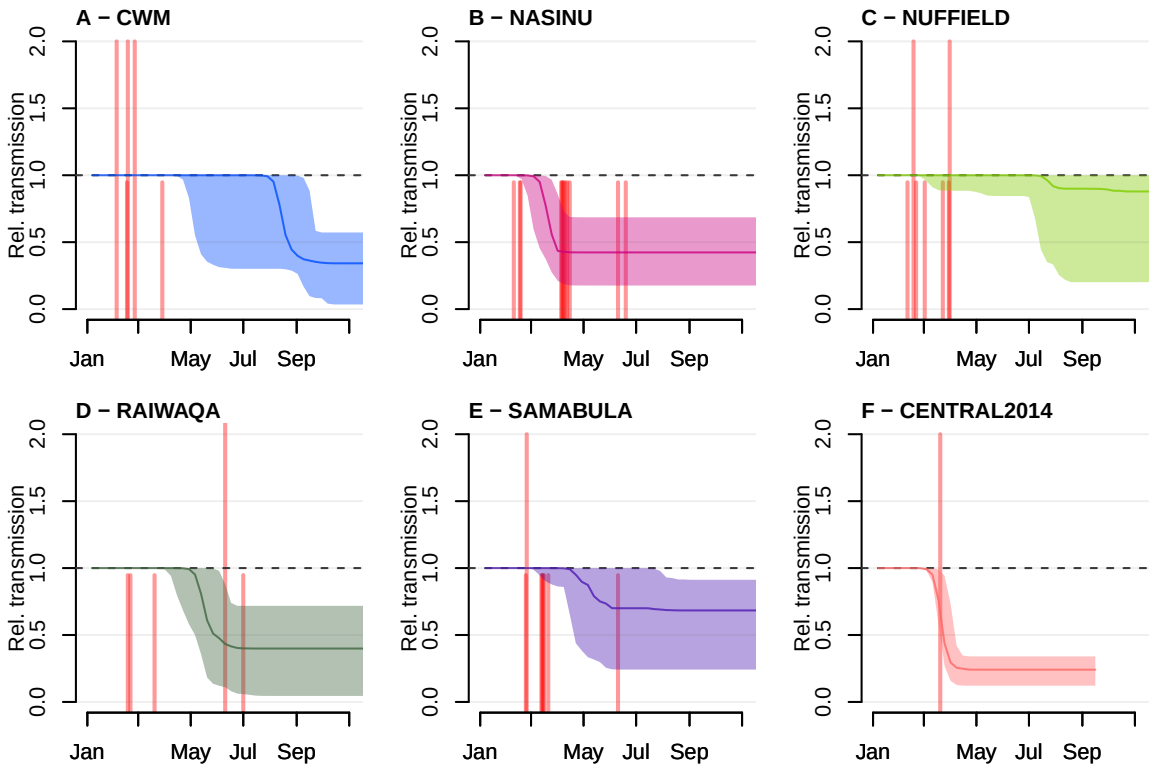

Figure 6.18: *Variable intervention strategies across the five regions which make up our study area. Coloured regions and line show the 95% CrI and median estimate of the 'control effect' (β_{base} in Equation 6.11). Transmission reduced relative to a baseline transmission rate according to these functions in the model (a value of 0.5 is equivalent to a 50% lower transmission rate at the corresponding time point). Vertical red lines show the number of vector control interventions in each region*

6.4 Discussion

In this study I analysed DENV outbreaks in 2013-14 and in 2017 by combining both surveillance and serological data. The same model was used for two purposes; forecasting outbreak dynamics in real-time and estimating the effect of interventions after the outbreak. I found that we were able to accurately recreate outbreak dynamics in terms of the number and timing of peak cases and the duration of the epidemic. As the 2017 outbreak emerged our model was able to accurately forecast the transmission dynamics before the peak of the epidemic had occurred by jointly fitting to the early case data, an estimate of pre-outbreak immunity and the full 2013-14 DENV-3 epidemic. This model was able to help in outbreak response and could be used in the same way for future

arbovirus outbreaks in Fiji. Additionally, using complete data after the DENV-2 outbreak I found that herd immunity and seasonal forcing of infection were not sufficient to explain the size and timing of the outbreak for both of the 2013-14 or 2017 DENV epidemics. I found evidence that an additional reduction in transmission was necessary to accurately recapture the transmission dynamics of the outbreak. However, I was unable to find conclusive results when analysing the relationship between interventions and reductions in transmission across the Fijian capital city of Suva.

With a model that can characterise a DENV outbreak in Fiji I was able to predict transmission dynamics accurately in real-time during the 2017 DENV-2 outbreak. The DENV-2 outbreak started in January 2017 and the final cases were recorded in September with 755 confirmed cases in total. Our model was able to accurately predict the scale and dynamics of the outbreak from as early as March 2017 when only 68 cases had been reported. Our model results were used during the outbreak to inform the response from the Ministry of Health to reduce uncertainty and fears that the outbreak would grow to the scale of previous large DENV outbreaks in Fiji.

After the outbreak I found that the increase in herd immunity and the decrease in transmission intensity as the temperature decreased in Fiji contributed to slowing the spread of DENV-2. However, by including an additional reduction in transmission in the model I was better able to recreate the 2017 DENV-2 transmission dynamics, both in terms of the scale of the outbreak and the timing of the decline in confirmed cases. These findings, combined with similar results from a modelling analysis of the 2013-14 DENV-3 outbreak, add to the very limited evidence base for the effectiveness of public health interventions in Fiji during arbovirus outbreaks. For this study I collected detailed data on incident cases, seroprevalence, and information on when and where interventions on the adult vector population were implemented. Despite this amount of data, I was unable to draw reliable conclusions when comparing transmission dynamics in the different regions using our model. This study highlights how difficult it is to accurately assess the effectiveness of interventions during an outbreak.

This study was heavily reliant on seroprevalence estimates in the Suva region, both when assessing the performance of the real-time forecast and when evaluating the role

of interventions in the outbreak. When looking at small regions within the Suva-Nausori region sample sizes were small and as a result some of the estimates of seroprevalence lack precision with wide confidence intervals. In addition, the timing of sera collection in June 2017 is not ideal with an ongoing outbreak and our assay tested for the presence of long term IgG antibodies against a variety of viruses. The potential for variable antibody responses during an outbreak introducing uncertainty into our seroprevalence estimates cannot be discounted. However, the model fitting should capture some of this uncertainty since I included the seroprevalence estimates in our likelihood with a binomial probability density. This means I was not reliant on a single point estimate and more precise seroprevalence estimates – such as for the whole Suva-Nausori region – will carry more weight in the model fitting than less precise estimates with wider confidence intervals and less information.

Modelling during an outbreak is inherently limited by the rapid evolution of outbreaks. People change their behaviours in response to an outbreak as they become aware of the danger directly through public health campaigns or indirectly through news reports (*Bausch and Edmunds*, 2018). This rapid evolution of outbreaks limits the conclusions I could draw from this model of the 2017 DENV-2 outbreak. I concluded that the outbreak was small – sera collected in June 2017 showed an estimated 28.9% (95% CI: 22-36.6%) of the population were seropositive – and that transmission slowed rapidly in June 2017 (Figure 6.14). However, it is hard to separate the mechanisms by which this was made possible. We know that herd immunity increased over the outbreak, that transmission likely slowed as temperatures declined and that an additional reduction in transmission best explains the observed dynamics. However, I was unable to separate the effect of several simultaneous interventions such as chemical spraying, health seeking behaviour in the community, people closing windows and wearing more insect repellent. A key limitation is therefore that I am not inferring a causal relationship between chemical spraying and a reduced burden of cases. Future research should be designed proactively to assess the performance of different interventions before a DENV outbreak in Fiji. This is especially important to get a baseline measure of effectiveness before new interventions are introduced such as *Wolbachia* infected mosquitoes that cannot transmit DENV when they bite a human (*Lambrechts et al.*, 2015).

In this study I chose a level of model simplicity that reflected the data we had available. I chose simple mechanisms to represent the effects of climate and interventions in the transmission model. It is well established that there is a strong relationship between temperature and DENV transmission (*Brady et al.*, 2014; *Descloux et al.*, 2012). In Suva, temperature typically fluctuates between a peak of 30°C and a low of 24°C (Figure 6.9) and recent research showed that maximal DENV transmission from *Aedes aegypti* occurs between 26-29°(*Mordecai et al.*, 2017). As a result, I used a simple sine wave to introduce seasonal forcing on DENV transmission. Similarly, because of the lack of evidence on the effectiveness of interventions on DENV transmission in Fiji I used a simple and flexible sigmoid function to capture any reduction in transmission and did not specify which interventions these could be from. This function assumes that any reduction occurs once, at a fixed rate to a diminished transmission rate from which it does not recover during the outbreak. A more complex model which more closely represents the reality of a mosquito-borne disease outbreak could deconstruct the baseline transmission rate β_H into component parts (*Funk et al.*, 2016; *Lourenço et al.*, 2017; *Pandey et al.*, 2013). However, I chose a more parsimonious approach due to the lack of robust entomological data in Fiji which would have made estimating more mosquito specific parameters a challenge. In both cases, these simpler mechanisms seemed justified at the expense of realism. This was especially true since real-time forecast results had to be communicated to an audience of decision makers mostly naïve to, and occasionally sceptical of, the benefits of modelling disease outbreaks. In the case of temperature and seasonal forcing, a more complex model incorporating daily changes in temperature and humidity could have produced a more detailed mechanism but the principle I wanted to capture was that transmission was not constant over the year and this was achieved with the sine curve. Our work on the impact of interventions demonstrates how hard the effectiveness of interventions is to measure at a population level, let alone when trying to compare different strategies.

This study was unable to draw conclusions about differing levels of effectiveness from heterogeneous intervention strategies in the Suva-Nausori region. The most likely limitation in our data was the use of health care facility reporting as the location of a case, not home address which was unavailable. As a result, the majority of cases (55%)

were located in CWM however it seems unlikely that all cases reported at CWM were infected in this region. This could potentially explain why I have a well-defined signal for the additional reduction in transmission for the Suva-Nausori region as a whole but not for individual regions within it. I was limited by ignoring the spatial component of this outbreak and consequently our model assumed that the walls between the 5 defined regions were barriers that disease could not cross. While it seems likely that infected mosquitoes would not travel far enough to blur these boundaries with a range of 200m (*Turell et al.*, 2005) it is likely that people – especially those seeking health care – would travel out of their home region to report in a neighbouring region. This highlights the need for more complete epidemiological data in routine surveillance, especially information on home residence and place of work or school so that it is possible to more accurately infer the likely location of infection in future studies.

There are limitations in generalising the findings from this study and the approach used. I clearly benefited from the availability of good quality representative serological data. The study setting of Fiji, as an island state in the South Pacific with epidemic outbreaks of arboviruses rather than ongoing endemic transmission, made characterising population immunity simpler. The potential for cross-reaction in serological assays if multiple virus were co-circulating at a high level would be greater in endemic regions. This feature of distinct invasive outbreaks makes island states such as Fiji valuable for research and hypothesis generation but limits the generalisability of conclusions from such studies.

Assessing this study post-outbreak I identified an area of clear missed opportunity which was to look at forecasting over a longer time period, even when the priority was the short-term course of the 2017 outbreak. I had the model and the ability to test whether re-emergence of DENV-2 was likely when temperatures increased. DENV transmission did continue into 2018 (*World Health Organisation*, 2018), and our model could have highlighted the risk of this happening which could have proved valuable. This highlights the need to focus on the long-term, even when forecasting an outbreak and preoccupied with the short-term predictions. As forecasting methods improve and the predictive ability of modelling improves, we should answer more interesting questions in real-time.

This study demonstrates the power of jointly fitting and combining data in model fitting. In terms of real-time modelling, the forecasts produced if we fitted to 2017 real-time data alone are much worse and estimate a larger outbreak. I included the 2013-14 outbreak in our model fitting and as a result borrowed information on certain common parameters from these data. Even within a single outbreak, the value of including the serological data is clear. An estimate of pre-outbreak population immunity sets realistic initial conditions for the model. Estimating post-outbreak seroprevalence gives a strong estimate of the size of the outbreak, which can improve parameter estimation in the model. The joint fitting and combination of data in our model were essential in establishing an accurate and timely prediction of the outbreak and in untangling different mechanisms that affected DENV transmission dynamics in post-outbreak analyses.

I collated a large amount of data for this study. I was able to estimate an additional reduction in transmission when vector control interventions were implemented when analysing Central Division. However, results on the effect of vector control interventions at a finer spatial scale were inconclusive. There is very little evidence available globally to demonstrate that current vector control tools such as fogging are effective at reducing DENV incidence (*Achee et al.*, 2015; *Bowman et al.*, 2016; *Heintze et al.*, 2007). Guidelines are available to design a study to fill this evidence gap and suggest that a cluster randomised control trial is an optimal study design (*Wolbers et al.*, 2012). These trials are expensive so I attempted to use modelling to analyse vector control interventions in Fiji. This study demonstrates that even with good quality data from multiple sources this is a very challenging application of modelling. The most notable omission from my study was spatial data on home locations of reported cases. Recent research has demonstrated that with fine scale spatial data on cases and interventions it is possible to estimate the effect of interventions on local DENV transmission (*Abdur Rehman et al.*, 2020). Modelling will be a valuable tool in improving DENV control (*Ferguson*, 2018) and it is important to test the effect of these interventions in real-world settings. This study implies a baseline of data requirements to conduct such a study. Data on seasonal factors, local immunity, infections, population size, and the timing and location of interventions were insufficient to produce a reliable estimate of the effect vector control. More accurate spatial data on the home location of cases is

a necessity, information on work location and mobility for these individuals would be beneficial. This is a large data burden but could still prove to be a more efficient route to improving DENV control in Fiji compared to establishing a large trial.

Outbreak response can clearly benefit from having a good model prediction at the start of the outbreak. In this study I demonstrated the value of embedding a modelling analysis in outbreak response to improve preparedness and most efficiently use resources. Working closely with local health officials over the course of two DENV outbreaks in 2013-14 and 2017, we were able to collate good data and therefore predict the dynamics of an emerging outbreak. The importance of evidence from a previous outbreak is clear in this study so there are great benefits to be gained from making outbreak research and previous forecasts accessible. Not only does this aid transparency but it has the potential to improve future forecasting efforts.

Our study added to the very limited evidence base for the effectiveness of current control measures in DENV. Especially in Fiji, there is a real question as to whether these chemical spraying efforts provide value for money. Previous work had shown that herd immunity and seasonal forcing alone could not recapture transmission dynamics in the 2013-14 DENV-3 outbreak. Our results in this study corroborate the same finding during the 2017 DENV-2 outbreak, adding to the evidence that interventions during DENV outbreaks do help to reduce transmission. However, this remains indicative and not proof of causality. Further, we do not know which intervention could reduce transmission most effectively. In the short-term this supports current strategies for outbreak control in Fiji. In the long-term, further study into current control strategies should be conducted to differentiate the effects of different interventions on the vector population and DENV transmission, especially as outbreaks become more frequent.

Through this study we have demonstrated the benefit of real-time modelling and the effectiveness of combining data with a relatively simple model to predict outbreak dynamics. This study has also shown that vector control interventions are likely having a negative effect on transmission in Fiji. In Fiji before this study there was extremely limited information on the effectiveness of vector control and the trajectory of ongoing outbreaks were not forecast. Through systematic incorporation of evidence from a

previous outbreak and combination of multiple data sets, mathematical modelling can help address both of these gaps.

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Chapter 7

Discussion

This research had two primary aims; the first was to improve the understanding of population immune dynamics following arbovirus outbreaks. Secondly, to identify the determinants of arbovirus transmission dynamics in island outbreaks. This aim led to the following five objectives:

1. Conduct a serological survey in Fiji, resampling as many participants of previous surveys in Central Division as possible
2. Analyse longitudinal serological data to determine the burden of ZIKV infection in Fiji between 2013 and 2017
3. Evaluate the population level immune response to ZIKV from serological data following outbreaks in Fiji and French Polynesia
4. Develop a mathematical model of arbovirus transmission and use it to explain different transmission dynamics of recent arbovirus outbreaks in Fiji
5. Inform control strategies for DENV outbreaks in Fiji by estimating the effect of vector control on DENV transmission

At the start of these studies Zika virus (ZIKV) was an emerging public health threat and, in the absence of better information, the majority of mathematical models for ZIKV transmission assumed that the virus acted similarly to dengue virus (DENV) (*Champagne et al.*, 2016; *Ferguson et al.*, 2016; *Funk et al.*, 2016; *Kucharski et al.*, 2016; *Netto et al.*, 2017; *O'Reilly et al.*, 2018). DENV and ZIKV share the same primary vector (*Musso and Gubler*, 2016) and are antigenically similar (*Priyamvada et al.*, 2016). However, it was still unknown whether these viruses did result in similar immune dynamics in a population following an outbreak. Similarly, as arboviruses including DENV, ZIKV and to a lesser degree chikungunya virus (CHIKV) began to circulate more frequently in the Pacific over the past decade there was a pressing need to understand the determinants of arbovirus outbreak dynamics. Outbreaks in the region were often short, large, single-season epidemics (*Cao-Lormeau et al.*, 2014b; *Dupont-Rouzeyrol et al.*, 2015; *Lowe et al.*, 2018a; *Roth et al.*, 2014), but there is increasing evidence of persistent low level circulation over several years (*Kama et al.*,

2019; *Ruchusatsawat et al.*, 2019). The factors that determine these contrasting outbreak dynamics in Fiji and the wider Pacific are not well understood.

This doctoral project has demonstrated distinct differences in the immunological response to outbreaks of both of these viruses in the Pacific. I found that ZIKV neutralising responses waned in adults within two years of a ZIKV outbreak, whereas DENV neutralising responses were maintained over the same time frame (Chapter 4). Overall, I found a diverse range of outbreak dynamics across closely related arboviruses in the same location which were revealed through a longitudinal seroepidemiological study (Chapter 3).

The diverse range in dynamics on tropical islands were shown to be highly dependent on the timing of the emergence of a virus. I developed a mathematical model that showed that if ZIKV was introduced to Fiji earlier in the year then it could have caused a large single season epidemic in Fiji, comparable to the 2013-14 DENV-3 epidemic (*Kucharski et al.*, 2018) or ZIKV outbreak in French Polynesia (*Cao-Lormeau et al.*, 2014b) (Chapter 5). Despite this range of outbreak dynamics, it was possible to reliably and accurately forecast outbreaks in real-time by combining a mathematical model with multiple data sources and knowledge from previous outbreaks (Chapter 6).

In Chapter 3 I presented an overview of a 2017 seroepidemiological data collection study I led. My objective was to resample as many participants as possible from previous serological surveys conducted in Fiji (*Kama et al.*, 2019; *Kucharski et al.*, 2018; *Lau et al.*, 2016; *Watson et al.*, 2017). The study design was effective and we successfully resampled 320 participants. Using these data I showed that seroprevalence against DENV-1 was highest in Fiji, but that seroprevalence against ZIKV and CHIKV remained low despite confirmed circulation of these viruses in 2016 (*Kama et al.*, 2019). By collating longitudinal samples between 2013 and 2017 from the same individuals I was able to demonstrate the wide variety of serological dynamics in a population against different circulating arboviruses during the study period. Seroprevalence by MIA increased following outbreaks of CHIKV and DENV-2 between 2015 and 2017 but seroprevalence declined for ZIKV despite recorded cases over the same period.

This seroepidemiological study was designed to specifically focus on ZIKV seroprevalence in Fiji between 2013 and 2017 by analysing longitudinal serological data in Fiji to determine the burden of ZIKV infections. Between 2013 and 2015 ZIKV seroprevalence in Fiji had increased from 7% in 2013 to 22% in 2015 (*Kama et al.*, 2019). In Chapter 3 I presented evidence that ZIKV seroprevalence as measured by MIA actually declined in Fiji between 2015 and 2017 to 12.5%. The proportion of the Fijian population with evidence of past ZIKV infection remained low, unlike other outbreaks of ZIKV in the Pacific (*Aubry et al.*, 2017a) and beyond (*Flamand et al.*, 2019; *Gallian et al.*, 2017; *Netto et al.*, 2017; *Rodriguez-Barraquer et al.*, 2019; *Zambrana et al.*, 2018). If the three serological studies conducted in Fiji between 2013 and 2017 had been designed as a cross-sectional studies then exploring the causes of this pattern of seroprevalence would have been limited. Since both studies in 2015 and 2017 included the additional complexity of re-contacting previous participants, I was able to analyse sera in the same 189 individuals in Fiji between 2013 and 2017.

Chapter 4 presented the results of an analysis of the three longitudinal serological studies conducted in Fiji, combined with five cross-sectional serological surveys from French Polynesia. The objective of this analysis was to evaluate the population level immune response to ZIKV from serological data following outbreaks in both countries. The main finding of this analysis was that seroprevalence declined in adults within 18 months of ZIKV transmission in each country. Seroprevalence in children in both countries remained stable over the study period. A subset of samples collected in Fiji were also tested for neutralising antibodies and we found that individual-level antibody titres against ZIKV, as well as overall seroprevalence, decreased over time (*Henderson et al.*, 2020).

Chapter 5 presents the main mathematical model of arboviruses in this thesis. I used the aforementioned serological data combined with surveillance and molecular data to evaluate different transmission dynamics of recent arbovirus outbreaks in Fiji. In particular, I wanted to compare a large single-season epidemic of DENV-3 in 2013-14 with the low level circulation of ZIKV between 2013 and 2017. I developed a mathematical model that included information on seasonal variation in temperature, reduced trans-

mission from a vector control campaign in March 2014 and estimated virus introduction date from a phylogenetic analysis. I fitted this model to surveillance and serological ZIKV data to evaluate the determinants of these diverse arbovirus outbreak dynamics. I found that virus introduction date, combined with a strong seasonal forcing on transmission, was sufficient to explain the diverse outbreak dynamics in Fiji. This analysis can be used to define a period of high epidemic risk in Fiji.

In the final results chapter of this thesis, Chapter 6, I present results from a modelling analysis of a DENV-2 outbreak in Fiji in 2017. The original objective of the study presented in this chapter was to estimate the relative contribution of vector control interventions during the outbreak on transmission. Previous research had found evidence of reduced transmission intensity during a vector control campaign in Fiji in March 2014 (*Kucharski et al.*, 2018). I aimed to take this research further by combining more detailed information on the DENV-2 outbreak at a finer spatial resolution. I found evidence that an additional reduction in transmission helped explain DENV-2 outbreak dynamics in 2017 at a regional level but I found no evidence that stronger interventions in local areas led to weaker transmission. This work added to the very limited evidence base for vector control effectiveness in Fiji but more research is clearly needed to help guide the most effective intervention strategy during arbovirus outbreaks.

While I was in Fiji during the 2017 seroepidemiological study the DENV-2 outbreak was spreading and was a cause of concern for the Ministry of Health. I therefore used this model of DENV transmission to provide real-time forecasts of the DENV outbreak to help inform the public health intervention strategy used by the Ministry. After the outbreak, I wanted to analyse how accurate this forecast was and how early in the outbreak it would have provided a useful projection of the DENV outbreak dynamics. The DENV-2 outbreak resulted in 755 confirmed cases between January and September 2017 in Suva, the capital of Fiji. In Chapter 6 I demonstrated that this model could accurately predict the scale and dynamics of the DENV-2 outbreak from as early as March 2017 when only 68 cases had been reported. I found that modelling could inform and guide public policy during an outbreak, especially if the model is well calibrated to a previous outbreak in the same setting.

7.1 Strengths

A major strength of this project is the serological data collected in Fiji between 2013 and 2017 and the fact that these data were population representative and collected from the same individuals over time. The original study in 2013 collected a population representative sample of individuals from Fiji. Most other ZIKV seroprevalence studies are a result of convenience sampling which are more susceptible to biased estimates (*Gake et al.*, 2017; *Gallian et al.*, 2017; *Lozier et al.*, 2018; *Netto et al.*, 2017). Through systematic recontacting of participants in 2015 and 2017 we were able to maintain the distribution in our sample in terms of key demographics, particularly age. As a result I collated a longitudinal data set of population representative sera from the same individuals with up to three measurements. The serological data strongly influenced the size of the modelled outbreaks and without these data the plausible parameter space in the model fitting would have been less constrained. The ZIKV model for example would have estimated a larger ZIKV outbreak in a majority susceptible population (*Kucharski et al.*, 2016) and the DENV-2 estimates would have been less precise without a baseline estimate of the proportion of residents of Suva that were immune at the start of the outbreak. These longitudinal sera data were also combined with cross-sectional serosurveys from French Polynesia to describe the population immune response to ZIKV outbreaks in both countries. The data collected from French Polynesia were cross-sectional so provided weaker evidence for the waning of ZIKV neutralising antibodies over time, whereas the longitudinal data from Fiji facilitated analysis of changes in neutralising antibody titres over time.

This project has shown how mathematical models can be used with multiple data sources to infer underlying epidemiological and immunological dynamics, even in situations with imperfect data. The analyses in this thesis, especially in Chapter 5 about outbreak dynamics of ZIKV in Fiji, are reliant on combining multiple data sets with a mathematical model. Without this approach the potential to answer questions about the diverse outbreak dynamics of ZIKV and DENV in Fiji would have been limited. For example, I took two steps to facilitate this analysis and mitigate the limited available surveillance data with just 16 cases of ZIKV confirmed in Central Division. Firstly

I combined multiple data sets. The surveillance data suggested minimal ZIKV transmission but serological data showed evidence of widespread ZIKV transmission, albeit lower than other ZIKV outbreaks in the Pacific (*Cao-Lormeau et al.*, 2014b; *Duffy et al.*, 2009; *Funk et al.*, 2016). Combining these multiple data sources told us that a ZIKV epidemic was likely missed in Fiji. Secondly, I used these multiple data sets in a mathematical model that explicitly modelled the mechanisms that affect arbovirus outbreak dynamics. The results from this model showed evidence of when ZIKV infections, both detected and undetected, spread most and defined a period of high epidemic risk in Fiji. A theoretical model without data would have been able to propose hypotheses for the diverse range of arbovirus dynamics in Fiji. However, the conclusions in this thesis are drawn from models that were validated with real-world data. This project demonstrates that even if one data source provides limited information, including another data sources with a mathematical model can compensate for this.

The models used in this thesis have been used to evaluate outbreak dynamics, but have also been empirically tested in real-time outbreak response. The aim of this doctoral project was to better explain arbovirus outbreak dynamics in Fiji and the wider Pacific. The test of my ability to characterise outbreak dynamics in Fiji was to use the data in real-time to forecast the course of an arbovirus epidemic, which is what I did during a DENV-2 outbreak in 2017 (Chapter 6). This demonstrated that this model could provide valuable input in real-time, as well as used in post-outbreak analysis to evaluate dynamics of the epidemic. I used the model to demonstrate that joint fitting of the ongoing outbreak with a previous DENV outbreak was the key contributor to successfully forecasting the outbreak. This leads to a clear recommendation that having a well calibrated model for a particular disease can help forecast emerging outbreaks in Fiji and beyond.

Finally, I have aimed to work in a transparent and reproducible manner throughout this doctoral project. An attainable minimum standard in epidemiological research is 'reproducibility', whereby data and software are available to verify findings (*Peng et al.*, 2006). The gold standard for this is to make the data and code publicly available and able to run the analysis on open source software (*Peng*, 2011). Data should be publicly

available alongside analyses, while respecting the anonymity of study participants. De-identified data from Fiji – all demographic and location data removed, except age – have been made public when published (*Henderson et al.*, 2020). This is not only appropriate for validation of the findings in my research but maximises the value of the data collected. Study participants in Fiji contributed a lot to our data collection and if the data drawn from these studies are publicly available then this maximises the potential usefulness of these data. I have benefited from publicly available ZIKV sequences and it is possible that others will similarly benefit from the availability of the data I have collected. Data and code have been made available on `GitHub` and all code uses open source software `R` (*R Development Core Team*, 2011).

7.2 Limitations

The conclusions drawn in this thesis rely heavily on the quality of the serological data collected in Fiji. Cross-reactivity of antibodies is a problem for all seroprevalence studies, especially studies of flaviviruses (*Andrade and Harris*, 2018; *Beck et al.*, 2019; *Keasey et al.*, 2017; *Mansfield et al.*, 2011). Serological cross-reaction of viruses imply the sharing of closely related antigenic sites or epitopes (*Calisher et al.*, 1989). As a result of these close relationships antibodies acquired from natural infection with one virus may bind to a different but related antigen in a serological test. This cross-reaction can provide a false positive result and we assume that there has been more infection with a particular virus in a population than actually occurred. The specificity of the microsphere immunoassay (MIA) is greater than tests using enzyme linked immunosorbent assays (ELISAs) however cross-reaction is still a cause for concern (*Beck et al.*, 2015). This doctoral project consistently assumes that ZIKV circulated in Fiji between 2013 and 2015 because of an increase in seroprevalence (from MIA testing) from 7% to 22% (*Kama et al.*, 2019). It is possible that the increased seroprevalence in 2015 was a result of cross-reaction. This would imply that ZIKV seroprevalence did not wane between 2015 and 2017, but that the assay results were more specific in 2017. However, there were limited increases in DENV seroprevalence between 2013 and 2015, with the

exception of DENV-3 because of a large outbreak (Chapter 3). Analysis of correlation between changes in neutralisation titre between 2013 and 2015 in those initially seronegative to any ZIKV or DENV showed no association between change in ZIKV titre and any of the four DENV serotypes (Chapter 4). It is however unavoidable that serological cross-reaction is a limitation for the analyses in this project. To account for the problem when mathematical modelling I explicitly estimated the sensitivity and specificity of the seroprevalence estimates in Chapter 5. Instead of comparing the proportion infected in the model to the reported seroprevalence I calculated the expected seroprevalence given the underlying "true" number of infections. Observed seroprevalence was therefore compared to a proportion of those infected in the model (sensitivity) plus a proportion of those not infected in the model (1 minus specificity. From this model I estimated a false positive percentage and sensitivity of 6.3% (95% CrI: 4.4–8.6%) and 79% (52–98%) for ZIKV. These results adjust for the inevitable uncertainty in seroprevalence estimates when modelling arbovirus transmission dynamics and were also consistent with the previously reported assay specificity and sensitivity for ZIKV of 94.9% and 79.6% (*Henderson et al.*, 2020). To validate results I have analysed complimentary assays and examined seroprevalence trends within the same participants. Through this work I am confident that the observed pattern of increasing then decreasing ZIKV seroprevalence in Fiji is a result of real immunological dynamics and not merely a function of the assays used.

A second problem with interpreting serological data is the uncertain relationship between a participant's antibody response and how this correlates with protective immunity against that virus. A particular cutoff for positivity does not necessarily mean that all those that test positive are immune and those that test negative are susceptible. Therefore, relating a seroprevalence level in a population with immunity against that virus is complicated. I observed a trend of declining seroprevalence as measured by MIA in adults between 2015 and 2017 in Fiji but cannot conclude from this alone that the majority of the population is susceptible to ZIKV infection. Collaborators in Tahiti tested all 2017 samples with a plaque reduction neutralisation test (PRNT) so that we could more directly relate our serological data to protective immunity. These results showed that for ZIKV, neutralising antibodies (NAbs) declined between 2015 and 2017

but that a significant proportion of the population still had high levels of NAbs in 2017 (log titre ≥ 2). Work on DENV suggests that there is a relationship with NAbs and immunity with DENV (*Katzelnick et al.*, 2016). We found that ZIKV NAbs waned in adults between 2015 and 2017 but were still able to neutralise ZIKV. I therefore included waning detectable ZIKV antibodies (from the recovered compartment) in my model of ZIKV transmission but did not allow for reinfection within the study period (Chapter 5). It is important to acknowledge the uncertainty in the connection between seroprevalence and immunity. I have been careful throughout this thesis not to imply that the low ZIKV seroprevalence in Fiji in 2017 implies that the close to 90% of the country are susceptible to ZIKV infection. However, this is more of a limitation in how far results can be extrapolated, not in the finding itself.

An additional limitation to the findings of this thesis is that more advanced methods exist to analyse serological data than have been used in this project. Data augmentation can be used to obtain probabilistic assessments of changes in titre values and whether they are caused by actual infection or assay variability. This has been done effectively in DENV serology using a haemagglutination-inhibition (HI) assay on blood samples from a school-based cohort of 3,451 participants (*Salje et al.*, 2018). It has previously been shown that there is good agreement between HI assay titre values and PRNT (*Venturi et al.*, 2006). The seroprevalence estimates from MIA in this study did not allow for this extended analysis because titre values were not stable enough. The process used in the MIA results in an immunofluorescent (IF) reading even if there is no sera in the well during the test. This IF measure is referred to as the 'background' and a participant's MIA value is their IF reading for a particular minus this background value. This control step is essential to ensure that the IF reading for a particular virus is the result of actual antibody binding not inflated or deflated because of the plate being tested. However, this does mean that values are not stable and can be negative, which when log transformed can lead to a loss of data. This issue makes comparisons of values between samples much harder than handling titre values that are discretised, such as PRNT. Our PRNT data was however limited by a smaller sample size than the MIA data. In 2015 a subset of samples were tested with PRNT, mostly those positive by MIA, so the longitudinal PRNT sample was too small to be robust enough to estimate

infection times. Another limitation in both MIA and PRNT assays is the batch effect where non-biological factors affect the data from these assays. Our data were sampled at two different time-points as samples from 2013 and 2015 were analysed together, then 2017 samples were analysed two years later. These separate analyses could also have introduced random error into our results that were not associated with the outbreak dynamics.

A final major limitation in the serological survey data is potential sampling bias through loss to follow-up over the five-year study period. The original study in 2013 was well designed to be population representative however successfully resampling an individual in follow-up studies was not completely random. Some demographic groups and locations in Fiji were going to be more difficult to resample in the 2017 survey. It is possible therefore that trends in seroprevalence were a result of changing samples rather than underlying transmission dynamics. I was conscious of this potential problem throughout and when collecting 2017 samples I tracked the spatial and age distribution as data were collected to try and collect as balanced a sample as possible. Even after this, it is possible to calculate adjusted seroprevalence if a particular variable is of concern. For example the unadjusted estimated ZIKV seroprevalence in 2017 was 12.5% (95% CI: 9.1-16.6%) and, using weights from the 2013 survey, the estimated age-adjusted seroprevalence was 12.1% (95% CI: 8.7-16.7%). We can repeat this process for rurality, of particular interest in this study given the urban mosquito vector for ZIKV and DENV. Estimated ZIKV seroprevalence in 2017 adjusted for rurality (using the distribution from the 2013 sample) was 12.3% (95% CI: 8.8-16.8%). Doubtless the follow-up surveys were not as representative of the population as the 2013 survey, but there was no evidence that this bias was significant enough to change the findings of this study. Where possible, as an additional step to mitigate this bias, I used a constant sample over time of those participants sampled at each survey.

In addition to the limitations with the data in this thesis, there were limitations in the modelling in this project. The major modelling analysis in this thesis analysed the dynamics of unobserved ZIKV transmission in Fiji before cases were recorded in July 2015. This analysis rested on the assumption that there was one single introduction

event. From this modelling I concluded that ZIKV was likely introduced in late 2014 and conclusions about waning ZIKV seroprevalence in Chapter 4 are affected by this conclusion. There is evidence elsewhere that there were multiple introductions of ZIKV (*Griffin et al.*, 2019; *Grubaugh et al.*, 2017, 2019), including in island settings (*Black et al.*, 2017). In Fiji, the three ZIKV sequences isolated in Central Division did not form a distinct cluster in phylogenetic analysis (*Kama et al.*, 2019). It is therefore possible that there were multiple introduction events; an earlier one that failed to seed a large outbreak, and a later one that led to the observed cases between 2015 and 2017. However, there is clear evidence of a close relationship between two sequences isolated in 2015 and 2016 in Central Division, consistent with persistence of the virus in the same location. Separate introduction of the virus from the same location could also explain this clustering. However there was further evidence that ZIKV spread at a low level for multiple years in seroprevalence data between 2013 and 2015 which suggests there was widespread transmission. To capture some of the uncertainty about the introduction dynamics, I modelled ZIKV introductions as a flexible and continuous process rather than relying on a single instantaneous introduction of infected individuals. This function could capture the flow of infections to the model over several months, but not several years. I concluded that there was sufficient evidence in the molecular and serological data that ZIKV circulated at a low level after a single introduction event and this is further supported by my modelling analysis, which fits the data well (Chapter 5). All conclusions in this project about ZIKV transmission dynamics in Fiji rely on the explicit assumption that there was one introduction event that seeded ZIKV transmission in Fiji.

In the introduction to this thesis I outlined the trade-off at the heart of any modelling strategy between transparency and detail. The models used in Chapters 5 and 6 were designed to find a balance in this trade-off, but as a result they could also be criticised for being both too complex or too simple. The finding with greatest direct impact from these models was likely the ability to predict the course of an epidemic in real time in 2017, which could have been possible with a simpler model. There is a lot of research into predicting DENV outbreaks from historic DENV incidence and climate data using statistical models (*Descloux et al.*, 2012; *Hii et al.*, 2012; *Johansson et al.*, 2016, 2019;

Lowe et al., 2018b). These have proven very effective at predicting the burden and timing of DENV outbreaks. It is fair to ask if the additional complexity I included – building a compartmental model – was justified? While statistical models can be very accurate for predicting case burden one of the aims of this research project was to evaluate the determinants of different transmission dynamics. This meant that these different drivers needed to be explicitly modelled. A key strength of this project was the ability to actually estimate the contribution of different mechanisms, not just provide approximate scenario analyses. For example, to estimate the relative contribution of a targeted vector control campaign I needed to model this mechanism. I was also not able to rely fully on one reliable source of data. I needed to combine multiple data sources to model these outbreaks. Both the model design and model fitting process had to be flexible enough to accommodate this, which is why I opted for a more complex model structure.

The models used in this thesis were therefore complex enough to capture the mechanisms of transmission but were still simple compartmental models. The simplicity in the models did rely on several assumptions about outbreak dynamics, especially concerning the mosquito population. I assumed that the mosquito population and biting rate were constant and that mosquitoes and humans mixed homogeneously. More explicit modelling of the mosquito population, and interaction with humans, could have improved model realism (*Hladish et al.*, 2016). Modelling human behaviour is crucial when modelling other diseases, especially respiratory infections (*Danon et al.*, 2009; *Mossong et al.*, 2008; *Munday et al.*, 2018; *Read et al.*, 2014) and human movement has an impact on arbovirus transmission as well, even at fine geographic scales (*Adams and Kapan*, 2009; *Salje et al.*, 2012). At larger spatial scales, movement of people globally could impact transmission dynamics (*Brockmann and Helbing*, 2013; *Colizza et al.*, 2006; *Semenza et al.*, 2014; *Tian et al.*, 2017). It is the omission of these spatial components that likely limited the conclusions drawn from the modelling of vector control effects on DENV-2 transmission in 2017 (Chapter 6). I could also have directly modelled the impact of secondary DENV infections on the severity of infection and therefore the probability of being recorded as a case of ZIKV or DENV (*Andrade et al.*, 2019; *Cummings et al.*, 2005; *Esteva and Vargas*, 2003; *Recker et al.*, 2009; *Wikramaratna*

et al., 2010). However, I judged this to be an inefficient complication of the models given the limited amount of available surveillance case data for DENV-2 in 2017 and ZIKV between 2013 and 2017.

As explained above, designing the models used in this project inherently involved a trade-off between detail and transparency. I believe these models present the best balance to meet the objectives set out at the start of the project. The complexity was needed to evaluate determinants of transmission dynamics. The simplicity of models was mostly a result of data availability. This simplicity also helped when communicating results with Ministry of Health officials in Fiji. It was important to be able to explain why DENV-2 transmission was likely to be low in 2017 compared to DENV-3 in 2013-14. By directly modelling the effect of seasonal forcing on transmission I could provide projections and the rationale behind them.

A final limitation is how generalisable these findings are. One of the principal findings of this work is that outbreak dynamics are heavily dependent on setting, population immunity and the timing of the outbreak. Island outbreaks are an ideal study site for modelling arbovirus transmission dynamics because of their unique isolation, small population size and well characterised outbreak history. My work is not the first to demonstrate the importance of setting on outbreak dynamics of DENV and ZIKV (*Funk et al.*, 2016). This project aimed to better understand island outbreak dynamics. Further work is needed to extend these methods to other settings where the immunological profile of the population is more complex, viruses transmit endemically or multiple viruses transmit simultaneously.

7.3 Implications and future work

Long term immune dynamics for ZIKV

The first aim of this doctoral project was to improve understanding of population immune dynamics following arbovirus outbreaks. The data I collected in Fiji provided

early evidence that ZIKV-specific antibodies and ZIKV neutralising antibodies wane in adults after a ZIKV epidemic. A recent study in Salvador, northeastern Brazil, similarly found a rapid decline in ZIKV NS1 antigen-specific antibodies (*Moreira-Soto et al.*, 2020). This is important because this trend could continue to be observed in other locations, particularly in the Americas where there were large ZIKV outbreaks several years later than in Fiji and the wider Pacific. However, my results show that seroprevalence estimates collected shortly after a ZIKV outbreak may underestimate the true burden of ZIKV infection. If researchers then fit models to post-outbreak seroprevalence data then this could lead to false conclusions about ZIKV dynamics. These findings should also help dispel the notion that ZIKV "could be considered as a fifth member of the DENV serocomplex" (*Dejnirattisai et al.*, 2016). Despite similarities between the two viruses ZIKV immunity following infection does not necessarily act in the same way as DENV.

This doctoral project has demonstrated the advantage of collecting longitudinal serological data but more studies are needed to fully understand the immune response to ZIKV, especially in the presence of co-circulating flaviviruses. I presented an indication that waning ZIKV NAbs could be related to prior DENV exposure in Chapters 3 and 4. However, these results were inconclusive and further research is needed into this area. Do those with more prior flavivirus infections lose ZIKV neutralising antibodies faster? Does the reduction in quantity of NAbs affect immunity, or are the remaining antibodies sufficient to protect from infection and disease? These were not questions I was able to answer with the data collected in this study but carry large implications on the likelihood and dynamic of future arbovirus outbreaks.

Efficiently combining data sources with mathematical modelling

Another novel aspect of this research is the framework I developed to include multiple data sets and models into a model of ZIKV transmission dynamics in Fiji. Combining multiple data sets in a model is not new (*Birrell et al.*, 2011; *Goubar et al.*, 2008; *Presanis et al.*, 2014; *Sweeting et al.*, 2008; *Welton and Ades*, 2005) nor is the ability to model outbreak dynamics from molecular data (*Faria et al.*, 2016; *Grenfell et al.*, 2004;

Grubaugh et al., 2019; *Jombart et al.*, 2014; *Koelle et al.*, 2006, 2010; *Ratmann et al.*, 2012). However, conclusions on outbreak dynamics drawn from phylogenetic analysis can have a wide range of uncertainty. For example, in the analysis of molecular data in Chapter 5 the estimated time of ZIKV introduction to Fiji spanned a wide period. Therefore, I developed a framework that could incorporate the uncertainty from the phylogenetic analysis in an outbreak dynamic mathematical model as prior information. Without this phylogenetic analysis, it was more difficult to differentiate between an early ZIKV introduction with longer circulation at a lower level, or a later shorter epidemic. By providing more information on the introduction time I was able to demonstrate clear support for a later introduction of ZIKV to Fiji and demonstrate the marked effect that virus introduction timing could have on transmission dynamics. This also demonstrated the flexibility of mathematical models and the ability to combine multiple data sets.

Combining data was essential to this project but there is a lot of methodological work that could be done into ensuring this process is efficient. Throughout this project I have combined the log likelihood of surveillance and serological data when fitting models to data. However, the contribution to the likelihood from seroprevalence data is much less than the contribution from a time series of surveillance case data. A potential area of future study would be whether this method is creating unbalanced contributions from each of the data sets and whether a more efficient method is available. It would also be interesting to investigate how best to fit a model to surveillance data when the start time is of particular interest and is estimated in the model. We know that transmission spreads before cases are reported, especially for novel infections. However, a model with an earlier introduction date will be penalised more heavily than a later introduction, since the model will estimate infections when no cases were reported. A method that can test the effect of this potential bias and adjust for it could be valuable. Combining data with mathematical models clearly has great potential but there are elements of the process that would benefit through validation with simulation studies.

Real-time modelling of arbovirus outbreaks

The importance of modelling in public health is growing, especially in outbreak response (*Bausch and Edmunds*, 2018). In this project I have demonstrated how a relatively simple model was able to make an important contribution to outbreak response to a DENV-2 epidemic in Fiji. The fear at the onset of the epidemic was that the peak would be as large as the 2013-14 DENV-3 epidemic. Using a simple model I was able to provide evidence that transmission of the DENV-2 outbreak would likely be lower due to seasonal forcing of DENV transmission in Fiji. This was a model simple enough for me to run in the first year of my PhD and was able to support the government response to an outbreak. The most important factor in the accuracy of the model predictions, as demonstrated in Chapter 6, was the joint fitting with a previous DENV epidemic. I think it is clear that if outbreak dynamics can be well characterised with a simple model this can greatly improve real-time modelling accuracy if a new, comparable outbreak emerges.

The biggest potential improvement to real-time modelling in Fiji and the wider Pacific would be to better categorise the cycling of viruses in the region. I have said many times throughout this thesis that arbovirus outbreaks in Fiji are becoming more common. I have presented details of four outbreaks in Fiji between 2013 and 2017. Previous perceived wisdom suggested for DENV there was a 4-5 year gap between outbreaks, then a 12 year period before re-emergence of the same serotype (*Cao-Lormeau et al.*, 2014a). The question remains, is this pattern changing in the region? This is hard to say but local data availability is improving which could help investigate this. Work has been done in French Polynesia to characterise historic DENV infections since 1970 (*Teissier et al.*, 2020). The ability of collaborators at Institut Louis Malardé to diagnose viruses from blood stored on filter paper from the region means there is an increasing data base for circulating viruses in the Pacific (*Aubry et al.*, 2012). This is already being used to identify potential future outbreaks such as DENV-2 in French Polynesia, a prediction in 2017 (*Aubry et al.*, 2017b) that came to fruition in 2019 (*Aubry et al.*, 2019). This doctoral project has demonstrated the potential for combining multiple data sets with mathematical modelling. It may be possible to learn how these arboviruses spread and

interact with each other if novel data sets can be included in models for a variety of locations across the Pacific.

7.4 Concluding remarks

I aimed to improve understanding of underlying serological dynamics and explain arbovirus outbreak dynamics in Fiji and the wider Pacific using mathematical modelling. There is much more to discover in this field, especially about how flavivirus infections interact and affect the long-term immune response. It is clear from this analysis of serological data collected in Fiji and the wider Pacific that these interactions are complex and that ZIKV does not necessarily cause the same long-term immune response in a population following an outbreak as DENV. It is also evident from modelling transmission of these viruses that we can characterise outbreaks well enough to predict their future dynamics in real-time, but that they are sensitive to a variety of factors that can shape outbreak dynamics. A change as simple as the month of virus introduction could have had a dramatic effect on the ensuing ZIKV outbreak dynamics in Fiji. Given the uncertainty surrounding DENV and ZIKV transmission globally, island outbreaks – combined with mathematical models – present a unique opportunity to gain insights into these diseases.

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Appendix A

Ethical approval from LSHTM

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Observational / Interventions Research Ethics Committee

Mr Alasdair Henderson
LSHTM

9 February 2017

Dear Alasdair

Study Title: Serosurvey to study Zika and related arboviruses in Central Division, Fiji

LSHTM Ethics Ref: 12037

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant. Please send evidence of local approval to ethics@lshtm.ac.uk once received.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Investigator CV	CV Alasdair Henderson	17/12/2016	1
Protocol / Proposal	MAIN Data collection form Zika 2017	22/12/2016	2
Protocol / Proposal	Main study protocol V3 zika 2017	22/12/2016	3
Covering Letter	12037 Response letter Zika 2017	06/02/2017	1
Protocol / Proposal	MAIN Information and consent form Zika 2017 v3	06/02/2017	3
Information Sheet	MAIN Information and consent form Zika 2017 v3	06/02/2017	3

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

[redacted]

Professor John DH Porter
Chair

ethics@lshtm.ac.uk

Appendix B

Ethical approval from FNHRERC (May 2017)

MINISTRY of HEALTH & MEDICAL SERVICES
Shaping Fiji's Health

Fiji National Health Research and Ethics Review Committee

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29th May 2017

Alasdair Henderson
Faculty of Epidemiology & Population Health
London School of Hygiene & Tropical Medicine
London

Project Title: "Serosurvey to study Zika and related arboviruses in Central Division, Fiji"

FNHRERC Number: 2017.20.MC

Primary Investigator(s): Alasdair Henderson, EPH, LSHTM, London.

Principal Supervisor(s): Adam Kucharski, EPH, LSHTM, London
Conall Watson, EPH, LSHTM, London
Mike Kama, MOHMS, Fiji
Van Mai Cao-Lormeau, ILM
Colleen Lau, UQ, Australia
John Edmunds, EPH, LSHTM, London

Dear Alasdair,

This is to inform you that the Fiji National Health Research Ethics Review Committee (FNHRERC) has granted scientific, technical and ethical **approval** to your proposal titled *"Serosurvey to study Zika and related arboviruses in Central Division, Fiji".*

As the Principle Investigator, it is **your responsibility to ensure that all the people associated with this particular project area aware of the conditions of this approval and copy of the final report is also submitted to the Ministry of Health and Medical Services at the conclusion of your project for our records.**

The following conditions apply to your approval. Failure to abide by these conditions may result in suspension or discontinuation of approval and/or disciplinary action.

1. **Variation to the project:** Any subsequent variation s or modifications you may wish to make to your project must be notified formally to the Chair, FNHRERC for further considerations and approval. If the Chair considers that the proposed changes are significant, you may be required to submit a new application for approval of the revised project.

2. **Incidence or adverse events:** Researchers must report immediately to the Chair FNHRERC anything which may affect the ethical acceptance of the protocol including adverse effects on subjects or

unforeseen events that may affect continued ethical acceptability of the project. Failure to do so may result in suspension or cancellation of approval.

3. **Monitoring:** Projects are subject to monitoring at any time by the Committee

4. **Annual/Final Report:** You must submit a progress report at 6 months of your study and an annual/final report at the end of the year or at the conclusion of the project if it continues for less than or more than a year. Also you are to present the evidence back to the participating institutions.

Please quote the FNHRERC number and the name of the project in any future correspondence.

If you have any further queries or require any additional information, please do not hesitate to contact the Secretariat on telephone: (679) 3306177 ext. 340170 or email: rosimina.tubuitamana@govnet.gov.fj.

We wish you all the best in your study.

[redacted]
Mr. Shivnay Naidu
Chairperson
Fiji National Health Research Ethics Review Committee

Appendix C

Ethical approval for ethics amendment from FNHRERC (October 2018)

MINISTRY of HEALTH & MEDICAL SERVICES
Shaping Fiji's Health

Fiji National Health Research and Ethics Review Committee

Level 2 Dinem House
88 Amy Street, Toorak.
Box 2223, Govt. Building
Suva, Fiji

Phone : (679) 3306177 / 3221 424
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23rd October 2018

Alasdair Henderson
Faculty of Epidemiology & Population Health
London School of Hygiene & Tropical Medicine
London

Project Title: "Serosurvey to study Zika and related arboviruses in Central Division, Fiji"

FNHRERC Number: 2017.20.MC

Primary Investigator(s): Alasdair Henderson, EPH, LSHTM, London.

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Van Mai Cao-Lormeau, ILM
Colleen Lau, UQ, Australia
John Edmunds, EPH, LSHTM, London

Dear Alasdair,

This is to inform you that the Fiji National Health Research Ethics Review Committee (FNHRERC) has granted scientific, technical and ethical **approval** to your amendment application submitted on 22nd August 2018, on *"Serosurvey to study Zika and related arboviruses in Central Division, Fiji"*.

As the Principle Investigator, it is **your responsibility to ensure that all the people associated with this particular project area aware of the conditions of this approval and copy of the final report is also submitted to the Ministry of Health and Medical Services at the conclusion of your project for our records.**

The following conditions apply to your approval. Failure to abide by these conditions may result in suspension or discontinuation of approval and/or disciplinary action.

1. **Variation to the project:** Any subsequent variation s or modifications you may wish to make to your project must be notified formally to the Chair, FNHRERC for further considerations and approval. If the Chair considers that the proposed changes are significant, you may be required to submit a new application for approval of the revised project.

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Please quote the FNHRERC number and the name of the project in any future correspondence.

If you have any further queries or require any additional information, please do not hesitate to contact the Secretariat on telephone: (679) 3306177 ext. 340170 or email: rosimina.tubuitamana@govnet.gov.fj.

We wish you all the best in your study.

[redacted]

Mr. Shivnay Naidu
Chairperson
Fiji National Health Research Ethics Review Committee

Appendix D

Informed consent and questionnaire

Dengue fever in Fiji – study of signs in the immune system

Information for people who may like to volunteer

We are doing research to prevent infectious diseases in Fiji. Many thanks for giving a blood sample before to help this research. Your blood sample is helping Ministry of Health see who is at risk of infection with dengue fever, typhoid and leptospirosis.

We are now also investigating Zika, a disease mostly spread by mosquitoes. In particular, we want to find out if people were infected with Zika in 2016. We think many people may have been infected without becoming ill. Understanding this will help the Ministry of Health to prepare for future epidemics.

You are being invited to take part because you are a member of the general public, and helped with a previous research project. We would like to take a blood sample to compare it with the blood sample you gave in 2013, **and possibly in 2015 as well**. We are not testing to see if you are currently infected, but will be able to see if your immune system fought off Zika during the epidemic, or if you ever had Zika before. At the same time, **we** will be able to test for other diseases that may have been spread, such as dengue. This helps the government decide how best to stop Zika in the future, as well as related diseases.

If you have any questions, please ask the research team. We are very grateful for your assistance.

Vinaka vaka levu.

Questions and answers

What is Zika?

Zika is a disease caused by infection with the Zika virus. It happens after being bitten by a mosquito. Not all mosquitoes carry the virus, but they can spread it if they bite an infected person and then bite somebody else.

The first major outbreak of Zika was recorded in Micronesia in 2007. Since then it spread to other Pacific Islands, South America and South East Asia. There have also been Zika cases reported in Fiji during 2016.

Often Zika is mild. However, the infection is a risk for pregnant women because Zika can sometimes cause birth defects. Once your body fights off Zika, you are likely to be immune to that strain.

What is dengue fever?

Dengue fever is a disease caused by infection with the dengue virus. It happens after being bitten by a mosquito. Not all mosquitoes carry the virus, but they can spread it if they bite an infected person and then bite somebody else. There are usually around a hundred cases a year in Fiji, but during the 2014 epidemic, there were more than 20,000 cases. This happened when a new strain of dengue came to Fiji, that very few people had immunity to.

Often dengue fever is mild. Symptoms can include fever for 3 to 7 days, rash, headache, tiredness and sore muscles, back, eyes or joints. Sometimes dengue symptoms can be severe, and it can be life-threatening. Severe dengue can cause bleeding, and stop organs working. If dengue fever is severe, patients need treated in hospital. Dengue can be particularly dangerous for children.

Once your body fights off dengue, you are immune to that strain, but can be infected by one of the other strains of dengue. There are four main strains of dengue.

How can I protect myself and my family from Zika and dengueNOT the night ones

Black and white striped (tiger/zebra) mosquitoes

You can take steps to reduce the number of mosquitoes near where you live, and take steps to avoid getting bitten, particularly during outbreaks.

Mosquitoes lay their eggs in warm, still water. Two of the main sources are old car tires and open water containers, such as oil drums or plastic tubs and buckets, but they can also grow in puddles, plant pots, rubbish, old coconut shells and natural hollows. You and your

neighbours should get rid of any old tires and containers that are not needed. Those that you need should be covered by a lid or kept under shelter. Crush tins and other rubbish. Keep house gutters clean and seal rainwater tanks. Clear blockages in ditches, and fill in puddles. Water in plant pots should be changed every week. If there is water than can't be drained, a little vegetable oil or insecticide can stop mosquito eggs hatching.

To help avoid being bitten, you can burn mosquito coils in the evening and !. Wear insect repellant on exposed skin. Repair mosquito screens on doors and windows, and use them. Kill mosquitoes in your home with insecticide.

What do I have to do for this research?

We would like to take a small sample of blood from your arm. The sample is taken by a trained health professional who will put a small needle into a vein and collect one small tube of blood (about a teaspoonful). It will take about a minute and the needle may sting a little as it goes in.

We will also ask you some questions about whether you could have had Zika or dengue. This will take about 5 minutes.

Are there risks?

Taking a blood sample has a small risk of bruising, and a rare risk of infection or nerve injury. In the unlikely event that you experience any of these, please let the study team know and we can give further advice. You can also ring the study team after they have gone on the number below. Or you can seek advice from your medical doctor.

Are there benefits?

There is no direct benefit to you, though we hope the research will benefit everyone in Fiji by helping prevent Zika and dengue outbreaks.

Can I say "No"?

You can. You do not have to donate a blood sample for this study, and you do not have to answer any questions you do not want to answer.

What will happen to the information about me, and the blood sample?

This information will be kept confidential and only accessed by the study team and the Ministry of Health. **The samples will be analysed in specialist laboratories by Fijian scientists and their collaborators at Institut Louis Malard**. The samples will be kept at these laboratories and/or the Ministry of Health for at least 10 years as a public health research resource.

The samples and information may be used for other health research as determined by the Ministry of Health, who may get back in touch with you about further research. Your name will be removed from the data before it is used for research. You will not be identifiable in any research reports, and all data will be presented at a grouped level. The overall results of the study will be given to the Ministry of Health and shared with public health scientists through medical journals.

What will the results of the test mean for me?

We are not looking for current dengue infection and will not be in touch with results of the blood test. We are looking for signs that your immune system has fought off dengue. This is to help the Ministry of Health decide strategies for controlling dengue. If you are concerned about dengue fever, the advice about protecting yourself from mosquitoes may help.

Are there any costs or payments?

No.

Who is organising the research?

The research is organised by a joint team from the Ministry of Health, Institut Louis Malardé, and the London School of Hygiene & Tropical Medicine.

Who has reviewed the study?

The study has been approved by the ethics committees of the Fiji Ministry of Health and the London School of Hygiene and Tropical Medicine.

Many thanks for offering to take part in this research. Vinaka vaka levu.

If you have any concerns about this research you can contact the investigator,

Dr Adam Kucharski on telephone 912-5567 or email adam.kucharski@lshtm.ac.uk

Or the Ministry of Health Research Unit, Elina Veitamana

Telephone: +679 3215770 ext 340170 Email: elina.veitamana@govnet.gov.fj

Dengue fever in Fiji - signs in the immune system

Research Consent form for adult and child participants

Participant ID Number: **FJD CT**☐☐☐☐ (site / person)

I/we have read and understood the information sheet. I/we understand that participation is voluntary and I/we can withdraw assent/consent at any point without giving a reason.

I consent to the following: (please tick yes or no to each question)		Yes	No
1.	A sample of my/my child's blood may be taken and used for Zika and dengue research.		
2.	The answers I/we give to questions can be used for public health research, including Zika and dengue.		
3.	The Ministry of Health, or researchers working with the Ministry, can contact me/us again about Zika and dengue research.		
4.	My/my child's blood sample can be used for other health research.		
5.	The Ministry of Health, or researchers working with the Ministry, can contact me/us about other health research.		

Name of participant:(please print)	Age in years:
Signed by participant if age 12+:	Date (dd/mm/yyyy)
Name of PARENT or CARER for child participants (age 17 or less): (please print)	
Signed by parent/carer:	Date (dd/mm/yyyy)

Consent taken by research staff member: (please print name)	
Signed by research staff member:	Date (dd/mm/yyyy)

If the participant/parent/carer does not speak English:

Witnessed by: (please print name)	
Signed by witness:	Date (dd/mm/yyyy)

Data collection form

Part A: Participant information

(Selecting individuals both agreeing further sample testing and further contact)

Participant ID FJD CT☐☐☐☐

First name:_____ **Family Name :** _____ **Age:** _____

Part B: Visit record sheet

Date today: __ __ / __ __ / 20__ __ (dd/mm/yyyy)

Interviewer: _____

Bula vinaka! Thank you for agreeing to take part in this research. We would like to ask you a few questions to help us understand zika and dengue fever in Fiji. Please stop me and ask questions whenever you would like.

Have **you** had a fever in the last 2 years?
Circle: 1=yes 0=no, -1=don't know, -2=refused

Have **you** visited a doctor with a fever in the last 2 years?
Circle: 1=yes 0=no, -1=don't know, -2=refused

Have **you** had a rash in the last 2 years?
Circle: 1=yes 0=no, -1=don't know, -2=refused

Have **you** visited a doctor with a rash in the last 2 years?
Circle: 1=yes 0=no, -1=don't know, -2=refused

If YES to visited doctor	Fever/rash episode (or 1st episode if more than one)	2nd episode (if more than one)	3rd episode (if more than two)
When? Year (-1=don't know)			
Month, if known (-1=don't know)			
Did the doctor suspect it was zika? 1=yes, 0=no, -1=don't know, -2=refused			
Did the doctor suspect it was dengue fever? 1=yes, 0=no, -1=don't know, -2=refused			
Did the doctor do a blood test for zika?			

1=yes, 0=no, -1=don't know, -2=refused			
Did the doctor do a blood test for dengue fever? 1=yes, 0=no, -1=don't know, -2=refused			
Was the blood test positive for zika? 1=yes 0=no, -1=don't know, -2=refused			
Was the blood test positive for dengue fever? 1=yes 0=no, -1=don't know, -2=refused			
Were you hospitalised? 1=yes 0=no			

Have **any household members** visited a doctor with a fever and rash in the last 2 years?			
Circle: 1=yes 0=no, -1=don't know, -2=refused			
If YES, give details of most recent episode (for up to 3 household members)			
	Member 1	Member 2	Member 3
That person's age now			
When? Year (-1=don't know)			
Month, if known (-1=don't know)			
Did the doctor suspect it was zika? 1=yes, 0=no, -1=don't know, -2=refused			
Did the doctor suspect it was dengue fever? 1=yes, 0=no, -1=don't know, -2=refused			
Did the doctor do a blood test for zika? 1=yes, 0=no, -1=don't know, -2=refused			
Did the doctor do a blood test for dengue fever? 1=yes, 0=no, -1=don't know, -2=refused			
Was the blood test positive for zika? 1=yes 0=no, -1=don't know, -2=refused			
Was the blood test positive for dengue fever? 1=yes 0=no, -1=don't know, -2=refused			
Were you hospitalised? 1=yes 0=no			

Presence or absence at the house of:

Item	Yes	No	Could not find out
Mosquitoes			
Used car tires			
Open water containers (e.g. vase, bucket, basin, oil			

drum)			
Long lasting puddles of water (i.e. do not dry up within a day after rain stops)			
Other mosquito breeding grounds If yes, specify_____			

Check new telephone number if not previously recorded. Add new address GPS, if moved house. Check: is date of move recorded?

GPS coordinates of 1= front door of house, 2= community centroid,
South 0□□°□□.□□□' (circle) **East** □□□°□□.□□□'

Any other comments?

Vinaka vaka levu.