Epidemiology of Dengue, Chikungunya and Zika in a Naïve Population in St. Kitts, West Indies.

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Abstract

Arboviruses such as dengue (DENV), chikungunya (CHIKV) and Zika (ZIKV) are of increasing global health concern and have caused recent rapid outbreaks in the Americas. However, studies focusing on behavioural risk that would assist with the understanding of transmission factors are scarce.

This prospective study followed immunologically naïve adults in an arbovirus endemic environment to investigate disease detection, transmission and associated risk factors.

University students from non-endemic areas studying in St. Kitts and Nevis were recruited as volunteers in three cohorts of sentinels between September 2014 and May 2015 (n = 224). Plasma was collected at enrolment and every 4 months subsequently until September 2016 and assayed for anti-DENV and anti-CHIKV IgM and IgG ELISA antibodies. Additionally, specimens collected from suspected cases of acute arboviral infection within the wider island population and mosquitoes captured in neighbourhoods were analysed for DENV, CHIKV and ZIKV by RT-PCR. Epidemiological data gathered at each sampling were investigated using mixed effect models, generalised estimating equations, Bayesian techniques and Cox proportional hazards survival analysis.

Evidence of dengue infection was found in all (100%) the suspected cases born in St. Kitts but proof of recent infection was elusive. Chikungunya prevalence in sentinels was 12.7% (95% PI: 8.2-18.4%), whereas prevalence in suspected cases born in St. Kitts was 69.6% (95% CI: 47.1-86.8%). Zika prevalence was 39.1% (95% CI: 25.1-54.6%) and evidence of infection and vertical transmission were also found in *Ae. aegypti* mosquitoes. Climatic variables were significantly associated with transmission followed by socio-economic conditions, frequency of mosquito bites and exposure to *Ae. aegypti*.

Data suggested that arbovirus transmission in St. Kitts are epidemic and expire when climatic conditions become unfavourable for mosquito transmission or herd immunity reaches a critical threshold. These findings increase the understanding of arboviral transmission in small islands and can assist in more efficient outbreak response.

‘Less is more’
(Browning, 1855)

Dedication

To my wife for her support and her love, to my parents and to my little brother for our mutual admiration.

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- List of abbreviations

Abbreviation	Name
Ae.	Aedes
CHIKV	Chikungunya virus
CRS	Composite Reference Standards
Cx.	Culex
CPE	Cytopathic effects
DHF	Dengue Haemorrhagic Fever
DSS	Dengue Shock Syndrome
DENV	Dengue virus
DALY	Disability-Adjusted Life Years
D^-	Disease Negative
D^+	Disease Positive
ELISA	Enzyme-Linked Immunosorbent Assay
EDTA	Ethylenediaminetetraacetic acid
GDP	Gross Domestic Product
GNI	Gross National Income
HIA	Hemagglutination Inhibition Assay
HRP	Horseradish Peroxidase
IOL	Indian Ocean lineage
JE	Japanese Encephalitis
LCM	Latent Class Models
ML	Maximum Likelihood
MLE	Maximum Likelihood Estimation
MAYV	Mayaro Virus
ODA	Official Development Assistance
OECD	Organisation for Economic Co-operation and Development
PBS	Phosphate-Buffered Saline
PRNT	Plaque Reduction Neutralization Test
PCR	Polymerase Chain Reaction
RDT	Rapid Detection Test
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
RUSVM	Ross University School of Veterinary Medicine
SKN	Saint Kitts and Nevis

Se	Sensitivity
Sp	Specificity
SLEV	St. Louis encephalitis virus
SD	Standard Deviation
SDG	Sustainable Development Goal
T^-	Test Negative
T^+	Test Positive
VIF	Variance Inflation Factor
WNV	West Nile Virus
YFV	Yellow Fever Virus
ZIKV	Zika Virus
PHEIC	Public Health Emergency of International Concern

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Chapter 1. Introduction

1.1. Relevance of dengue, chikungunya and Zika in a global and regional context

Mosquito-borne diseases are an increasing global health concern (Gubler, 2014) and dengue is the most common cause of arboviral disease in the world (Gubler, 2002; Wilder-Smith and Schwartz, 2005; Runge-Ranzinger *et al.*, 2008). Dengue is mostly asymptomatic although it can progress into a mild flu-like course that may advance into severe dengue and a fatal outcome in some cases. It is estimated that over half of the world's population are at risk of contracting dengue (Brady *et al.*, 2012) and 390 million cases occur globally every year of which 96 million manifest clinical signs (Bhatt *et al.*, 2013). Dengue is endemic in more than 100 countries in Central and South America, South-East Asia and Western Pacific where the climatic conditions are favourable for the mosquito vector (Figure 1.1) (Brady *et al.*, 2012; World Health Organization, 2017c). The majority of the severe cases of dengue are reported in Asia where severe dengue is a leading cause of hospitalization and death among children (Gubler, 2002; World Health Organization, 2017d). In the American tropics, the severe form of the disease was rare before 1981 (Gubler, 2002), however, there has been a 4.6-fold increase in reported dengue cases in the Americas, rising from around 1 million cases during the 1980s to 4.7 million in 2000–2007 (L'Azou *et al.*, 2014 citing San Martin *et al*., 2010) and in 2010 all four dengue serotypes were found co-circulating within the Caribbean islands with crude fatality rates of 6 in Barbados, 4 in Jamaica, 3 in the Bahamas and 2 in Dominica (Chadee, Mahabir and Sutherland, 2012). Dengue is currently in a hyper-endemic state in most of the American continent and the continental Chile was the only country in Latin America that remained to be without indigenous transmission in early 2017 (Murray, Quam and Wilder-Smith, 2013; World Health Organization, 2017b).

The actual burden of dengue is difficult to measure. According to data submitted by national health authorities, dengue cases across the Americas, South-East Asia and Western Pacific exceeded 1.2 million in 2008 and over 3.2 million in 2015, of which 2.41 million cases of dengue were reported in the Americas, mostly in Brazil and Mexico, 12,490 cases were diagnosed as severe dengue and caused 1,354 deaths (World Health Organization, 2017b). The reasons for discrepancies between the figures submitted to WHO and the figures reported in the literature have been attributed to widespread underreporting of cases (Cash and Narasimhan, 2000; Schiøler and Macpherson, 2009; Gubler, 2011; Petersen and Powers, 2016;

Wearing, Robert and Christofferson, 2016), mainly as a consequence of the high rate of asymptomatic cases, misdiagnosis, poor surveillance, especially in locations with less developed public health systems, lack of uniform criteria to report cases of dengue to WHO, and the lack of cooperation by the tourism and other industries (Suaya, Shepard and Beatty, 2007; Suaya *et al.*, 2010; Gubler, 2011; Riou, Poletto and Boëlle, 2017; World Health Organization, 2017c).

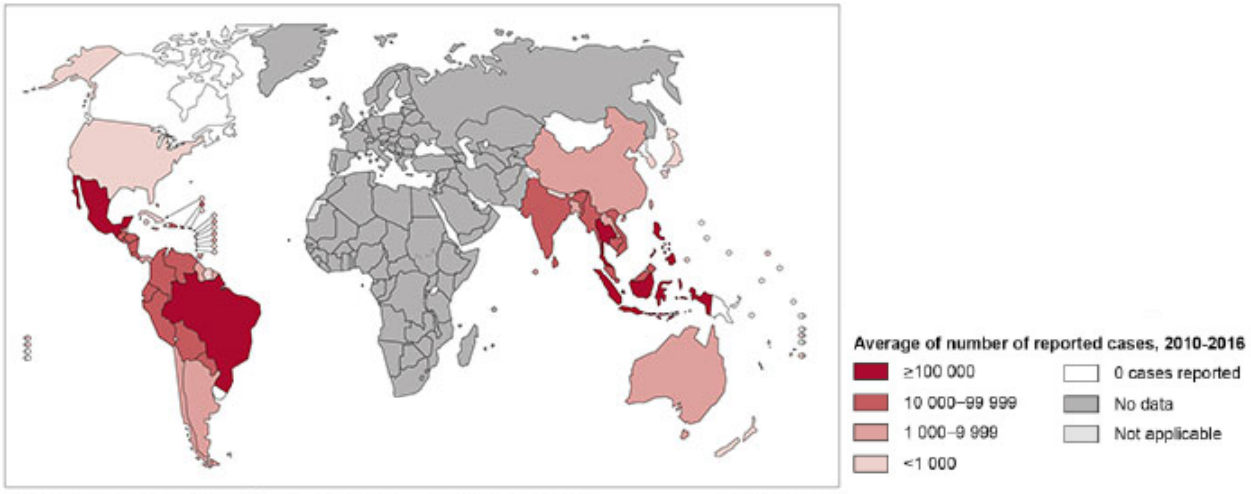


Figure 1.1 Distribution of dengue, worldwide, 2016. Source: World Health Organization (2017c)

There are also large discrepancies on the economic impact of dengue. Some studies have reported that dengue fever has a total impact of the same order of magnitude as malaria, tuberculosis or hepatitis (Gubler, 2002), with estimations of the cost of dengue in the Americas at $2.1 billion per year on average (in 2010 US dollars), and a range of $1–4 billion which exceeds that of other viral illnesses such as human papillomavirus or rotavirus (Shepard *et al.*, 2011). Other studies have reported that dengue fever has a total impact substantially lower than that of malaria, tuberculosis or hepatitis, with estimations accounting for 3.3%, 2.0% and 1.2% of the global disability-adjusted life-years (DALYs) burden respectively, whereas the cost of dengue accounts for 0.03% of the global disability-adjusted life-years burden (Murray *et al.*, 2012; Horstick, Tozan and Wilder-Smith, 2015). Similarly, a dengue study over a 5 year period in Kamphaeng Phet, Thailand, estimated a loss of 465.3 DALYs per million population per year, which accounted for 15% of DALYs lost to all febrile illnesses and dengue non-hospitalized patients represented 44–73% of the total DALYs lost to dengue each year with DENV-1 responsible for 9% of total DALYs lost, DENV-2 for 30%, DENV-3 for 29% and DENV-4 for 1%, though during large outbreak DALYs lost to dengue was three times greater than reported by the World Health Report for 2003 (World Health Organization, 2003; Endy, Yoon and Mammen, 2010).

Other mosquito-borne diseases have also received further interest in the Americas in recent years including chikungunya and Zika. Chikungunya was mostly present in Africa, south Asia and the Indian subcontinent until it spread to the Caribbean French territory of St. Martin in 2013 (Cassadou *et al.*, 2014), resulting in 1.7 million cases and 240 deaths communicated from 45 of the 53 countries or territories reporting to the Pan American Health Organization as of September 2015 (Petersen and Powers, 2016) with the burden of the disease shifting to Colombia and Brazil that reported the highest number of cases in 2015 and 2016 (World Health Organization, 2017a). Also, Zika virus was first reported as circulating in the north east of Brazil in early 2015 (Zanluca *et al.*, 2015) and by 2017 it had spread to 48 countries and territories in the American continent, with 27 of those reporting microcephaly and/or neurologic malformation cases and 25 Guillain-Barré syndrome (GBS) potentially associated with Zika virus infection (PAHO / WHO, 2017b). The potential association between microcephaly and other neurological disorders with Zika virus reported in Brazil, France, United States of America, and El Salvador prompted the World Health Organization to declare Zika a Public Health Emergency of International Concern (PHEIC) on 1st February 2016 (World Health Organization, 2016).

There is a general consensus among experts that mosquito-borne diseases will continue spreading in the future, including geographic expansion, greater incidence and reporting to WHO (Hales *et al.*, 2002; Wilder-Smith and Gubler, 2008; Wilder-Smith *et al.*, 2010; Gubler, 2011; Murray *et al.*, 2013). Dengue is spreading at an accelerating pace and some of the predictions made at the beginning of the century, as that approximately 50%–60% of the global population would be living in areas at risk of dengue transmission by 2085 (Hales *et al.*, 2002), are becoming obsolete. The reasons for dengue spread are varied and include population growth, urbanization in substandard living conditions, deficient vector control, international travel and climatic changes (Gubler, 1998a, 2002, 2011; Wilder-Smith and Schwartz, 2005; Åström *et al.*, 2012; L. P. Campbell *et al.*, 2015). Also, the incidence of dengue in international travellers is rising and there are concerns of importation into free areas (Wilder-Smith and Macary, 2014) as dengue is the second most diagnosed cause of fever among travellers returning to Europe from low and middle income countries after malaria (World Health Organization, 2017b). For instance, dengue was reported in France (La Ruche *et al.*, 2010) and Croatia (Gjenero-Margan *et al.*, 2011) in 2010, followed by Madeira and mainland Portugal in 2012 (European Centre for Disease Prevention and Control, 2013), which were the first local

transmission reports of dengue in Europe since the outbreak in Greece in 1927 (Papaevangelou and Halstead, 1977). Also, local transmission of chikungunya was recently reported in Italy in 2017 (European Centre for Disease Prevention and Control, 2017) and 2007 (Rezza *et al.*, 2007; Moro *et al.*, 2010), and in southern France in 2014 (Delisle *et al.*, 2015) and 2017 (ECDC, 2017). Moreover, the US has also seen outbreaks of dengue in Texas between 2003 and 2005 (Murray *et al.*, 2013), in Florida in 2009 (Radke *et al.*, 2012) and 2013 (Teets *et al.*, 2014), in Hawaii in 2015 (Effler *et al.*, 2005), and of Zika in Florida in 2016 (Likos *et al.*, 2016).

1.2. Arbovirus:

1.2.1. Structure and classification of principal human arboviruses

The most significant arboviruses causing human illness belong to genera in the families Flaviviridae, Togaviridae, and the former Bunyaviridae (Figure 1.2). More than 50% of all known *Flavivirus* (family Flaviviridae), containing the dengue virus (DENV), yellow fever virus (YFV), and Zika (ZIKV) among others, have been associated with human disease (Vasilakis and Weaver, 2008). *Flavivirus* are grouped taxonomically with regard to their vector and phylogenetic association into tick-borne, mosquito-borne, and viruses with no known arthropod vector, which have little clinical relevance (Fields, Knipe and Howley, 2013). *Flavivirus* are small (40-50nm), spherical, host-derived lipid enveloped with a single-stranded RNA positive sensed genome of approximately 11 kilobases that is made up of three structural protein genes (capsid, C, prM, the precursor of membrane protein, M, and envelope protein, E) and seven non-structural protein genes (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) (Vasilakis and Weaver, 2008).

The dengue virus has four different serotypes antigenically related (DENV-1, -2, -3, and -4) and several genotypes related on phylogenetic analysis (Table 1.1) (Vasilakis and Weaver, 2008). A new serotype antigenically different to the others, DENV-5, was discovered in 2013, however its epidemiological relevance remains unclear (Normile, 2013). Similarly, little is known about the genetic variation of the Zika virus but recent studies have revealed that ZIKV strains can be grouped into two major genetic lineages: African and Asian and that strains derived from the Asian lineage caused the 2015-2016 American epidemic (Yun *et al.*, 2016; Song *et al.*, 2017).

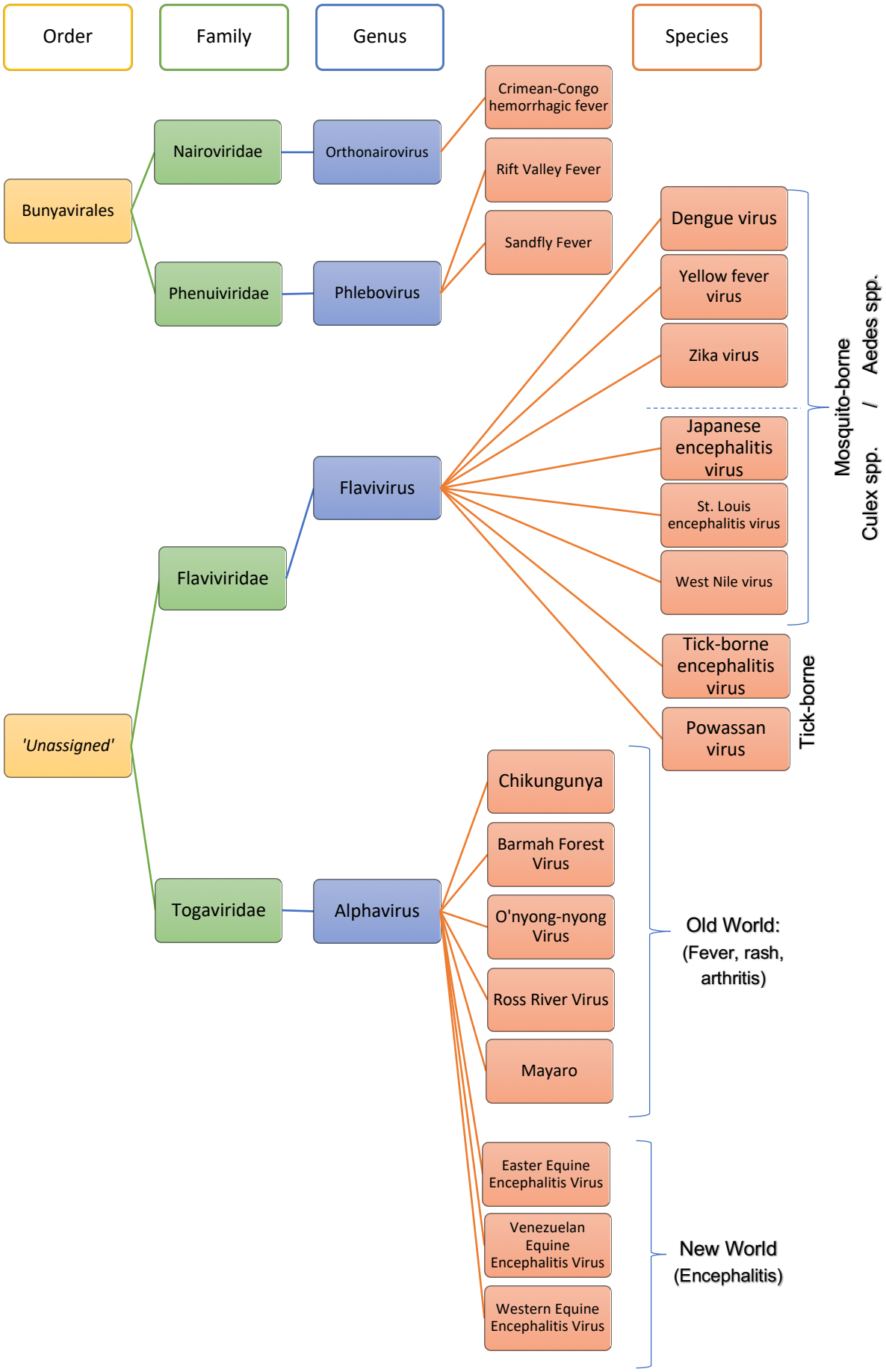


Figure 1.2. Schematic classification of principal human arboviruses

Table 1.1. Different genotypes present in America and sylvatic strains (Vasilakis and Weaver, 2008).

Serotype	Genotype DENV in the Americas	Genotype sylvatic DENV strains
DENV1	Genotype V	Genotype III
DENV2	Genotype 3 (older strains) / Genotype 4 (last 20 years)	Genotype 5
DENV3	Genotype IV	Not isolated
DENV4	Genotype II	Genotype IV

The family *Togaviridae*, to which chikungunya virus (CHIKV) belongs, comprises two genera: *Alphavirus* and *Rubivirus*, the latter containing a single non-arbovirus member: Rubella virus (Fields, Knipe and Howley, 2013). *Alphavirus* have been categorised into two groups based on their historical presence and main clinical presentations: Old World alphaviruses including chikungunya virus, characterised by fever, rash and arthritic symptoms and New World alphaviruses characterised by encephalitis (Figure 1.2). Chikungunya virus are small (60-70nm), spherical, host-derived lipid enveloped, with a single-stranded RNA positive-sensed genome of approximately 11.5 kilobases that is made up of five structural protein genes (capsid protein C, E1 glycoprotein that is responsible for membrane fusion during virus entry; precursor protein pE2, yielding glycoproteins E3 and E2, and peptide 6k) and four non-structural proteins (nsP1-4) (Strauss and Strauss, 1994; Jose, Snyder and Kuhn, 2009). Four different genotypes of chikungunya have been described: East Central South African (ECSA), West African, Asian lineage, responsible for the latest outbreak in the Americas, and the Indian Ocean lineage (IOL) carrying the mutation E1/226V responsible for an enhanced transmission by *Ae. albopictus* (Weaver and Forrester, 2015).

1.2.2. Epidemiology, methods of transmission and clinical symptoms

Arboviroses are diseases caused by different viruses and transmitted by different arthropods but all cause similar symptoms and characteristics that make them difficult to be diagnosed accurately. None of these diseases have effective treatment and only a few have safe vaccines including yellow fever, Japanese encephalitis and more recently dengue but only in particular cases (Sabchareon *et al.*, 2012; Fields, Knipe and Howley, 2013; Wilder-Smith, 2014).

Most arboviruses were first recognised in the 1940-50's, however, debilitating polyarthralgia with sudden fever onset diseases have been described in past

centuries with the earliest report found in China in 265-420AC (Vasilakis and Weaver, 2008). These diseases have traditionally been considered to be dengue but since arboviroses share many symptoms, historical outbreaks could have been caused by an array of viruses such as dengue, chikungunya, Mayaro virus or other undifferentiated tropical febrile illness (UTFI) (Carey, 1971; Kuno, 2009; Petersen and Powers, 2016; Mattar *et al.*, 2017).

Isolated for the first time in 1943 (*Nature.com*, 2017), dengue virus was believed to have originated in Africa, but recent evidence suggests that it originated in Asia and spread worldwide from the initial sylvatic location (Vasilakis and Weaver, 2008). The virus and mosquito vector were likely spread into the Americas in the 1600-1700's with the increase in the trade routes and created a number of epidemics (Appendix 1). Also known as 'break-bone fever' after an epidemic in Philadelphia in the late 18th century for the painful articular symptoms (Vasilakis and Weaver, 2008), the disease pattern associated with dengue-like illness from 1780 to 1940 was characterized by infrequent but large epidemics around port cities (Gubler, 1998a). The global expansion of dengue was favoured by the ecological disruption in South-East Asia and Pacific islands caused by the movements of population, personnel and equipment during World War II (Gubler, 1997). With an increasing concern for mosquito borne-viruses in the Americas and following earlier mosquito eradication programmes led by Oswaldo Cruz and the Rockefeller Foundation that targeted *Aedes aegypti* originally as the vector of yellow fever (Halstead, 2006), the World Health Organization initiated the Continental Eradication Plan in 1947 (Vasilakis and Weaver, 2008). The programme was deemed a success in 1962 when it achieved eradication of the mosquito *Ae. aegypti* in 18 countries except the United States and some Caribbean islands and only one dengue serotype remained in circulation in the continent: DENV2, genotype V (Gubler, 1997; Halstead, 2006; Vasilakis and Weaver, 2008). The abandonment of the control programmes led to the re-invasion of the mosquito vector in the 1970's and an increase in the number of cases and epidemics (Gubler, 1998a). In 1963, Asian DENV3 genotype V was introduced in the Caribbean and in 1981, both DENV-4 genotype I and DENV-2 genotype III were causing new outbreaks followed by DENV-4 that spread rapidly westward to other islands in the region (Gubler, 1997; Halstead, 2006). This trend accelerated in the 1990's creating a situation of hyperendemia (the co-circulation of multiple dengue virus serotypes) in the American continent with nearly all countries reporting cases of dengue fever or severe dengue by the early 2000's (Gubler, 1998a, 1998b;

Mackenzie, Gubler and Petersen, 2004; Rezza, 2014; Rodríguez-Morales, 2015; Wearing, Robert and Christofferson, 2016).

Another relevant arbovirus in the Americas in recent times, chikungunya virus (CHIKV), was first isolated in Tanzania in 1952 with human cases mainly restricted to numerous but small outbreaks in Africa, followed by massive outbreaks in Thailand and in India in the late 1950s through the 1970s (Weaver and Lecuit, 2015; Petersen and Powers, 2016). For unknown reasons, outbreaks in India abruptly stopped for 32 years until 2005 (Lahariya and Pradhan, 2006; Petersen and Powers, 2016). The name chikungunya, meaning 'that who bends up' in the Bantu language from the Makonde people, was given as a result of the contorted posture adopted by those who suffered the painful disease (Lumsden, 1955; Robinson, 1955). The eastern, central and southern African (ECSA) enzootic genotype was first introduced into Asia in the 19th century and originated from the distinct Asia lineage that have caused outbreaks in India and Southern Asia and continues to circulate in the latter region (Weaver and Lecuit, 2015). In 2004-2005, the ECSA lineage spread into Africa, Indian Ocean islands and India and, by several factors, including a mutation in the E1 envelope glycoprotein for an enhanced transmission by the mosquito *Aedes albopictus,* this new Indian Ocean lineage (IOL) caused explosive epidemics with high attack rates in Asia (Schuffenecker *et al.*, 2006; Weaver *et al.*, 2012; Weaver and Lecuit, 2015) and was also responsible for the brief outbreaks in Italy in 2007 (Rezza *et al.*, 2007; Moro *et al.*, 2010). The latest outbreak throughout the Caribbean and Central America in 2014-2015 was caused when an Asian-lineage chikungunya virus strain was introduced into St. Martin (Leparc-Goffart *et al.*, 2014; Weaver and Lecuit, 2015).

Zika virus, a virus closely related to dengue, remained unknown since its discovery in 1947 in the Ziika forest of Uganda with no significant outbreaks reported for 60 years (Weaver *et al.*, 2016). It re-emerged in 2007 and caused a series of epidemics in Gabon, Micronesia, French Polynesia and Oceania (Duffy *et al.*, 2009; Weaver *et al.*, 2016), and in late 2014 it was introduced to Brazil during an international athletic competition (Musso, 2015), from where rapidly spread to over 48 different countries causing over 170,000 confirmed cases as of November 2016 (Song *et al.*, 2017). Initially considered a relatively mild dengue-like disease, Zika was soon linked to an increase in the number of rare congenital microcephaly and Guillain-Barré syndromes (Weaver *et al.*, 2016; Song *et al.*, 2017) causing what some authors

described as ‘an unprecedented epidemic in Latin American threatening North America’ (Fauci and Morens, 2016).

Similar to other arboviruses, DENV, CHIKV and ZIKV circulate in two distinct cycles (Table 1.2): enzootic transmission among non-human primates by tree dwelling *Aedes* spp. mosquitoes in African and south-east Asian sylvatic foci and urban transmission among humans by *Aedes aegypti* and *Aedes albopictus* (Vasilakis and Weaver, 2008; Weaver and Reisen, 2010; Simon Djamel Thiberville *et al.*, 2013; Coffey, Failloux and Weaver, 2014; Petersen and Powers, 2016). Sylvatic and urban DENV strains are ecologically and evolutionary distinct lineages and there is insufficient evidence to link sylvatic cycles with major outbreaks of human dengue with the former confined to forest habitats that produce relatively mild illness (Vasilakis and Weaver, 2008). Infection of other species such as domestic dogs is rare (Thongyuan and Kittayapong, 2017).

Table 1.2. Geographical distribution of sylvatic and urban cycles of DENV, CHIKV and ZIKV (Vasilakis and Weaver, 2008; Althouse *et al.*, 2016)

Virus	Sylvatic cycle (non-human primates)	Urban cycle (human)
DENV	Africa, South-east Asia	Worldwide within the tropics
CHIKV	Africa	Asia, Indian Ocean, Africa, Southern Europe, America
ZIKV	Africa, Asia (?)	Africa, South-east Asia, America

The epidemiology of arboviruses is associated to the behaviour and ecology of their vector. Rainfall and temperature have a substantial impact in vector productivity and survival, viral replication and infective periods of DENV, CHIKV and ZIKV, and weather variability as a predictor of viral activity has been extensively studied (Gubler *et al.*, 2001; Patz and Reisen, 2001; Reiter, 2001; Wu *et al.*, 2007; Wilder-Smith and Gubler, 2008; Earnest, Tan and Wilder-Smith, 2012). Further, the impact of human and economic factors in the epidemiology of dengue has also been widely reported in the literature (Reiter *et al.*, 2003; Wearing and Rohani, 2006; David, Lourenço-de-Oliveira and de Freitas, 2009; Teurlai *et al.*, 2015). These characteristics of the epidemiology of arboviruses will be discussed in more detail in section 5.4.

The high rate of subclinical dengue infections, estimated to range between 20% to 90% (Grange *et al.*, 2014), has an important role in the epidemiology of dengue as asymptomatic individuals are significantly more infectious to mosquitoes and also

may be exposed to more mosquitoes through their undisrupted daily routines than symptomatic individuals (Grange *et al.*, 2014; Duong *et al.*, 2015). In addition, social connections and routine movement of people among the homes of family and friends also have a key role in the spread of infections (Stoddard *et al.*, 2013). Likewise, it has been suggested that vertical transmission of the dengue virus from a female mosquito to her progeny could sustain the infection in a geographical area during inter-epidemic periods (Martins *et al.*, 2012). Vertical transmission has been demonstrated in *Ae. aegypti* and *Ae. albopictus* mosquitoes with minimum infection rates for dengue of 1.01% to 1 per 2,755 mosquitoes (Fouque, Garinci and Gaborit, 2004; Le Goff *et al.*, 2011; Ciota *et al.*, 2017), of CHIKV in *Ae. aegypti* (Jain *et al.*, 2016) and recently ZIKV (Pereira-Silva *et al.*, 2017).

Although these diseases share many aspects of their epidemiology and all can be fatal to humans, their incidence rates are variable. It has been suggested that CHIKV may be less of a problem than DENV as lower incidence rates have been observed overall even in regions with lower herd immunity, indicating that the co-circulation of different DENV serotypes and the ability of immune enhancement probably give an edge to DENV as a human pathogen (Weaver and Forrester, 2015). The seroprevalence of dengue in adult populations in endemic areas has been estimated to be greater than 90% (Brown *et al.*, 2009; Morrison *et al.*, 2010; Leslie *et al.*, 2014). Whereas the seroprevalence of chikungunya in adult populations has been recorded as 68% in India (Kumar *et al.*, 2011) and as high as 70-85% in Bangkok, suggesting long-standing endemicity in those areas (Petersen and Powers, 2016).

Methods of transmission

The main method of transmission of DENV, CHIKV and ZIKV is through bites by the mosquitoes *Aedes aegypti* and/or *Aedes albopictus*. After ingestion of a blood meal from an infected host, the virus travel into the mosquito midgut (Figure 1.3, Nos.1 & 2). After several days, the virus disseminate into the haemolymph in the hemocoele (Figure 1.3, No. 3) to distal tissues infecting the salivary glands (Figure 1.3, No. 4) (Salazar *et al.*, 2007; Conway, Colpitts and Fikrig, 2014). The two genetic bottlenecks identified in the mosquito, midgut barrier and saliva gland barrier, occurring when a virus is unable to replicate in such cells, suggest that certain viral genotypes have been selected to infect these organs and become infectious to a host (Salazar *et al.*, 2007; Forrester *et al.*, 2012). Finally, infected mosquitoes inoculate virus-infected saliva into extravascular spaces in the dermis when probing

host skin for a source blood (Figure 1.3, No. 5). The time between ingestion of a virus and the moment when it will be infectious for the next vertebrate host is known as the 'extrinsic incubation period' (EIP) (Salazar *et al.*, 2007).

Mosquito saliva plays a significant role in the modulation of arbovirus infectivity and transmission (Conway, Colpitts and Fikrig, 2014). The bite of a mosquito and subsequent injection of saliva into a human will trigger a reaction that induces the migration of Langerhans cells and macrophages towards the site of infection. Langerhans cells are targets of DENV infection (Wu *et al.*, 2000) and, whereas macrophages may serve to control infection at this point (Fink *et al.*, 2009), induction of immune cells increases the probability of dissemination of virions in the host (Conway, Colpitts and Fikrig, 2014).

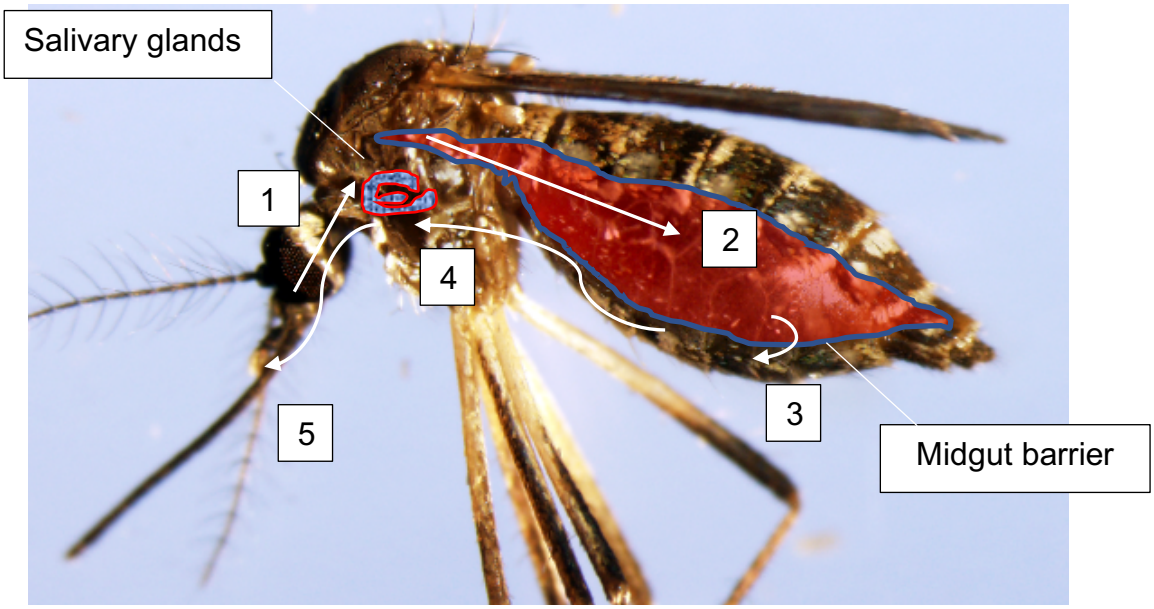


Figure 1.3. Arbovirus migration in Aedes aegypti: (1) Introduction of virus during blood ingestion. (2) Progression to midgut (3) Dissemination of virus into hemocoele (4) Introduction of virus into salivary glands (5) Infection of a new host via blood meal (Source: personal collection).

Non-vector transmissions of arboviruses are rare and the implications in their epidemiology are minimal. Some cases of DENV infection through blood transfusions have been reported (Stramer *et al.*, 2012; Rooks *et al.*, 2016), and of CHIKV through neonatal transmission which can lead to severe encephalopathy and long-term neurologic sequelae (Gérardin *et al.*, 2008; Weaver and Lecuit, 2015). Other rare forms of CHIKV transmission have been reported in laboratory immunosuppressed mice through saliva and the virus has also been isolated from saliva in macaques and humans with the rare haemorrhagic form (Gardner *et al.*, 2015). Direct human-to-human transmission of ZIKV has been documented to occur perinatally, from an infected mother to her foetus during pregnancy; sexually, with the male-to-female

transmission occurring more frequently than other combinations, and through breastfeeding or blood transfusion (Song *et al.*, 2017). In addition to semen, ZIKV has also been detected in urine, saliva, and nasopharyngeal swabs (Leung *et al.*, 2015; Atkinson *et al.*, 2016; Barzon *et al.*, 2016).

Clinical symptoms

Both DENV and ZIKV infections can be asymptomatic in up to 90% of the cases (Grange *et al.*, 2014) with a symptomatic to inapparent ratio of 1:8 (Burke *et al.*, 1988) in contrast with CHIKV where symptoms occur in 72-97% of infections (Brouard *et al.*, 2008; Moro *et al.*, 2010; Kumar *et al.*, 2011; Thiberville *et al.*, 2013), although in endemic areas asymptomatic infections may also be as high as 58% (Kuan *et al.*, 2016) or even 80% (Yoon *et al.*, 2015).

Dengue

The incubation period of dengue lasts around 4-7 days with a median incubation period of 5.6 days (Rudolph *et al.*, 2014). Dengue manifests a wide range of clinical presentations that last for about 7 days. The typical symptoms are summarized in Table 1.3 and comprise high fever of sudden onset (above 39^{o}C), muscular and articular pain, retro-orbital pain, headache, malaise, and on occasions, rash, nauseas and / or diarrhoea (World Health Organization, 2009). Acute dengue virus infections are rarely associated with neurological symptoms and direct dengue virus infection of the nervous system remains controversial (Sahu *et al.*, 2014).

Severe dengue, formerly known as dengue haemorrhagic fever or DHF (Horstick *et al.*, 2014) is principally associated with secondary heterotypic infection and occasionally with primary infection of children (Halstead, Nimmannitya and Cohen, 1970). Ethnicity has also been suggested to affect the development of severe dengue after significantly lower rates of severe dengue were observed in individuals of African ancestry than of other ethnic groups (Sierra, Kourí and Guzmán, 2007). Severe dengue develops after the fever phase and can be manifested by plasma leakage and haemorrhages in mucosal tissues (typically gums, nose, eyes and others) that may evolve into a dengue shock syndrome (DSS) requiring hospitalization and may lead to a fatal course in around 1-5% of the cases (World Health Organization, 2009). Severe dengue caused during a secondary infection with a different serotype is attributed to antibody-dependent enhancement (ADE), which occurs when non-neutralizing antibodies produced during a primary infection increase intracellular infection and leads to higher viral production, supress antiviral

immune response and generate inflammatory cytokines during infection (Halstead, 1979; Guzmán and Vázquez, 2010), a concern also raised during the development of dengue vaccines (Halstead *et al.*, 2005). Being able to discern between primary or secondary infections and the cause of infection is important for the prognosis and clinical management of a case and surveillance support (Guzmán and Kourí, 2004) as severe dengue can progress from a non-specific syndrome to irreversible shock and death within hours (Gubler, 2002).

Table 1.3. Criteria for dengue and severe dengue (World Health Organization, 2009)

CRITERIA FOR DENGUE		CRITERIA FOR SEVERE DENGUE
Probable dengue Live in /travel to dengue endemic area. Fever and 2 of the following criteria: • Nausea, vomiting • Rash • Aches and pains • Tourniquet test positive • Leukopenia • Any warning sign	Warning signs: • Abdominal pain or tenderness • Persistent vomiting • Clinical fluid accumulation • Mucosal bleed • Lethargy, restlessness • Liver enlargement • Laboratory: increase in HCT concurrent with rapid decrease in platelet count	One or more of the following: (i) plasma leakage that may lead to shock and/or fluid accumulation, with or without respiratory distress (ii) severe bleeding (iii) severe organ impairment.

Chikungunya

The incubation period of chikungunya lasts between 2-12 days with a median incubation time of 3.0 days (Rudolph *et al.*, 2014). Chikungunya is characterised by an abrupt onset of fever (39°C-40°C) and coincides with high viremia levels that can reach up to 10^9 viral genome copies per millilitre of blood. Shortly after the onset of fever, severe and painful myalgia and arthralgia develop in the large and smaller peripheral joints in arms and legs (Suhrbier, Jaffar-Bandjee and Gasque, 2012) and, occasionally, the vertebral column (Staikowsky *et al.*, 2009). The intensity of the infection correlates with viremic load, and symptoms usually lasts one week, until viremia ends (Staikowsky *et al.*, 2009; S D Thiberville *et al.*, 2013; Weaver and Lecuit, 2015). Maculopapular rash appears in 40-50% of the cases (Suhrbier, Jaffar-Bandjee and Gasque, 2012) although other authors suggest it can occur in as low as

19% (Powers, 2010) or as high as in 80% of chikungunya cases, typically on the trunk, but also on the face, arms, legs, soles or palms (Weaver and Lecuit, 2015). Severe chikungunya is rare but can be manifested with multi-organ failure, especially in older patients with coexisting medical conditions, requiring hospitalization and may result on a fatal course (Weaver and Lecuit, 2015). The disease and economic burdens of chikungunya result from the high attack rate and the long-term sequelae in the form of chronic pain in distal joints that can last for several months and lead to incapacitation and long-term treatment (Schilte *et al.*, 2013; Weaver and Lecuit, 2015; van Aalst *et al.*, 2017).

Zika

The incubation period of Zika lasts an average of 5.9 days (Lessler *et al.*, 2016). Summarizing Song *et al*. (2017) review of existing Zika studies, the most common symptoms of Zika are non-specific and resemble a flu-like infection, including mild fever, itchy rash, and non-purulent conjunctivitis; on occasions, arthralgia, retro-orbital pain, headache, and myalgia can be seen (Cao-Lormeau *et al.*, 2014; Musso, 2015). In rare cases, serious consequences may develop including Guillain-Barré syndrome (Sudre *et al.*, 2014), an autoimmune disease causing muscle paralysis that has also been associated in rare cases with infection by dengue (Carod-Artal *et al.*, 2013) and chikungunya virus (Wielanek *et al.*, 2007), and microcephaly in new-borns from Zika infected mothers, a neurological condition in which the brain of a baby remains undeveloped causing the head to be smaller than normal (Klase *et al.*, 2016).

1.3. Vector:

1.3.1. Main arboviral vectors

The main vectors of human arboviroses comprise the mosquitoes *Aedes* spp. and *Culex* spp. and ticks of the genus *Ixodes* spp. (Table 1.4). Similar to other arboviruses, humans are not dead-end hosts of dengue, chikungunya or Zika, but contribute to the transmission cycle by infecting *Aedes aegypti* mosquitoes (Vasilakis and Weaver, 2008; Petersen and Powers, 2016). The modern species *Aedes aegypti aegypti* evolved from tree dwelling mosquitoes in Africa (*Ae. aegypti formosus)*, and lives in close contact with people in urban settings by relying on artificial water containers for its larval habitats (Tabachnick and Powell, 1979; Vasilakis and Weaver, 2008). The latter sub-species uses tree holes as larval development habitats and currently encompasses both wild and domestic

populations across Africa, while *Ae. aegypti aegypti* has a nearly worldwide tropical and subtropical distribution, mostly between latitudes 35ºN and 35ºS, with high degrees of anthrophily in all locations (Brown *et al.*, 2011). These geographical limits correspond approximately to a winter isotherm of 10ºC (Brady *et al.*, 2013). Also, because of lower temperatures, *Ae. aegypti* is relatively uncommon above 1,000 metres (World Health Organization, 2009).

Table 1.4. Main human arboviruses, vectors and principal hosts (Conway, Colpitts and Fikrig, 2014; Kunze, 2016; Lednicky *et al.*, 2016)

Virus	Main Vectors	Main hosts	Endemic regions
Dengue virus	*Aedes* spp.	Primates, humans	Africa, Asia, South America, Pacific
Yellow fever virus	*Aedes* spp.	Primates, humans	Africa, South America
Chikungunya virus	*Aedes* spp.	Primates, bats, rodents	Africa, Asia, Americas
Zika virus	*Aedes* spp.	Primates, humans	Africa, Americas
West Nile virus	*Culex* spp.	Birds	Europe, North America, Africa, Asia
Japanese encephalitis virus	*Culex* spp.	Birds, pigs	Asia
St. Louis encephalitis virus	*Culex* spp.	Birds	Americas
Western equine encephalitis virus	*Culex* spp.	Birds	Americas
Venezuelan equine encephalitis virus	*Culex* spp., *Aedes* spp.	Rodents	Americas
Eastern equine encephalitis virus	*Culex* spp., *Aedes* spp.	Birds, rodents	Americas
Mayaro virus	Haemongus spp., *Aedes* spp.	Primates, birds, reptiles	Americas
Rift Valley fever virus	*Culex* spp., *Aedes* spp.	Ruminants	Africa, Asia
Tick-born encephalitis virus	Ixodes spp.	Birds, ruminants, humans	Central and North Europe, Asia

Another important mosquito vector is *Aedes albopictus,* also known as Asian tiger mosquito, an aggressive biting mosquito that can feed opportunistically from a wide range of hosts outdoors (Paupy *et al.*, 2009) and survive lower temperatures than

Aedes aegypti (Brady *et al.*, 2013) (Table 1.5). *Aedes aegypti* and *Aedes albopictus* are closely related and although they can sustain cross-species inseminations (Bargielowski, Lounibos and Carrasquilla, 2013), the species differ in their behaviour and biology and occupy different niches (Eisen and Moore, 2013; Kraemer, Sinka, Duda, A. Q. N. Mylne, *et al.*, 2015) which has commonly resulted in inter-species competition and the displacement of *Ae. aegypti* by *Ae. albopictus* (O'meara *et al.*, 1995; Juliano, Lounibos and O'Meara, 2004).

Table 1.5. Summary of main differences between the ecologies of Ae. aegypti and Ae. albopictus.

Ae. aegypti	*Ae. albopictus*
Tropical and subtropical	Tropics and temperate regions
Feeds almost exclusively on humans	Feeds opportunistically
Feeds mainly indoors	Feeds outdoors
Takes multiple blood meals within a gonotrophic cycle	Usually takes a single blood meal within a gonotrophic cycle
Exploits artificial water containers near houses as larval habitats	Uses artificial and natural larval habitats
Moderately susceptible to CHIKV	Moderately to highly susceptible to CHIKV

Finally, there have been some reports indicating the role of *Culex quinquefasciatus* as a competent vector for Zika (Guo *et al.*, 2016). However, several experimental studies have demonstrated that *Culex* spp. mosquitoes are incompetent vectors of ZIKV transmission, and conclusive evidence of natural transmission during outbreaks remains elusive (Vasilakis and Weaver, 2016). Other mosquito vector species are summarised in Table 1.6.

Table 1.6. Principal mosquito vectors of dengue, chikungunya and Zika (Coffey et al., 2014)

Mosquito species	Virus	Source	Reference
Aedes aegypti formosus	CHIKV	Africa	(Tabachnick and Powell, 1979)
Aedes albopictus	DENV	Laboratory	(Tesh, Gubler and Rosen, 1976)
Aedes albopictus	CHIKV	Italy	(Moro *et al.*, 2010)
Aedes albopictus	ZIKV	Gabon	(Wong *et al.*, 2013)
Aedes furcifer	CHIKV	South Africa	(Jupp *et al.*, 1981)
Aedes hensilli	CHIKV	Micronesia	(Ledermann *et al.*, 2014)
Aedes hensilli	ZIKV	Yap, Micronesia	(Duffy *et al.*, 2009)

Mosquito species	Virus	Source	Reference
Aedes polynesiensis	ZIKV	French Polynesia	(Musso, Nilles and Cao-Lormeau, 2014)
Aedes vexans	CHIKV	Italy	(Talbalaghi *et al.*, 2010)
		France	(Vazeille *et al.*, 2008)
Culex pipiens	CHIKV	Italy	(Talbalaghi *et al.*, 2010)
		France	(Vazeille *et al.*, 2008)
Culex quiquefasciatus	CHIKV	Zimbabwe	(Jupp *et al.*, 1981)

1.3.2. *Aedes aegypti* morphology and classification

There are over 3,600 species of the family Culicidae described divided in two subfamilies, the Anophelinae, which contains one genus: *Anopheles*, and the Culicinae, which includes the genera: *Aedes,* and *Culex,* amongst others (Wilkerson *et al.*, 2015). There are 11 tribes within Culicinae, which Aedini is the largest. Several re-classifications have been proposed for the mosquito *Aedes (Stegomyia) aegypti* since 2000 which was renamed *Stegomyia aegypti* (Reinert, Harbach and Kitching, 2004) although, the former denomination derived from Knight and Stone (1977) is currently accepted and used extensively (Wilkerson *et al.*, 2015).

Morphology and flight range

Mosquitoes are slender and relatively small (3-6mm in length). Adult *Ae. aegypti* can be distinguished by the characteristic white scales on the dorsum with the shape of a lyre over a black scutum and the particular patterns of white scales on the side of their thorax (Figure 1.4, A) represented by a pair of narrow sub-median lines composed of slender yellowish-white scales (Service, 2012). Other identifying features include a dark-scaled proboscis, short and dark palpi, tipped with silver-white scales and broad appressed silver white scales can also be seen on the clypeus (Figure 1.4, B) and an arrangement of black and white scales on the legs. (Carpenter and LaCasse, 1974; Darsie and Ward, 2005) (Figure 1.4, C). Although a potential flight range in *Aedes aegypti* of up to several kilometres has been described (Appendix 1), female mosquitoes will rarely fly further than 30-50m to their adjacent house (Trpis and Hausermann, 1986; Harrington *et al.*, 2001, 2005; Getis *et al.*, 2003) and only when houses are clustered further apart, or need to search for more suitable homes, mosquito dispersal will be larger (Tsuda *et al.*, 2001) suggesting that

people are the primary mode of virus dissemination rather than mosquitoes (Harrington *et al.*, 2005).

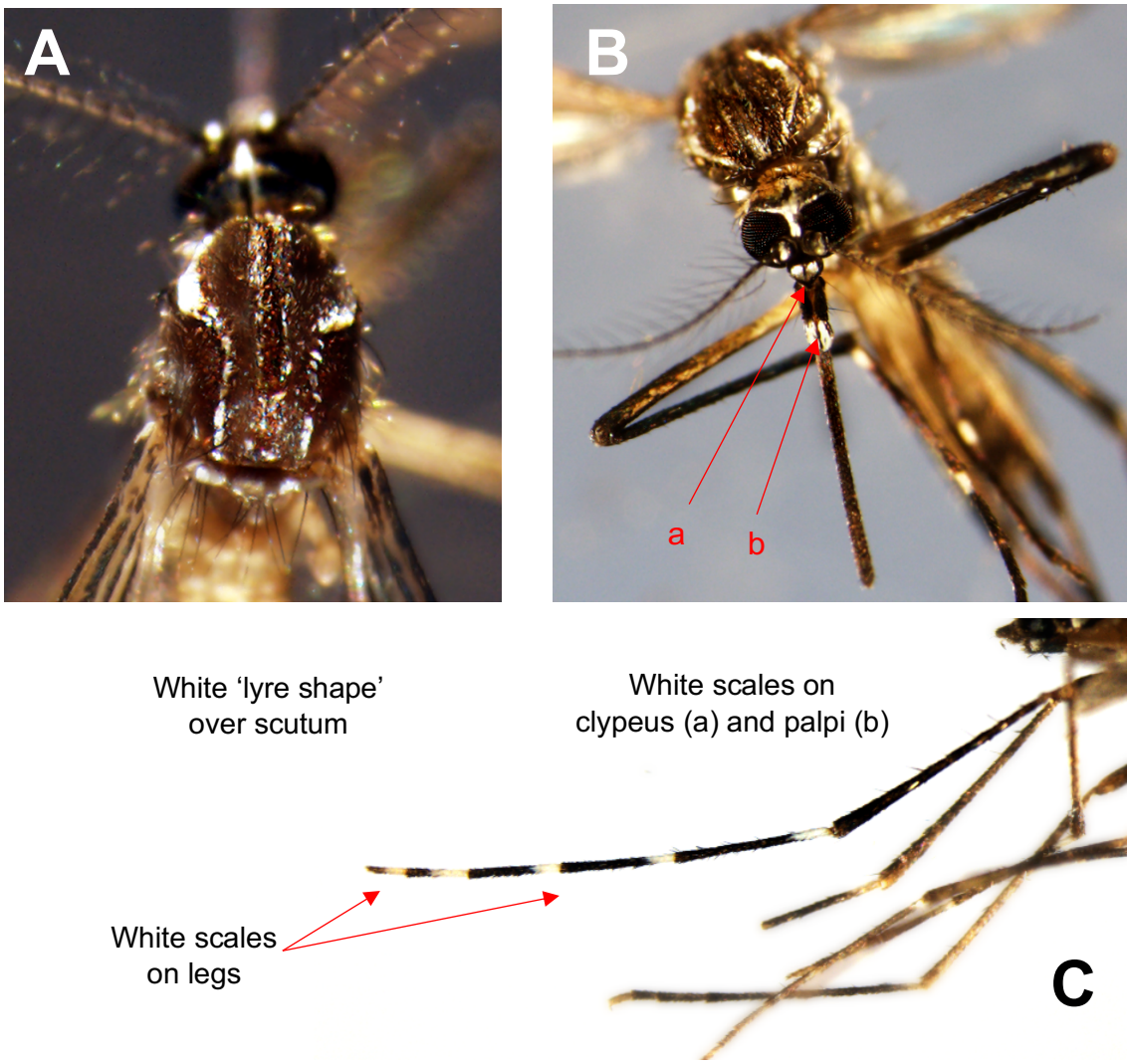


Figure 1.4. Morphologic characteristics of Ae. aegypti (Source: personal collection).

1.3.3. *Aedes aegypti* ecology and vectorial capacity

Vectorial capacity is the ability of a vector in transmitting effectively a virus, and depends on the vector competence, the interactions of the vector with the pathogen and with the host, the size of the vector population, their life span, feeding behaviours and daily activity (Turell, 2016). Vectorial capacity (V) was first expressed by Garret-Jones (1964) from the classic Ross-McDonald model, and later adapted by Gething *et al.* (2011) and modified by Brady *et al.* (2014) as:

$$V = \frac{m\,a^2}{g} \mathrm{e}^{-gn} \qquad (1)$$

where *m* is the ratio of adult female mosquitoes to humans, *α* is the human blood feeding rate, *g* is the instantaneous per-capita death rate of adult female mosquitoes and *n* is the length of the incubation period of the virus in the mosquito and all of the parameters are affected by temperature (Brady *et al.*, 2014).

Gonotrophic cycle

The vectorial capacity is strongly associated with human biting during the gonotrophic cycle (GC) which involves an unfed female taking a blood meal (blood-fed), progressive development of eggs (half-gravid), followed by full egg development (gravid) and oviposition (Service, 2012). The duration of each stage and the number of times each cycle occur within a female lifespan are influenced by temperature (Table 1.7 & Figure 1.5). Experiments in the Caribbean island of Guadeloupe demonstrated that the GC in *Ae. aegypti* can range from 4.9 days at 30ºC to 8.4 days at 24ºC, the number of GC per female peaked at 27ºC, and that the duration of the first GC was significantly longer than the second GC (Goindin *et al.*, 2015). Other authors have demonstrated that the duration of the GC also varies with body size: experiments in Penang, Malaysia, showed that the mean duration of the GC for large mosquitoes was around 2.5 days and of 3 days for small ones and, although one individual achieved eight GC in the experiment, most died at the fourth GC (Saifur *et al.*, 2012). Furthermore, female *Ae. aegypti* field parity rates and fecundity increased at mean temperatures of 27ºC, producing a greater number of progeny (Goindin *et al.*, 2015). Shorter cycles and a greater number of cycles increase the capacity of infected mosquitoes to transmit viruses.

Table 1.7. Duration of GC at different temperatures. GC: gonotrophic cycle. Field parity rates: proportion of females having already deposited a batch of eggs. Fecundity: number of eggs deposited per female (Goindin et al., 2015).

	24ºC	27ºC	30ºC
Duration GC	8.4 days (±2.7)	7.1 days (±3.4)	4.9 days (±1.1)
Females 2GC	28%	43%	39%
Females 3GC	0	10%	3%
Field Parity rates	35.8%	70.6%	64.2%
Fecundity	54	62	53

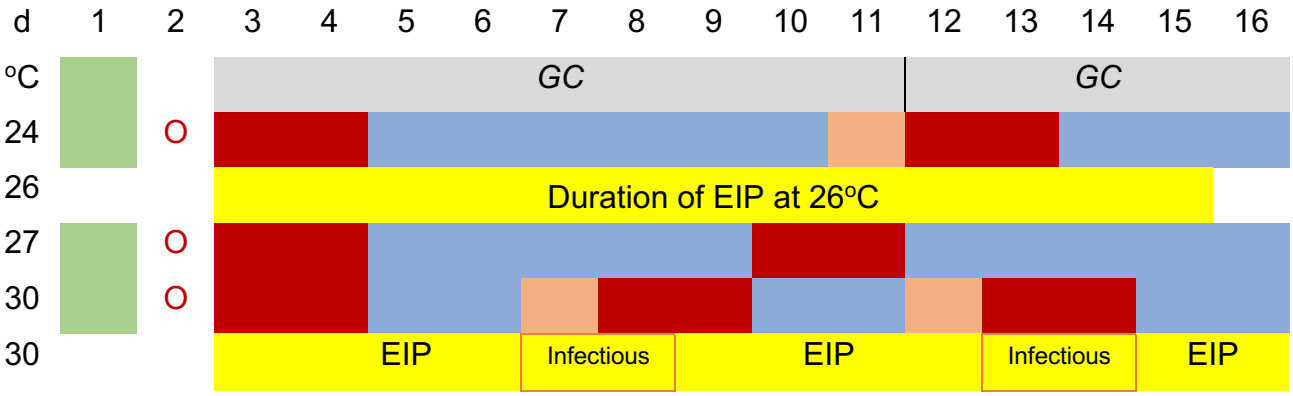


Figure 1.5. Schematic representation of the duration in days of Ae. aegypti Gonotrophic Cycle (GC) and Extrinsic Incubation Period (EIP) of DENV at different temperatures. Green cells: Emergence of adult mosquitoes from pupa. Red circles: Mating. Red cells: Blood feeding. Blue cells: Resting period. Orange cells: Oviposition. Yellow boxes: approximate duration of extrinsic incubation period at 26ºC and 30ºC (Goindin et al., 2015).

The GC commences after emergence from the pupa stage when a female mosquito must bite a suitable host and take a blood meal to obtain the proteins for the development of her eggs (Klowden, 1995). *Ae. aegypti* has a strong preference for feeding on human blood which is low in the amino acid isoleucine and confers a fitness advantage for *Ae. aegypti* (Harrington, Edman and Scott, 2001) resulting in an estimated probability of feeding on a human of between 0.83 in French Guiana (Fouque, Garinci and Gaborit, 2004) and more than 0.90 in Thailand (Scott, Clark, *et al.*, 1993). Female *Ae. aegypti* can bite multiple times before oviposition as experiments in Thailand and Puerto Rico demonstrated in which *Ae. aegypti* took 0.76 and 0.63 human blood meals on average per day respectively, and in Puerto Rico, 32% of the females took double meals and 2% took triple meals during the same GC (Scott, Chow, *et al.*, 1993; Scott, Clark, *et al.*, 1993; Scott, Amerasinghe, *et al.*, 2000). It has been reported that *Aedes* has a low susceptibility to dengue and Zika viruses (Reiter, 2007; Chouin-Carneiro *et al.*, 2016) but the reliance on human blood for its energetic needs and its endophilicity result in contacts with multiple hosts during a single gonotrophic cycle (Harrington, Edman and Scott, 2001) suggesting that these behavioural and ecological traits rather than its susceptibility to an arbovirus contribute to a high vectorial capacity of *Ae. aegypti*'s and effective dissemination of the virus as an endemic and epidemic vector (Reiter, 2007; Vasilakis and Weaver, 2008).

Female *Ae. aegypti* will preferably feed indoors and during the day (Harrington, Edman and Scott, 2001) attracted by carbon dioxide, lactic acid, fatty acids, ammonia emanating from their host breath, body odours and warmth (Bosch, Geier and

Boeckh, 2000), and will rest primarily inside the house (mainly in bedrooms and living rooms) until they are ready to oviposit (Scott *et al.*, 2000; Maciel-de-Freitas, Eiras and Lourenço-de-Oliveira, 2006; Reiter, 2007). Female *Ae. aegypti* peak activity coincides with dawn and dusk (Figure 1.6) although atypical blood feeding outside these times has also been reported (Chadee, 2013). *Ae. aegypti* prefer resting over black or dark surfaces but vision plays a minor role in host orientation (de Ázara *et al.*, 2013; Conway, Colpitts and Fikrig, 2014).

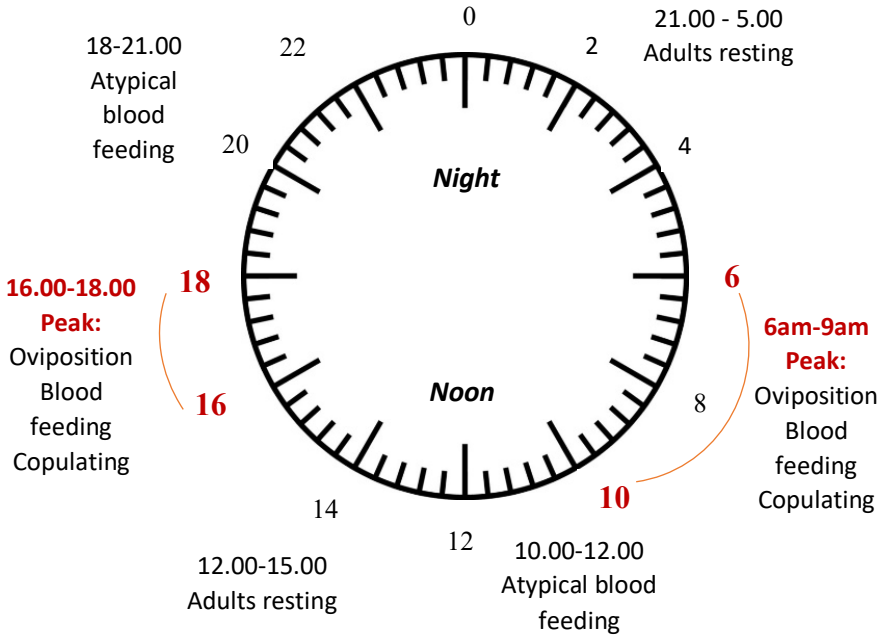


Figure 1.6. Aedes aegypti circadian rhythms in Trinidad. (Adapted from Chadee, 2013).

Female *Ae. aegypti* will preferably lay their eggs outdoors on the surface of artificial sun-exposed containers, rather than shaded, such as water drums, buckets, tyres, water tanks and similar (Focks *et al.*, 1993; Kearney *et al.*, 2009). The type of preferred containers or 'key containers' has extensively been studied and it will determine the productivity of larvae and the body size of adult mosquitoes (Tun-Lin, Kay and Barnes, 1995; Chadee, 2004; David, Lourenço-de-Oliveira and de Freitas, 2009). Females deposit small numbers of eggs in several breeding containers, a process known as 'skip oviposition' (Reiter, 2007) with a peak egg laying activity observed at 26°C (Carrington *et al.*, 2013). The eggs are oval and black in colour and can remain viable for several months in the absence of water contributing to the spread of *Aedes* spp. into new territories by the international trade in used tyres (World Health Organization, 2009). When eggs are submerged in water, they will hatch into larvae and after 6 – 11 days at temperatures of 30°C or 20°C (Saifur *et al.*,

2012) will moult into pupa. Two to three days later adult mosquitoes sexually differentiated into male and female will emerge and repeat this cycle several times during her adult life (Service, 2012).

Mosquito survival and infective life

Vectorial capacity is also influenced by the mosquito survival rate and the duration of infective life (Garrett-Jones, 1964). The optimal range of temperatures for adult mosquito survival is 15ºC to 30ºC (Yang *et al.*, 2009) within a range of 10ºC to 35ºC (Brady *et al.*, 2013). Experiments in the Caribbean island of Guadeloupe demonstrated that half of female *Ae. aegypti* survived more than 38 days at 27ºC whereas half of female *Ae. aegypti* survived less than 25 days at 24ºC or at 30ºC (Goindin *et al.*, 2015). Other field experiments in French Guiana observed a mean longevity of female *Ae. aegypti* of 25.12 days (Fouque *et al.*, 2006). Consequently, the duration of infective life, which is the number of days the mosquito can transmit the disease, is also strongly influenced by temperature and can be as high as 24 days at the optimum temperature of 27ºC and when 70% of the female are parous (Table 1.8) whereas at 20ºC, infective life remains low for low parity rates, but at 30ºC, long infective life of up ot 20 days was correlated with high parity rates (Goindin *et al.*, 2015). Fluctuations of temperature, or diurnal temperature range (DTR), also affects mosquito survival and vector competence of DENV transmission by *Ae. aegypti*. At high DTR, mosquito infection and transmission potential are lower than when the daily temperature is constant or with low variation. (Lambrechts *et al.*, 2011; Carrington *et al.*, 2013).

Table 1.8. Effects of temperature on Ae. aegypti ecology. (Goindin et al., 2015).

	24ºC	27ºC	30ºC
Life expectation at 10 days	24.5 days	32.8 days	20.3 days
Infective life (low – high parity rates)	10 – 15 days	18 – 24 days	10 – 20 days

Although most models use a constant daily survival rate throughout the mosquito life ranging from 0.51 to 0.95 (Table 1.9), it has been suggested that the daily survival rate of *Ae. aegypti* females is significantly lower after day 13 (Harrington *et al.,* 2001), and the survival of both larval and adult forms is U-shaped (Yang *et al.*, 2009).

Table 1.9. Daily Aedes aegypti survival rates (NLE: Non-linear estimates).

Location	Females	Males	Reference:
China	0.763	0.52	Tsuda *et al.*, 2001
French Guiana	0.80-0.95 (mean = 0.913)		Fouque *et al.*, 2006
Rio de Janeiro, Brazil	0.607-0.704 (exp model) 0.659-0.721 (non-linear model)		David, Lourenço-de-Oliveira and de Freitas, 2009
Bootstrap based on NLE	0.509-0.701	0.370-0.541	Buonaccorsi, Harrington and Edman, 2003
Puerto Rico	3-13 days old: 0.735 13-23 days old: 0.356		Harrington *et al.*, 2001
Thailand	3-13 d. old: 0.814-0.843 13-23 d. old: 0.834–0.805		
Australia	0.91 0.86	0.57 0.70	Muir and Kay, 1998

Temperature also enhances virus transmission due to shorter extrinsic incubation period. The EIP for DENV has a variable duration of about 10 days depending on temperature (Fouque *et al.* (2006) citing Watts *et al.* (1987)) although the time until 50% of females carry infectious DENV (EIP_{50}), has been estimated to range between 29.6 days at 20ºC and 5.2 days at 30ºC (Table 1.10) (Lambrechts *et al.*, 2011), and for ZIKV it has been estimated between ~10 days (Hayes, 2009) and 14 days (Chouin-Carneiro *et al.*, 2016).

Table 1.10. Estimated duration of DENV EIP_{50} (Lambrechts et al., 2011).

Temperature	Days
20ºC	29.60
26ºC	11.10
30ºC	5.16
35ºC	5.16

Additionally, rainfall also affects mosquito survival. Alto and Juliano (2001) simulated precipitation at different temperatures and concluded that higher temperatures and no drying conditions increased the number of adults but higher temperatures and drying conditions reduced the number of adults produced, with the exception of 22ºC

when there was no effect of precipitation (Alto and Juliano, 2001). In contrast, Hugo *et al.* (2014) reported higher mosquito survival up to 12 days (an age when mosquitoes were able to transmit dengue) during the dry seasons (92% in the cool season and 64% in the hot season) but survival was reduced to 29% during the wet/cool season in Central Vietnam. Some authors have reported lower mosquito survival at very low and very high rainfall (Fouque *et al.*, 2006), whereas others have suggested that extreme rainfall may have contributed to sustaining high abundance of *Ae. albopictus* during the chikungunya outbreak in France (Roiz *et al.*, 2015) and the dengue outbreak in Guangzhou, China, in 2014 (Cheng *et al.*, 2016). Wong *et al.* (2011) citing Mellor and Leake (2000) reported that the abundance of Aedes mosquitoes increased with rainfall but decreased with higher rainfall and suggested that rain can help build breeding sites but very heavy or prolonged rain may disrupt the sites and wash the eggs and larvae away or kill the mosquitoes (Wong *et al.*, 2011).

Vector control

It has been suggested that despite nearly a century of research, the only tools presently available to combat dengue target mosquito populations and rely mostly on insecticides and larval source reduction (Stoddard *et al.*, 2013). The World Health Organization (2009) outlined the methods of control of mosquitoes based on three broad approaches: environmental management to reduce breeding sites, chemical control of larvae and/or adults and biological control. However, there are concerns of toxicity, effectiveness of application and resistance to chemical substances, and biological control based on the introduction of organisms that prey upon, parasitise, compete with or reduce populations of the Aedes mosquito have been reported as successful in reducing mosquito populations and transmission of dengue and chikungunya, in addition to infections of mosquitoes with the bacterium Wolbachia that reduce the reproductive capacities of the mosquito and increase their resistance to arboviruses. (McMeniman *et al.*, 2009; Moreira *et al.*, 2009; Kamtchum-Tatuene *et al.*, 2017). Finally, integrated vector management tailored to local eco-epidemiological and sociocultural settings and combined with educational programmes has been reported as the most effective method to reduce entomological indices (Erlanger, Keiser and Utzinger, 2008).

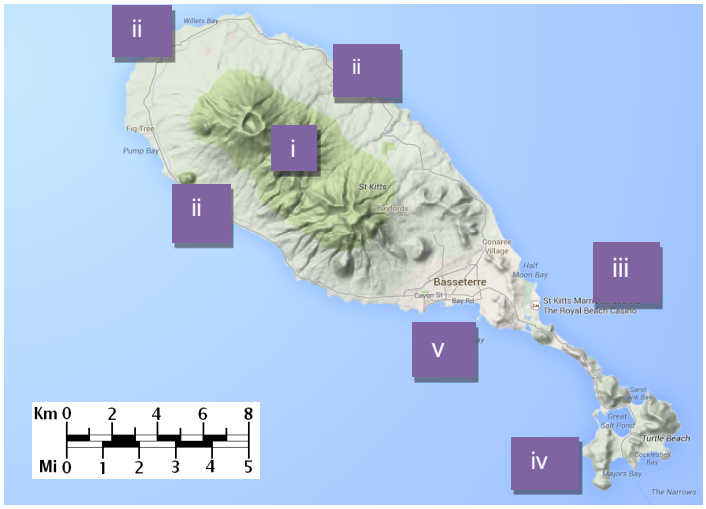
1.4. Saint Kitts and Nevis

The twin island Federation of Saint Kitts and Nevis (SKN) is located in the Leeward Islands in the Caribbean region about 200 miles ESE from Puerto Rico. It has an extension of 68 square miles and a population of less than 50,000 inhabitants mostly living in the capital town of Basseterre.

1.4.1. Island ecosystems and weather.

Ecosystems

Saint Kitts is a small island that has a relatively simple ecology and five distinct ecosystems (Figure 1.7). The main ecosystem of interest in the transmission of human arboviruses is urban and sub-urban where the domestic mosquito Aedes thrives. Urban areas in St. Kitts are typically one or two story high buildings made of concrete and/or wood with one to several rooms, a patio and/or garden. The capital town has some modern buildings housing offices, shops and government offices a few stories high. The use of mosquito screens or protective barriers in windows and doors is scarce with most public buildings having open air ventilation (including the hospital and health centres, police stations and most administrative buildings).



(i)	rain forest covers the virtually uninhabited tops and sides of the volcanoes (1,156 m);
(ii)	rural areas derived from the former cane fields abandoned in 2005. these have largely been left and not turned over to farming or property development;
(iii)	mangrove areas associated with salt ponds and beaches frequented extensively by tourists;
(iv)	the largely uninhabited dry savannah-like peninsula
(v)	urban areas where most of the population live, consisting of the capital, Basseterre, and a string of parishes/villages around the coast of the main island, characterized by high-density housing, shops, and businesses.

Figure 1.7. Five distinct ecologic areas in St. Kitts (i-v). (Google Maps, 2017)

Weather

Saint Kitts and Nevis experiences a tropical climate with hot and humid weather constant during the year with small daily temperature variation that provides ideal conditions for the multiplication and persistence of mosquitoes (Lambrechts *et al*., 2011; Carrington *et al*., 2013). The annual average temperature between 2010 and 2016 recorded by the St. Kitts Meteorological Services weather station located at the Robert L. Bradshaw International Airport was 27.6ºC and the yearly minimum and maximum mean temperatures were 23.6ºC and 30.3ºC, respectively. During the period of study, the absolute minimum and maximum temperature ranged between 19.3ºC and 34.1ºC. The coolest months of the year with a mean temperature below 27ºC were December through May which broadly coincides with the dry season. The other main tropical season, the rainy season, presents frequent and torrential downpours and it lasts from June to December (Figure 1.8). The average yearly rainfall between 2010 and 2016 was 1,242mm with a daily mean of 3.40mm. The amount of rain has been decreasing steadily in the last years and reached its lowest point in 2015 (Table 1.11) attributed to the effects of the meteorological phenomenon El Niño-Southern Oscillator (ENSO) during 2015-16 which also made 2015 the warmest and the driest in the Atlantic side (Banu *et al.*, 2015; Muñoz *et al.*, 2016) and caused less favourable conditions for the breeding and survival of mosquitoes affecting the incidence of dengue (Earnest, Tan and Wilder-Smith, 2012; Zambrano *et al.*, 2012).

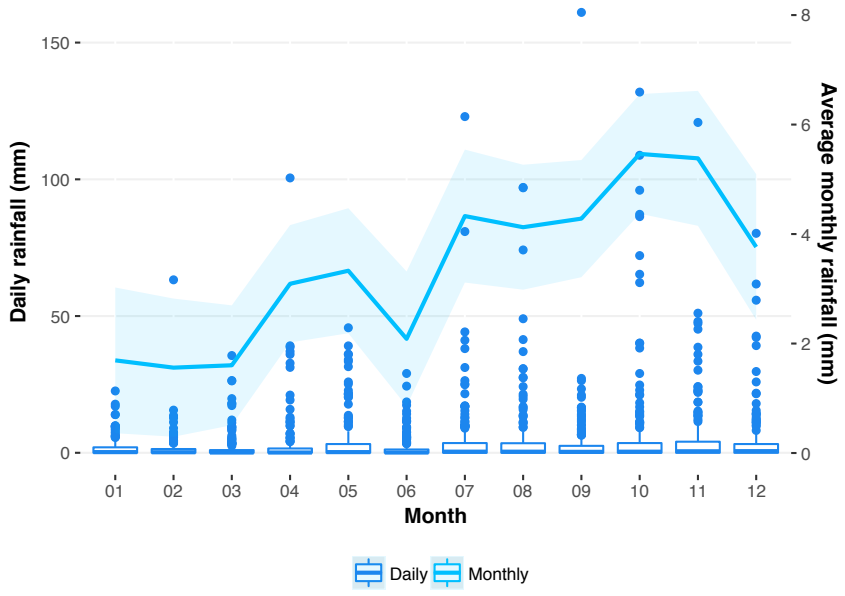


Figure 1.8. Daily (dark blue boxplots and points) and monthly (light blue solid line with 95% C.I. shaded area) average rainfall between 2010 and 2016. The top line of the boxplot indicates the upper quartile or 75% of rainfall data and the dots above the whiskers represent outliers (data provided by SKN Meteorological Services).

Table 1.11. Average rainfall, temperature (temp.) and relative humidity (RH) in St. Kitts between 2010 and 2016 (data provided by SKN Meteorological Services)

Year	Daily mean rainfall	max daily rainfall	Yearly rainfall	Yearly Mean temp	Yearly Mean max temp	Yearly Mean min temp	Mean RH	Max RH	Min RH
2010	5.43	122.94	1,980.58	27.7	29.9	23.1	-	-	-
2011	4.38	161.04	1,597.70	27.3	31.3	23.8	-	-	-
2012	4.04	131.90	1,477.29	27.4	29.8	23.2	-	-	-
2013	2.83	55.80	1,033.92	27.5	30.9	23.3	-	-	-
2014	2.75	120.80	1,003.20	27.5	30.0	24.1	76	94	55
2015	1.91	40.10	693.70	27.9	30.8	24.5	74	90	60
2016	2.49	96.90	910.80	27.8	29.8	23.3	75	91	55

1.4.2. Socio-economic factors

In addition to weather, socio-economic factors including gross domestic product (GDP) have been reported to impact the epidemiology of dengue and chikungunya (Reiter *et al.*, 2003; Åström *et al.*, 2012; Teurlai *et al.*, 2015; Kuan *et al.*, 2016). Although Saint Kitts and Nevis had the highest GDP in the Caribbean at US$13,350 in 2013, this figure showed a distorted picture as it omits the inequality in the distribution of wealth and the heavy dependency of the island's economy on external financial pressure (Pan American Health Organization, 2012; Vassell, 2014). According to the Caribbean Development Bank (2016), the federation of SKN exhibits high levels of poverty and has been designated as 'less developed' along with some other island states and territories in the region considered extremely vulnerable to exogenous sources due in part to their small size, location, openness and exposure to natural hazards (Caribbean Development Bank, 2016). With a poverty line estimated in 2007 at US$2,714 per year per person, the poverty rate for St. Kitts was 23.7% with 13.5% of the households in the island below the poverty line, 16.5% of the households lacked access to piped water and 14.6% lacked access to water 7 days per week (Pan American Health Organization, 2012). The parishes with highest poverty levels were St. John and St. George in Basseterre West (Pan American Health Organization, 2012), which usually comprise highly compacted residences made mostly out of wood with open windows and doors, and accumulation of debris in the vicinity that can serve as mosquito breeding containers. In contrast, the affluent neighbourhoods along the peninsula and the areas of Frigate

Bay and Half Moon Bay typically comprise highly disperse residences with regular private cleaning and gardening contractors, close-fitting windows with mosquito screens, and extensive air-conditioning coverage, all factors reported to affect arboviral infections (Gubler, 2011).

1.4.3. Population

The population of St. Kitts can be divided into three broad groups based on their country of birth, cultural background and socio-economic factors as native Kittitians, long term foreign-born residents including university students and short term residents including tourists.

Kittitians

The Federation of SKN has around 46,000 inhabitants of which 35,000 resided in Saint Kitts, women slightly outnumbered men (96.95 men to 100 females) and had a life expectancy at birth in 2016 of 75.7 years overall (73.3 for males and 78.2 years in females) according to the census of 2011 (Pan American Health Organization, 2016). A total of 17,425 households were identified in the Federation with the average household size being 2.7 (Statistics Department, 2012; Vassell, 2014). Kittitians normally reside in the medium and lower range of properties in Basseterre downtown and the various parishes alongside the main road around the island.

Long term residents

There is also a regular inflow of foreign-born residents to St. Kitts primarily associated to the US-based university campuses: Ross University School of Veterinary Medicine (RUSVM), which is the largest one housing around 1,000 students, University of Medicine and Health Sciences (UMHS) and Windsor University. Additionally, a small sub-population of retiree ex-pats and of contractors employed in the construction of new touristic resorts also live on St. Kitts. Ex-pats are usually of both sexes and mostly originate from N. America or Europe and reside in properties in the affluent areas of Frigate Bay and Half Moon Bay and the south-east Peninsula. Contractors and construction workers are mainly male from diverse origins and interact with other ex-pats and tourists in their activities, behaviours and areas of residence. The regular introduction of susceptible hosts from non-endemic areas into a small territory can create foci of susceptible population that may maintain the endemic cycle as opposed to islands where the population is stable and

transmission is stopped once a level of herd immunity in the population has been reached.

Short term residents

In addition to offshore-banking, tourism is the main pillar of the economy on the island, principally day tourists from cruise ships. The total annual number of tourists that visited SKN in 2014 was over 816,000, of which 85% arrived on 371 cruise ships (St. Kitts Statistics Office, 2015) resulting in an estimated 1,870 passengers per ship equivalent to over 5% of the island population. The impact of cruise ships visitors on small islands is unclear. Cruises generally originate form ports in the US or the larger Caribbean islands and visit the smaller islands for a week. Tourists wander around the visited island during the day and return to their ship in the evening to repeat the process the next day on a different island. It is possible that this may also be a mechanism of spreading pathogens between islands and of entry into the US.

1.4.4. Presence of arboviruses on St. Kitts.

Dengue virus (DENV)

In addition to reports indicating the endemicity of dengue (PAHO / WHO, 2018b), between 1996 and 2016, there were 441 suspected cases of dengue reported in St. Kitts of which 295 were confirmed, 5 developed to severe dengue and no deaths associated with the virus were reported. The majority of dengue cases have been described in the last decade (Figure 1.9. and Table 1.12) and since 2011, all four DENV serotypes have been reported on St Kitts (World Health Organization, 2017d). Outbreaks show periodicity occurring every 3 to 5 years but with an increasing number of cases over time (San Martín *et al.*, 2010). Most cases are reported by public health clinics and the general hospital based on clinical symptoms and the information passed on to the Caribbean Public Health Agency (CARPHA). Passive surveillance is conducted by the Health Information Unit of the Ministry of Health of St Kitts. A selection of samples from suspected cases during outbreaks is tested by the CARPHA official laboratory in Trinidad and Tobago to confirm the pathogenic agent.

*Table 1.12 Number of DENV cases reported on St. Kitts and Nevis. * Incomplete data provided. Weeks: number of weeks worth of data provided. NA: Not Available. Source: WHO/PAHO*

Year	Weeks	Suspected cases	Confirmed cases	Annual incidence confirmed cases (per 100,000 population)	DENV serotype	Severe cases	Deaths
1996	52	NA	6	NA	NA	-	-
1997*	36	NA	-	-	NA	-	-
1998	51	NA	-	-	NA	-	-
1999*	22	NA	1	NA	DENV3	-	-
2000*	40	NA	5	12.82	DENV2	-	-
2001	52	NA	89	234.21	DENV2	4	-
2002	52	NA	20	52.63	DENV2	-	-
2003	52	2	2	5.26	NA	-	-
2004	52	4	3	7.89	NA	-	-
2005	52	-	-	-	NA	-	-
2006*	42	1	-	-	NA	-	-
2007*	41	0	-	-	NA	-	-
2008*	48	49	49	128.95	NA	-	-
2009	52	2	2	5.26	DENV3	-	-
2010*	24	19	19	50	DENV1,2	-	-
2011	52	47	43	11.16	DENV1,4	1	-
2012	52	1	1	2.04	DENV4	-	-
2013	52	100	45	91.84	DENV4	-	-
2014	53	75	9	17.31	NA	-	-
2015*	24	5	1	1.92	NA	-	-
2016*	46	136	-	-	NA	-	-
Total		441	295			5	0

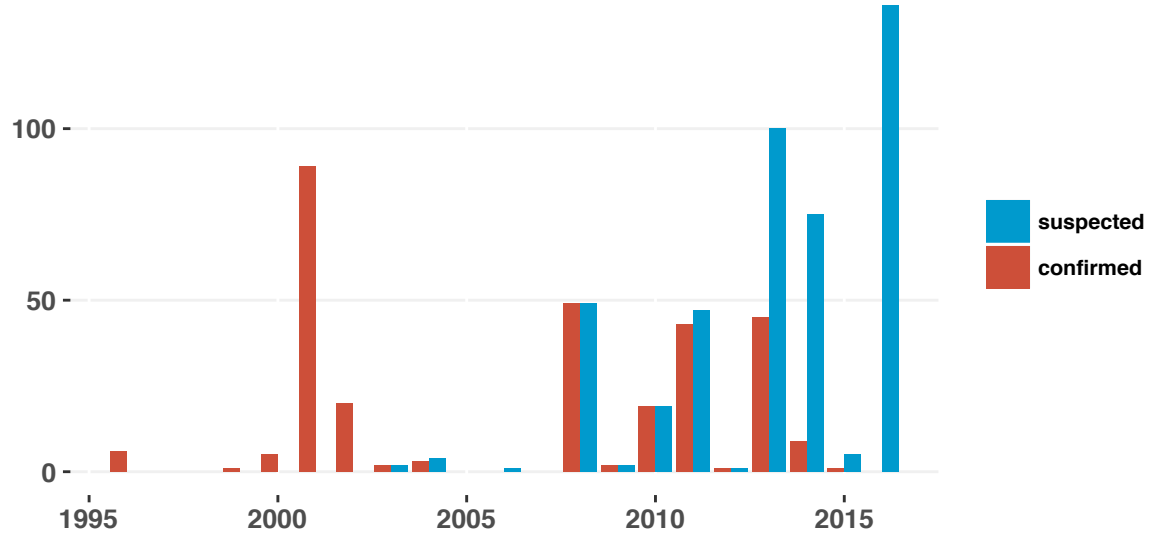


Figure 1.9. Cases of DENV reported on SKN (Data source: (World Health Organization, 2017d)*)*

Chikungunya virus (CHIKV)

The first case of chikungunya in the Caribbean was detected in the French territory of St. Martin in December 2013, although it was demonstrated that the virus had been circulating since the beginning of October (Cassadou *et al.*, 2014). The first case of chikungunya in St. Kitts was reported on 20th February 2014, and by November 2014 there were 402 suspected cases identified and a further 195 were reported in February 2015 reaching a total of 625 suspected cases and 28 confirmed cases reported by the St Kitts and Nevis Ministry of Health (CARPHA, 2015).

Zika virus (ZIKV)

The first case of Zika in the Caribbean was detected in the French territory of Martinique in November 2015 (Daudens-Vaysse *et al.*, 2016) and it rapidly spread to other islands. The first case of Zika in St. Kitts was reported in September 2016, although there were indications that the virus may have been circulating for several months before. A total of 554 suspected cases and 33 confirmed cases were reported by April 2017 (Figure 1.10), although only five suspected cases were reported after December 2016 (PAHO / WHO, 2017b).

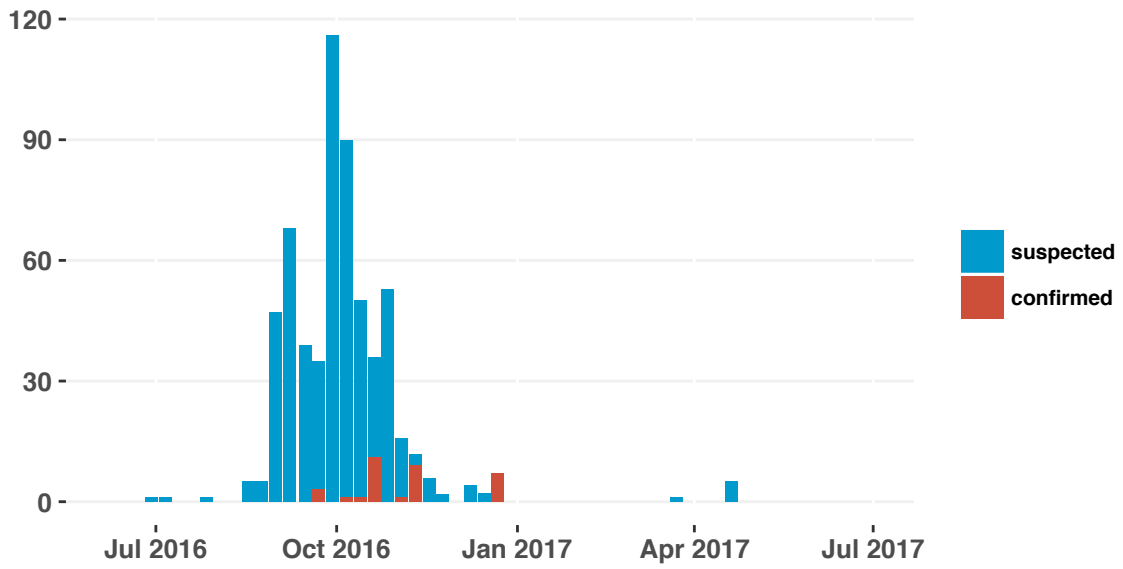


Figure 1.10. Cases of Zika reported in St. Kitts. Data provided by the Saint Kitts and Nevis Ministry of Health, Social Services, Community Development, Culture and Gender Affairs. Adapted from PAHO / WHO (2017a).

1.5. Gaps in existing knowledge.

Most dengue studies in Central America and the Caribbean are based on cross-sectional studies and mainly describe differing prevalence rates and variable factors of infection in the autochthonous population. Wong-McClure, Suárez-Pérez and Badilla-Vargas (1999) as cited by Torres *et al.* (2017) reported a seroprevalence in children in Costa Rica between 2.9-36.9% and found a significant association between infections and temperature, altitude and poverty. Later, Yamashiro *et al.* (2004) demonstrated a dengue seroprevalence of 98% in adults and 56% in children in Santo Domingo, Dominican Republic, finding no significant differences in the prevalence by age, sex and residential area. Similar high dengue prevalence were found in Trinidad, with rates in pregnant women of 94.4% from cord blood samples (Campbell *et al.*, 2007) and, in Jamaica, 100% prevalence was reported from 277 samples from asymptomatic adults (Brown *et al.*, 2009). Further studies in healthy adults in the French Caribbean indicated 90.7% prevalence of dengue in Martinique and 96.2% in Guadeloupe of which 80% presented antibodies to all four serotypes and the only significant differences were age and birth on the islands as opposed to birth in continental France (L'Azou *et al.*, 2015), slightly higher than 83.3% reported in the neighbouring island of St. Eustatius of which 62% presented antibodies to all four serotypes and the only significant factor associated with seroconversion was length of residence on the island (Leslie *et al.*, 2014).

Furthermore, there are only a few studies targeting non-residents in endemic areas (Grange *et al.*, 2014) and most of the studies investigated seroconversion rates after

return to their countries of origin. For instance, a serosurvey amongst German citizens indicated acute infections in 1.1-4.7% travellers (Wichmann *et al.*, 2005), similar to 2.9% probable infection in Dutch travellers returning from endemic areas in Asia (Cobelens *et al.*, 2002). Analogous studies in returning longer-term residents have indicated 6.7% prevalence in Israeli adults but no significant factors of infection were identified (Potasman, Srugo and Schwartz, 1999), and 19% prevalence in US travellers, of which 12% had antibodies by PRNT and length of residence and self-reported history of dengue were the only significant factors identified (Sanchez-Vegas *et al.*, 2013). The reported rates of dengue infection in NGO workers have ranged from 4.3% (Jänisch *et al.*, 1997), 32% (Gambel, Drabick and Martinez-Lopez, 1999) and up to 50% (Salyer *et al.*, 2014), whereas studies in U.S. military personnel deployed in endemic countries reported rates of dengue infections of 1.5% (Hesse *et al.*, 2017) and 7.7% (Sharp *et al.*, 1995).

In St. Kitts, a cross sectional study conducted in 2009 collected 118 serum samples from RUSVM students, faculty and local staff shortly after the last DENV3 outbreak (Mohammed *et al.*, 2012). Overall, 44.1% of the study population showed anti-DENV antibodies and seroprevalence ranged from 30% in the students group to 100% in the local staff group. The time for half of the students and faculty to become seropositive was calculated to be 1.5 years and duration of residence on St Kitts was the only factor found to be significantly associated with seropositivity with odds of infection increasing 21.0% for each year of residence. Other relevant factors considered were age, ethnicity and birth in endemic countries, and it was suggested that during inter-epidemic periods students may still become infected with dengue (Mohammed *et al.*, 2012). A separate study that collected serum samples from healthy pregnant women in Caribbean countries reported an overall prevalence of 83% (±3.5%) across the region and 100% seropositivity on the samples from St. Kitts (Wood *et al.*, 2014).

Also, mathematical models of arboviral transmission have been proposed aiming to quantify transmission, to understand temporal and spatial patterns of disease transmission, the serotype/strain structure and to estimate the potential impact of control methods (Perkins *et al.*, 2014). However, the majority of transmission and epidemiological models proposed in the literature are aimed at large scale settings and there are still deficiencies in our knowledge of the transmission models at a finer scale (Wearing, Robert and Christofferson, 2016). These deficiencies include multivariate analysis of the dynamics between *Ae. aegypti* and people, ecological, behavioural,

household, work, recreational and environmental factors influencing infections in small geographical areas (Brady *et al.*, 2014; Kraemer, Sinka, Duda, A. Q. N. Mylne, *et al.*, 2015; L. P. Campbell *et al.*, 2015; Messina *et al.*, 2016).

The limitation of cross-sectional studies from the autochthonous population and returning residents and large scale agent-based models is that information on the environment, individual behaviours, and climatic during the stay on the endemic area is scarce and to determine the time of seroconversion and the relationship of different factors associated with infection at a local level is challenging. To provide this information, prospective longitudinal studies are needed with ready access to clearly defined and readily followed cohorts of immunologically naive individuals in areas that are susceptible to invasion of emerging arboviruses and will shade light on the interaction of multiple factors. Such conditions are available on the island of St Kitts in the Caribbean.

1.6. Characteristics of St. Kitts for the study of arboviruses.

Transmission and spread of arboviral infections are determined by the interplay of multiple factors including presence of the virus, abundance of mosquito, contact between human and mosquito population and sustained environmental conditions to support continued transmission (Wearing, Robert and Christofferson, 2016). St. Kitts has several characteristics that make it suitable for the study of human arboviruses. Firstly, dengue is considered endemic on the island (Mohammed *et al.*, 2012; PAHO / WHO, 2018b) and confirmed cases of dengue, chikungunya and Zika have been reported over the past few years (CARPHA, 2015; PAHO / WHO, 2017b). Secondly, the principal vector of dengue, *Ae. aegypti*, is ubiquitous in urban areas of St. Kitts (Belkin and Heinemann, 1976; Smith *et al.*, 2011; Mohammed *et al.*, 2015). Thirdly, a number of universities with base in the US have their campus on St. Kitts, of which Ross University School of Veterinary Studies (RUSVM) is the largest and has been on the island for over 30 years and is an important part of the economy and life on the island with near 1,000 veterinary students, faculty and administration staff mainly from North America who are expected to be immunologically naïve to dengue on arrival. The academic course at RUSVM is divided into 3 annual semesters of 16 weeks which start in the first week of January, May and September. Approximately, 175 new students enrol each semester and are required to arrive at least one week before their due date of commencement for their orientation week for which frequent group activities are organized around the island with little awareness for mosquito

borne diseases and stay on St. Kitts for the duration of their pre-clinical studies during 7 semesters (28 months) and allows a good opportunity to study a population of immunologically naïve adults to dengue in an endemic environment (Mohammed *et al.*, 2012). In addition, many students do extra-curricular activities or volunteer work in Central and South American countries which could put them at risk of importing diseases on their return to St. Kitts.

Until this study, there were no data on seropositivity expected in inter-epidemic periods in St. Kitts but reports from nearby islands (Campbell *et al.*, 2007; Chadee *et al.*, 2007; Kumar, Gittens-St Hilaire and Nielsen, 2013) and local experience suggested that dengue may be circulating (PAHO / WHO, 2017b) at an increased incidence during the rainy season (July to December). Understanding the factors associated with the development of inapparent dengue infection increases our understanding on the pathogenesis of this disease (Endy *et al.*, 2011) and the presence of university students facilitates the collection of specimens and data regarding human factors in three main areas: environmental, mosquito exposure associated with indoor and outdoor behaviours and frequency of mosquito bites. Therefore, St. Kitts provides the opportunity to study the dynamics in the spread of several arboviruses in the presence of only one vector within a restricted and well defined geographic area.

1.7. Main hypothesis and structure of the thesis

When the study was first conceived, dengue was the only known arbovirus on St. Kitts. However, since the introduction of chikungunya in 2014 and Zika in 2016, these viruses were also incorporated in the study and the three formulated research hypotheses amended accordingly:

I. The incidence of dengue, chikungunya and Zika in a naïve population in an endemic setting varies with (a) duration of residence; (b) seasonality; (c) individual behaviour; (d) exposure to the Aedes vector; (e) presence of Aedes vector.
II. The dynamics of different serotypes of dengue virus on St. Kitts is a key factor for determining outbreaks of symptomatic disease.
III. There is geospatial variation in the pattern of dengue serotypes prevalence in the naïve population.

This thesis is presented in six chapters. The first chapter introduces the main characteristics of arboviruses, the epidemiology and clinical symptoms of the study

arboviruses and the principal mosquito vectors, their morphology, ecology and vectorial capacity. Also, this chapter identifies knowledge gaps and relevant socio-geographic conditions of St. Kitts in the epidemiology of arbovirus, and outlines the research hypothesis of this study.

Chapter 2 describes the characteristics of the study population which comprised three cohorts of asymptomatic sentinels and a number of suspected cases of acute arboviroses. The chapter explains the recruitment of sentinels, the collection of samples and epidemiological data, and the analysis of their personal characteristics, environment and behaviours that affected exposure to mosquito bites and potential risk factors of infection.

Chapter 3 describes the materials and laboratory methods employed in the analysis of the samples collected. This chapter also illustrates the challenges in arboviral diagnosis, validation of the laboratory tests employed and adapts an existing latent class Bayesian model for diagnosis of arboviruses.

Chapter 4 describes a survey of the mosquito population performed in urban and semi-urban areas in St. Kitts and a series of models are proposed to describe the abundance of *Ae. aegypti* in urban and semi-urban areas of St. Kitts at a fine scale and the prevalence of arboviruses in mosquitoes.

Chapter 5 describes the analysis of epidemiological data collected and the association of risk factors with arbovirus infection in the study human population. This chapter also describes a time to event analysis, models of infection and overall discussion on arboviroses in St. Kitts.

Chapter 6 summarises the final conclusions of the hypothesis outlined in the first chapter and briefly discuss some final remarks regarding arboviruses and neglected tropical diseases.

Ethical approval: This study was approved by the Institutional Review Board (IRB) of Ross University School of Veterinary Medicine and the Ministry of Health of Saint Kitts and Nevis.

Chapter 2. Analysis of the study population

2.1. Introduction

Numerous cross-sectional arboviral studies performed in Central American and the Caribbean have been described in the literature (Yamashiro *et al.*, 2004; Campbell *et al.*, 2007; Chadee, Doon and Severson, 2007; Brown *et al.*, 2009; Mohammed *et al.*, 2012; Kumar, Gittens-St Hilaire and Nielsen, 2013; Leslie *et al.*, 2014; L'Azou *et al.*, 2015; Torres *et al.*, 2017). However, although cross-sectional studies are rather uncomplicated to conduct, the strength of proof of causal association and the relevance to extrapolate findings to real-world situations are only moderate (Dohoo, Martin and Stryhn, 2012). The limitations of cross-sectional studies stem from the fact that the outcome measure is prevalence and it is difficult to unravel the factors associated with the development and persistence of the outcome, and to determine if exposure occurred before the outcome (Dohoo, Martin and Stryhn, 2012). In contrast, detailed prospective studies of cohorts of sentinels that are followed over time in endemic areas can be used to determine the incidence rates of inapparent dengue infection, the effects of certain risk factors and to establish the time of infection within a known interval (Endy *et al*., 2011; Dohoo, Martin and Stryhn, 2012).

Previous prospective dengue studies conducted in Kamphaeng Phet, Thailand, in 1998-2002 and in 2004-08 (Endy, Yoon and Mammen, 2010), in Maracay, Venezuela, in 2001 (Comach *et al.*, 2009), and in Managua, Nicaragua, between 2001-03 and 2004-08 (Balmaseda *et al.*, 2006, 2010) followed large cohorts of children and reported highly variable spatial and temporal diversity of infections attributed to seasonality and herd immunity that were dependent on environmental and mosquito factors (Endy, Chunsuttiwat, *et al.*, 2002; Endy, Nisalak, *et al.*, 2002; Balmaseda *et al.*, 2006, 2010; Endy *et al.*, 2011; Biswas *et al.*, 2012), in addition to strong clustering of cases within 100 metres associated with deficient piped water, lack of screened windows and high *Ae. aegypti* pupal indices (Mammen *et al.*, 2008).

Fewer similar studies have been conducted in adults. Prospective studies in West Java, Indonesia, between 2000 and 2002, reported higher incidence of inapparent than symptomatic dengue infections in areas of high disease transmission, and a year-round circulation of all serotypes, although cases increased at the beginning of the dry season which were attributed to increased storage of water and the creation of additional mosquito breeding sites (Porter *et al.*, 2005). Further research in Iquitos, Peru, between 1999-2005, indicated that dengue prevalence increased with

age and that there was significant geographic variation (Morrison *et al.*, 2010), whereas studies in Costa Rica, between 1999-2007, demonstrated that temperature, altitude and the human poverty index were risk factors of dengue (Mena *et al.*, 2011), and in Managua, Nicaragua, in 2014-15, higher prevalence of chikungunya was associated with lack of water availability, household size and low socio-economic status (Kuan *et al*., 2016). Other reports in the literature have correlated arboviral transmission with mosquito ecology and mosquito biting risk, which is influenced by climatic and anthropological factors such as age and greater surface area, and behaviours related to occupation, social habits, outdoor recreational activities or socio-economic status determined by housing infrastructure, screened window, air-conditioning, density of and distance to breeding containers (Reiter *et al.*, 2003; Sissoko *et al.*, 2008; Wearing, Robert and Christofferson, 2016).

A regular complication encountered in longitudinal studies is missing data and ignoring the mechanisms that caused the missing data can cause bias and incorrect inferences (Rubin, 1976). Examples of missing data comprise variables not observed, non-responses in questionnaires because of non-contact, refusal or other reasons, or subjects dropping out prior to the end of the study (Ackroyd and Hughes, 1981; Little and Rubin, 2002). Patterns of missing data can emerge as univariate, when missingness is confined to a single variable, file-matching when two sets of variables were never jointly observed, latent variables that are never observed, monotone patterns due to item non-response and attrition in longitudinal studies, and, the most commonly found in longitudinal studies, missing values in a haphazard or 'general' pattern (Little and Rubin, 2002). With regard to the mechanisms of missingness, Little and Rubin (2002) classified missing data as missing completely at random (MCAR), when the missingness does not depend on the data values, missing at random (MAR), when the missingness depends only on components that are observed, and not missing at random (NMAR), when the missingness depends on the missing values themselves. Later, Gelman and Hill (2007) proposed that data NMAR can also depend on unobserved predictors or on the missing values.

Shortfalls arising from data missing at random can be addressed more straightforward and with less risk of bias than data not missing at random (Little and Rubin, 2002). Simple approaches to handling missing data such as analysis of complete cases only or analysis of data in different subsets excluding the missing data are discouraged due to the loss of information and the potential introduction of

biases (Little and Rubin, 2002; Gelman and Hill, 2007). Other methods proposed include single imputation of the mean or the mode, random single imputation, and single or multiple imputation by logistic regression modelling or by a Bayesian approach amongst others, of which the later methods are more desirable as these retain most of the information and variability of the data with negligible bias (Gelman and Hill, 2007). Imputing predicted responses based on modelling missing data can be applied robustly to MCAR and MAR, however, imputation of NMAR should be addressed with care as while it can be possible to predict missing values based on the other variables in the dataset, the nature of the missing-data mechanism may cause the models to extrapolate beyond the range of the observed data (Gelman and Hill, 2007).

This chapter describes the recruitment and collection of samples and epidemiological data from three cohorts of asymptomatic sentinels and from individuals suspected of suffering an acute arbovirosis with the aim of developing a longitudinal study of arbovirus infection in adults in an endemic area in the Caribbean. The chapter goes on to discuss the challenges of collecting reliable information in longitudinal studies and analyses the personal characteristics, environment and behaviours of the study population that are associated with exposure to mosquito bites and risk of arbovirus infection.

2.2. Methods of sentinel recruitment, collection of samples and collection of epidemiological data.

2.2.1. Recruitment of sentinels

University students commencing at RUSVM at one of the intakes in September 2014, January 2015 and May 2015 were invited to act as surveillance sentinels for arboviral infections during their stay on St. Kitts. A minimum sample size of 40 sentinels from each of the three cohorts with planned observations of 7, 6 and 5 samplings covering an observation period of 28, 24 and 20 months, respectively, was calculated to be sufficient to detect arboviral seroconversions and relevant risk factors of infection in the student population. These estimations were based on the conclusions from preliminary studies conducted at RUSVM (Mohammed *et al.*, 2012) and calculated following methodology described by Hsieh and Lavori (2000). The sentinels were required to provide a blood sample at the beginning of each academic semester every four months and to complete an *Informed Consent form for Student Sentinels in the Epidemiology of Dengue/Chikungunya/Zika on St Kitts* (Appendix 3) and a

Questionnaire for Student Sentinels - Epidemiology of Dengue/Chikungunya/Zika on St Kitts (Appendix 4) each time until September 2016. The inclusion criteria required the sentinels to be uninfected with dengue prior to the study and reportedly healthy at the time of the sampling, and the samples were analysed to detect subclinical arboviral infections by the methods later described in chapter 3. The epidemiological questionnaires provided sentinels' personal and medical information, housing environment and personal behaviours that influenced their exposure to mosquitoes and were analysed to establish associations between arboviral infection and risk factors by the methods described later in chapter 5. Also, faculty members and other post-graduate students arriving within the enrolment dates were invited to participate as sentinels and allocated to one of the cohorts. When sentinels dropped out of the study, calls were made to replace them through presentations, poster signs and popular social media.

Additionally, health practitioners at RUSVM Health Services and at the JHF General Hospital were invited to submit samples from consenting adult patients within the wider university and the island population suspected of an acute arboviral infection along with an *Informed Consent form* and a *Questionnaire for Acute Cases - Epidemiology of Dengue/ Chikungunya/Zika on St Kitts (Appendix 6*). Samples from suspected cases were received at any time between September 2014 and December 2016.

2.2.2. Collection of samples

Blood samples were collected from the sentinels by a team of phlebotomists using standard techniques to draw whole blood from the medial cubital vein into Vacutainer BD tubes containing K3 EDTA 15% Solution, 0.081mL (BD Vacutainer, USA) to separate plasma. Additionally, at the sampling call in May 2016, blood was also collected into Monoject™ Blood Collection Tubes (Covidien, Medtronic, USA) with no additives to separate serum. The collection of sera in addition to plasma was used to validate the performance of serologic tests for the use of both specimens and will be described later in section 3.2.3. Further blood samples were collected from suspected cases by RUSVM Health Services and JHF General Hospital following the same technique and materials described. After collection, the blood tubes were transported to RUSVM Research Laboratory and left at room temperature for up to 2 hours. Plasma and sera were separated by centrifugation at 4,000g for 5 minutes and aliquoted into 1.5ml and 0.5ml Eppendorf tubes and the specimens were stored

at +4°C for two days and at -80°C for longer periods of time. Also, after Zika transmission was declared in St. Kitts in September 2006, urine samples were collected from some suspected patients by RUSVM Health Services in Medi-Pak sterile specimen containers (McKesson, USA) and transferred without delay to the research laboratory. Urine was aliquoted into 2ml and 0.5ml Eppendorf tubes and stored at -80°C for further analysis described in chapter 3.

2.2.3. Collection of epidemiological data

Epidemiological data from the sentinels was collected through the *Questionnaire for Student Sentinels - Epidemiology of Dengue/ Chikungunya on St Kitts (Appendix 4*). A shorter version of the questionnaire (Figure 2.1) was designed for new intakes and provided background personal information, previous travel to areas at risk of dengue (World Health Organization, 2009) and medical history before arriving to the island. A longer version of the questionnaire (Figure 2.1) was completed at subsequent samplings and provided further information on the main risks of arboviral infection that broadly comprised household environment and presence of physical barriers to mosquito entry, socio-economic factors such as presence of air-conditioning, water supply, construction materials, (Reiter *et al.*, 2003; Brunkard *et al.*, 2007; Wearing, Robert and Christofferson, 2016), and presence of mosquito breeding sites in the environment (Chadee, 2007). Also, the questionnaires provided information on individuals' behaviours indoors and outdoors that increased the exposure to the mosquito vector and protective measures to prevent mosquito bites (Wearing, Robert and Christofferson, 2016), places frequented, particularly where mosquito bites may occur such as bars, restaurants, beaches, churches, grocery stores and RUSVM and frequency of mosquito bites and times and location they occurred (Vanwambeke *et al.*, 2006; Wearing, Robert and Christofferson, 2016). The questionnaires were developed with the R package '*shiny'* (Chang *et al.*, 2015) and made accessible on-line for completion, which can be accessed on this link: https://dengueross.shinyapps.io/App-Sentinel_Questionnaire2/. Also, a paper copy of the questionnaire was also available as a back-up and the data imputed manually in the sentinel database.

Similar epidemiological information was gathered from suspected cases advised by medical practitioners who completed the analogous *Questionnaire for Acute Cases - Epidemiology of Dengue/ Chikungunya/Zika on St Kitts (Appendix 6*) at the time of sample collection.

Figure 2.1. Schematic representation of the information collected from sentinel questionnaires.

2.2.4. Methods of analysis of sentinel behaviours, dropouts and missing data.

Sentinel behaviours were analysed by generalised estimating equations (GEE), an extension of generalized linear models (GLM) to longitudinal data that is considered the most appropriate model to analyse longitudinal data with a relatively small number of observations and less assumptions than other mixed effect models (Zuur *et al.*, 2009). A further discussion on GEE's is detailed in chapter 5. Dropout patterns were analysed by Generalized Linear Models (GLM) where the probability of dropout was assumed to follow a binomial distribution against predictors considered to affect sentinel attendance, i.e.: age, gender, cohort, academic semester, and previous results of dengue and chikungunya tests considered as a continuous variable (i.e. index values of the ELISA reading), and as a categorical variable (i.e. 1 for positive results and 0 for negative and inconclusive results, and 1 for positive and inconclusive results and 0 for negative results). Following the assumption that missing data hid true values that were meaningful for analysis (Little and Rubin, 2002), missing data and erroneous entries were first attempted to be re-entered in the questionnaires by contacting the sentinel for clarification or by single imputation based on logical rules when possible (e.g. when missing data related to a constant response variable, the missing question was filled with an earlier answer). Next, missing data were analysed by binomial GLM fitted to the variables cohort, length of stay of the sentinel on the island, gender and age for each of the sampling calls for the corresponding cohort to explore significance of patterns. Little's MCAR test, an established method to assess randomness, was discarded as this test is more appropriate with monotone pattern of missing data and quantitative variables (Little, 1988) as opposed to the general haphazard missing pattern of the data which also included categorical variables. Continuous data with MCAR and MAR were imputed by predictive mean matching and categorical data by logistic regression using the 'mice' package (Buuren and Groothuis-Oudshoorn, 2011) in R. Variables with data NMAR or with a percentage of missingness greater than 25% were removed from the analysis (Little and Rubin, 2002).

2.3. Results

Overall, 888 specimen samples were collected (Table 2.1) of which 798 (89.8%) samples of plasma originated from 224 asymptomatic sentinels obtained at the scheduled sampling calls and 90 (10.1%) samples of plasma, serum and urine were

collected from individuals suspected of having an arboviral infection presenting to RUSVM Health Services (78.9%) or the local JHF General Hospital (21.1%).

Table 2.1. Number of samples per cohort and sampling call.

	Cohort	Sep.14	Jan.15	May.15	Total samples
	Sampling	n	n	n	n
No. 1	Sep. 2014	69	-	-	69
No. 2	Jan. 2015	49	55	-	104
No. 3	May 2015	36	49	62	147
No.4	Sep. 2015	43	42	61	146
No. 5	Jan. 2016	39	32	57	128
No. 6	May 2016	33	27	46	106
No. 7	Sep. 2016	37	23	38	98
-	Suspected cases	-	-	-	90
	Total samples	306	228	264	888

2.3.1. Results: Dropout analysis and missing data analysis

Sentinel attendance was irregular as sentinels enrolled at different times and others missed one or more samplings and returned at later callings (Figure 2.3) originating occurrences of interval censoring, when infections happened at an unknown time within periods of time of different length and will be discussed in section 5.2.2. Attendance in earlier samplings was higher than in later samplings and the number of dropouts increased as the study progressed, although those sentinels who attended three consecutive samplings were significantly more likely to remain in the study until completion ($p < 0.001$). Overall, 43 (19.2%) sentinels dropped out after their first sampling, 18 from cohort Sep.14 (24.7%), 11 in Jan.15 (16.2%) and 14 in May.15 (16.9%), and 56 (25.0%) sentinels provided only one baseline sample. Across the three cohorts, 142 sentinels (66.4%) were sampled at least three times (Table 2.2). A comprehensive sentinel pathway showing the number of individuals tested and lost to follow up is detailed in Figure 2.2.

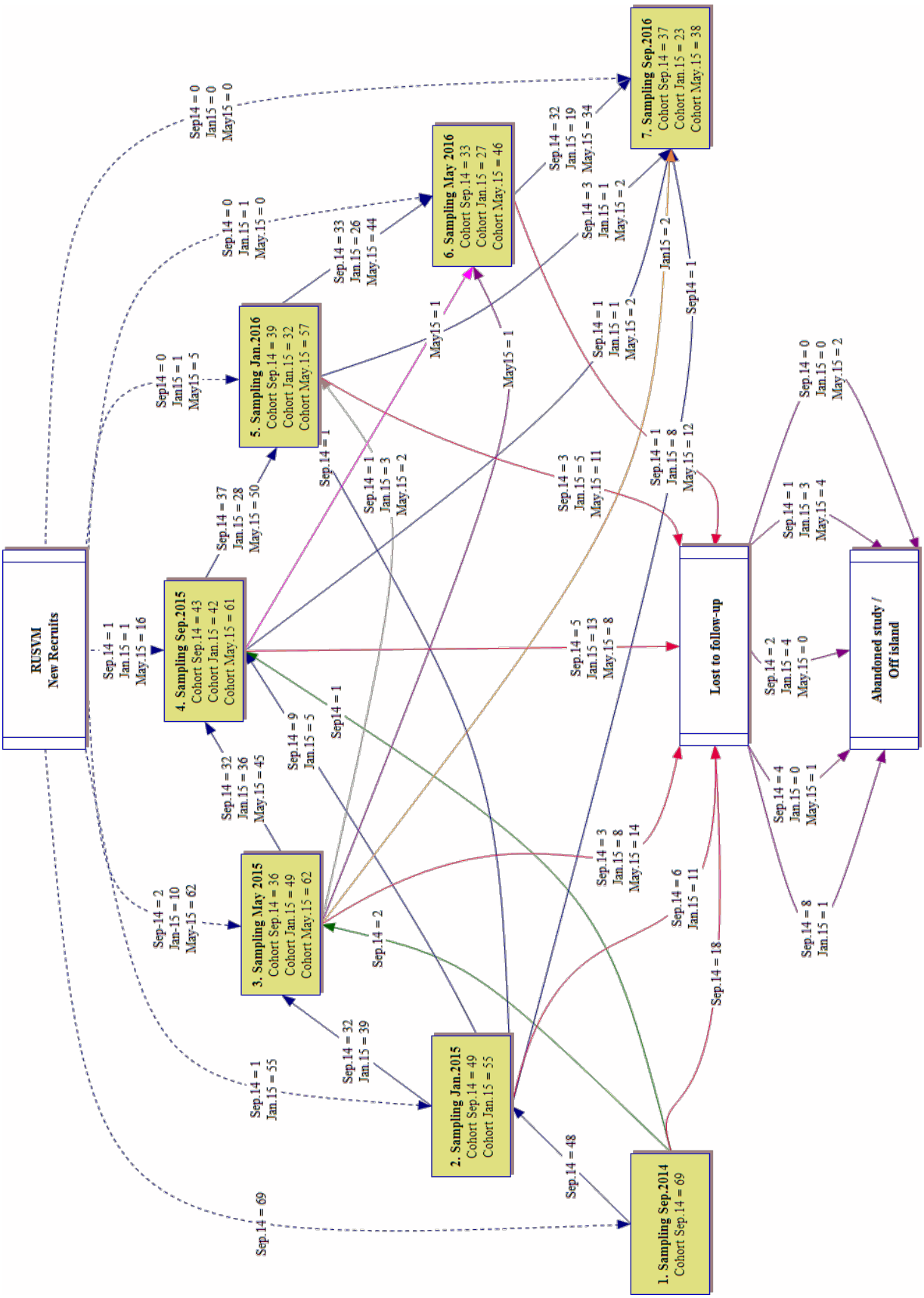


Figure 2.2. Sentinels pathway showing new recruits, number of sentinels sampled and lost to follow up.

Statistical analysis failed to identify significant factors that affected the probability of sentinel dropout which was concluded to occur randomly. Only the results of one of the dengue laboratory tests (i.e. InBios DENV DetectTM IgM Capture ELISA that will be described later in chapter 3) for cohort *Jan.15* at the sampling in May 2016 suggested that the results of a test might have had a statistically significant negative effect (p = 0.048) on the sentinel attendance for the following sampling call. However, this borderline finding was considered non-significant and will be discussed further in section 2.6.

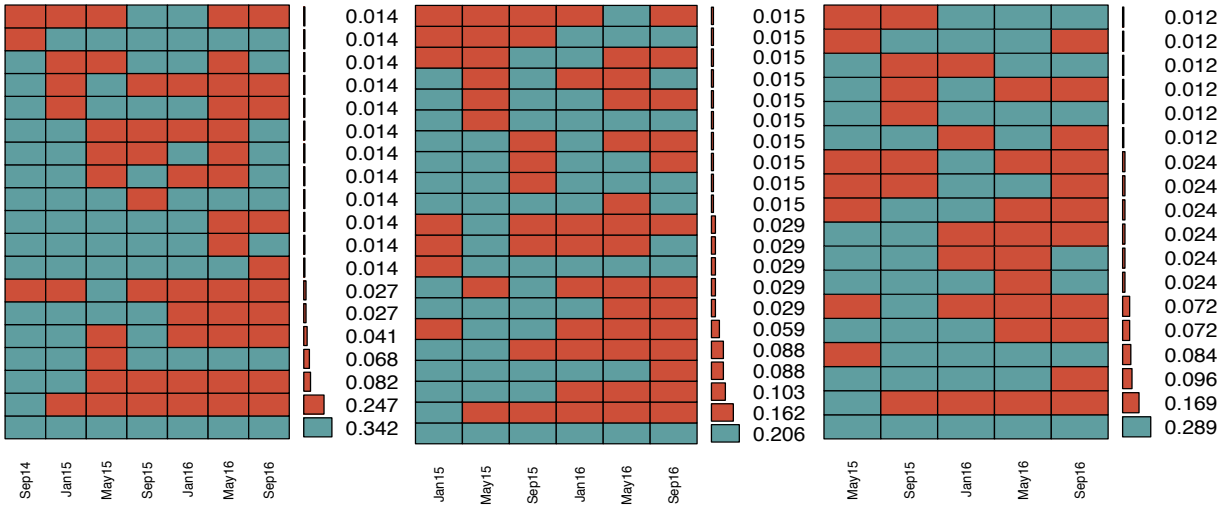


Figure 2.3. Patterns of sentinel dropouts for each cohort (from left to right: Sep.14, Jan.15 and May.15) Green cells represent attendance and red cells represent a missed sampling. The figures on the right column indicate the proportion of sentinels within each pattern. (34.2% of the sentinels in cohort Sep. 14, 20.6% from Jan.15 and 28.9% from May15 attended all of their sampling calls).

Table 2.2. Number of samples provided by sentinels during regular samplings.

No. of times sampled	Sep.14	Jan.15	May15	No. of sentinels
1	20	14	22	56
2	8	15	7	30
3	4	11	12	27
4	6	3	18	27
5	1	10	24	35
6	9	15	-	24
7	25	-	-	25
Total:	**73**	**68**	**83**	**224**

The rate of questionnaire completion was 91.1% across all sentinel cohorts and sampling calls with a higher number of non-completed questionnaires on the last sampling for cohorts '*Sep.14*' and '*May.15*' for which the reasons are unclear (Table 2.3).

Table 2.3. Number of questionnaires missing by cohort and sampling.

Sampling	Sep.14	Jan.15	May.15	Total
Sep. 2014	0	-	-	0
Jan. 2015	0	5	-	5
May 2015	1	0	2	3
Sep. 2015	0	0	0	0
Jan. 2016	1	1	0	2
May 2016	0	0	0	0
Sep. 2016	6	0	4	10
Total	8	6	6	20

A few questions drew high rates of missing data. The frequency of pest control was largely unanswered with 50.5% of non-responses overall, followed by the time spent at RUSVM (39.7%), attendance to and frequency of biting at a church (23.2%), frequency of biting at grocery stores (14.1%) and hiking (13.4%). The complete figures of the 10 most commonly unanswered questions are detailed in Appendix 7. The high rate of non-responses may be caused by unfamiliarity with local pest control practices, unawareness of mosquito bites or reluctance to disclose the amount of time spent at RUSVM by some sentinels. Since the rate of missingness of frequency of pest control and hours spent at RUSVM are substantially high and potentially NMAR, the artificially imputed data would be unreliable and, therefore, these questions were removed from further analysis (Gelman and Hill, 2007).

Statistical analysis of the remaining variables for the probability of missing data suggested occasional episodes of significance ($p < 0.05$) for the variables length of stay and cohort that were associated with scattered questions (Appendix 7) with no discernible pattern that raised little meaning as a whole. In summary, missing data on the remaining variables were considered to be MCAR and MAR and new data was imputed by predictive mean matching or by logistic regression (Buuren and Groothuis-Oudshoorn, 2011).

2.3.2. Sentinels characteristics

The study population was considered representative of the source population at RUSVM. The majority of the sentinels (87.0%) were female and half of the sentinels were between 23.2 to 27.3 years of age ($\bar{x}$ = 26.3), although the overall age extended between 20 and 55 years (Figure 2.4). Nearly ninety per cent of the sentinels (88.4%) originated from North American countries (i.e. Canada, US and US territories including Puerto Rico and U.S. Virgin Islands) while the remaining 11.6% came from 14 different countries in Central America, Europe and Asia (Figure 2.5). Although most sentinels arrived from dengue-free areas, over half of them (59.4%) had travelled to areas declared at risk of dengue (WHO, 2009) before coming to St. Kitts. The most popular countries visited were Mexico, Puerto Rico and Costa Rica plus another 30 territories in Central and South America, the Caribbean and Australia, with the trip duration fluctuating from one week to several years. Sentinels that have lived or visited 'at risk' areas before coming to St. Kitts could have been exposed to the dengue virus and show seroconversion on their baseline sampling. Furthermore, a number of sentinels had lived in areas in the US where West Nile Virus (WNV) and St. Louis Encephalitis virus (SLEV), Flaviviruses similar to dengue, are present (Mackenzie, Gubler and Petersen, 2004). This needs to be considered in the analysis as it may compromise the accuracy of DENV testing due to the known cross reactivity observed between these viruses (Kuno, 2003).

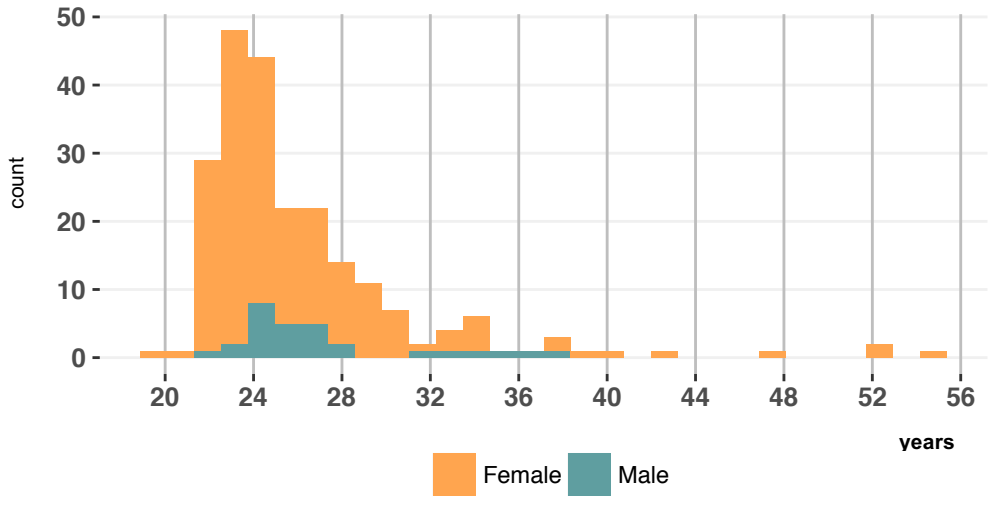
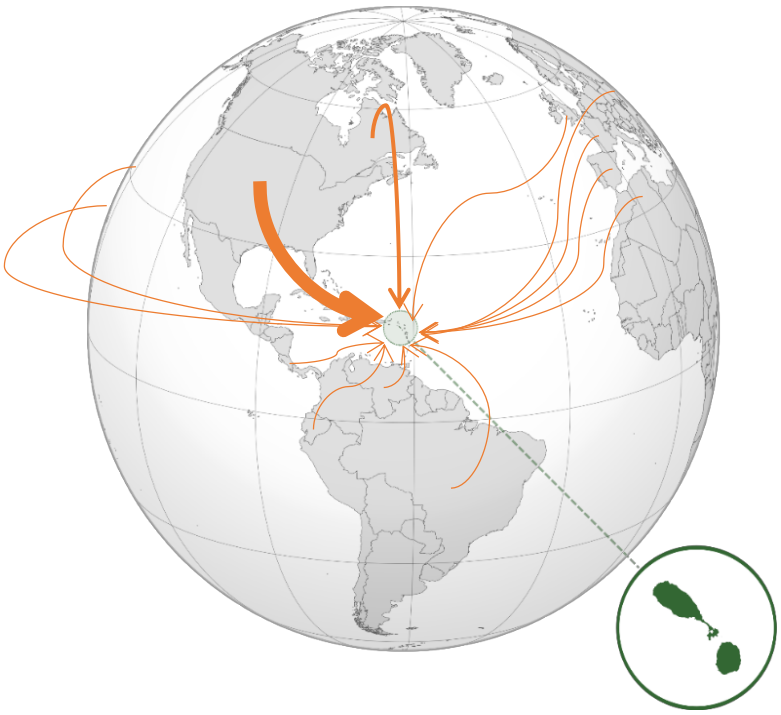


Figure 2.4. Distribution of sentinels by age and gender.

Map of countries of origin of the sentinels



	n
USA	197
Canada	10
Costa Rica	2
India	2
South Korea	2
Algeria	1
Brazil	1
Ecuador	1
France	1
Lithuania	1
Mexico	1
Poland	1
Spain	1
St. Croix	1
UK	1
Venezuela	1
Total	**224**

Figure 2.5. Countries of origin of the sentinels. Adapted from 'Orthographic map of Saint Kitts and Nevis centered at 17° N, 62° W', Wikipedia, Public Domain.

Sentinels medical information and previous arboviruses history:

Two sentinels reported having been diagnosed with dengue before coming to St. Kitts although neither the year of diagnosis was specified nor confirmatory diagnosis performed in either case. No sentinels reported having been diagnosed with WNV, YF, JEV or CHIKV before coming to St. Kitts, but 17 sentinels reported having been vaccinated against YF, some as recently as 2014 (6 sentinels), 2013 and 2012 (2 sentinels each year). Three sentinels were also vaccinated against JEV in 2009, 2004 and 1997. Furthermore, out of the 177 sentinels sampled within 30 days of arrival to St. Kitts, 50 (28.2%) reported a pre-existing medical condition (Table 2.4). The conditions most commonly reported were respiratory (generally asthma) and rheumatologic or pain such as joint and muscle pain and migraines, and only 4 were of an infectious nature.

Table 2.4. Medical conditions reported by sentinels before arriving to St. Kitts.

Medical condition	N	%
No medical condition reported	127	71.8
Respiratory	11	6.2
Rheumatologic	11	6.2
Endocrine	6	3.4
Other	5	2.8
Infectious	4	2.3
Allergies / Anaphylaxis / Autoimmune	3	1.7
Anxiety	3	1.7
Reproductive	3	1.7
Anaemia	2	1.1
Blood pressure	1	0.6
Flu-like symptoms	1	0.6

During their residence on St. Kitts, 19 sentinels reported rheumatologic symptoms (i.e. muscle or joint pain), of which 6 described newly conditions with unspecific symptoms, another 6 sentinels reported an infectious condition diagnosed as either fungal, parasitic or pre-existing and up to four sentinels reported flu-like symptoms during their stay that could be typical of an arbovirus. In particular, sentinel no. 124 reported unspecific fever, pneumonia and chills in May 2015 and muscle pain in the next sampling in September 2015 which are compatible with chikungunya symptoms (Figure 2.6). Other medical conditions reported involved symptoms and diagnosis that were unrelated to arboviroses (i.e. asthma, anxiety, diabetes, allergies, blood pressure, anaemia and IBS).

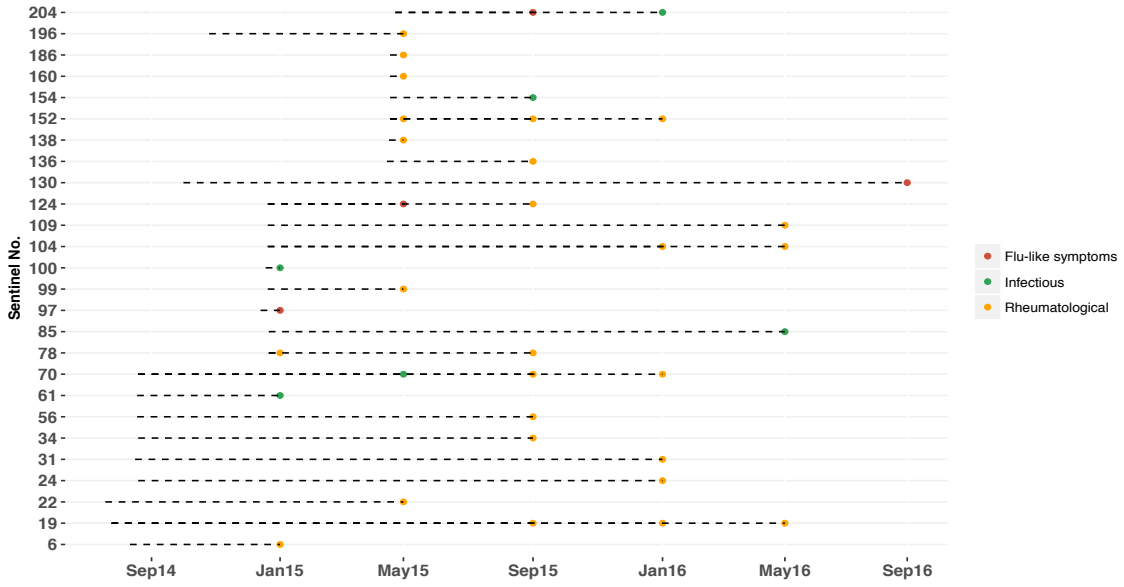


Figure 2.6. Relevant medical conditions reported by sentinels during their residence on St. Kitts. The dashed lines represent residence in St. Kitts until a medical condition was described.

Household environmental factors

Sentinels resided in 8 distinct neighbourhoods during the period of study in addition to RUSVM dormitories. On arrival to St. Kitts, students moved predominantly into RUSVM (80.3%) or West Farm (7.9%) but after their initial semester, nearly half of the sentinels (45.8%) moved to the popular area of Frigate Bay, 42.5% to Bird Rock, Half Moon Bay, Camps or West Farm areas and the remaining 11.7% of the sentinels moved into Monkey Hill, Mattingley, Boyd's or Conaree (Figure 2.7). Virtually all the sentinels remained in the same neighbourhood for the duration of the study and a few relocated into the most popular neighbourhoods and into smaller accommodation ($p < 0.05$). In terms of socio-economic status, the areas of Frigate Bay, Half Moon Bay, Peninsula and RUSVM were classified as 'high', whereas Bird Rock, Camps, Mattingley, West Farm and Monkey Hill were considered 'medium' and Conaree, Boyds, Trinity, Bladens, and Basseterre city were classified as 'low'.

Over a third of the sentinels (36.9%) reported that the screens on windows and doors were in good condition and only 25.9% reported that they had no screens on windows and doors or that all were damaged and constituted a hazard for mosquitoes accessing their residence. Air-conditioning (AC) was present in 98% of the sentinels' residences, mostly as individual units and only occasionally as central-AC (8%). More than half of the sentinels (56.9%) lived in concrete residences, 42.1% lived in residences made of concrete and wood, and only three sentinels (1.3%) lived in a house built entirely of wood. No significant differences between neighbourhoods and socio-economic status were observed. The average sentinel household had 3 rooms (two with AC) and 2 residents although it varied from 1 to 8 rooms, (1 to 5 with AC) and between 1 and 12 residents.

Largely, the majority of the sentinels (80.1%) had a constant supply of piped water although restrictions were imposed across neighbourhoods by the authorities in 2015 and 2016 following the large decrease in annual rainfall. Regular water supply decreased gradually until its lowest point in May 2016 when 41% of the sentinels experienced interruptions in their water supply which were highly significant at the end of 2015 and during 2016 and correlated with the periods of lower rainfall (Figure 2.8). Sentinels in Monkey Hill, Mattingley and Frigate Bay had a significantly more consistent water supply than the rest of the areas ($p < 10^{-4}$).

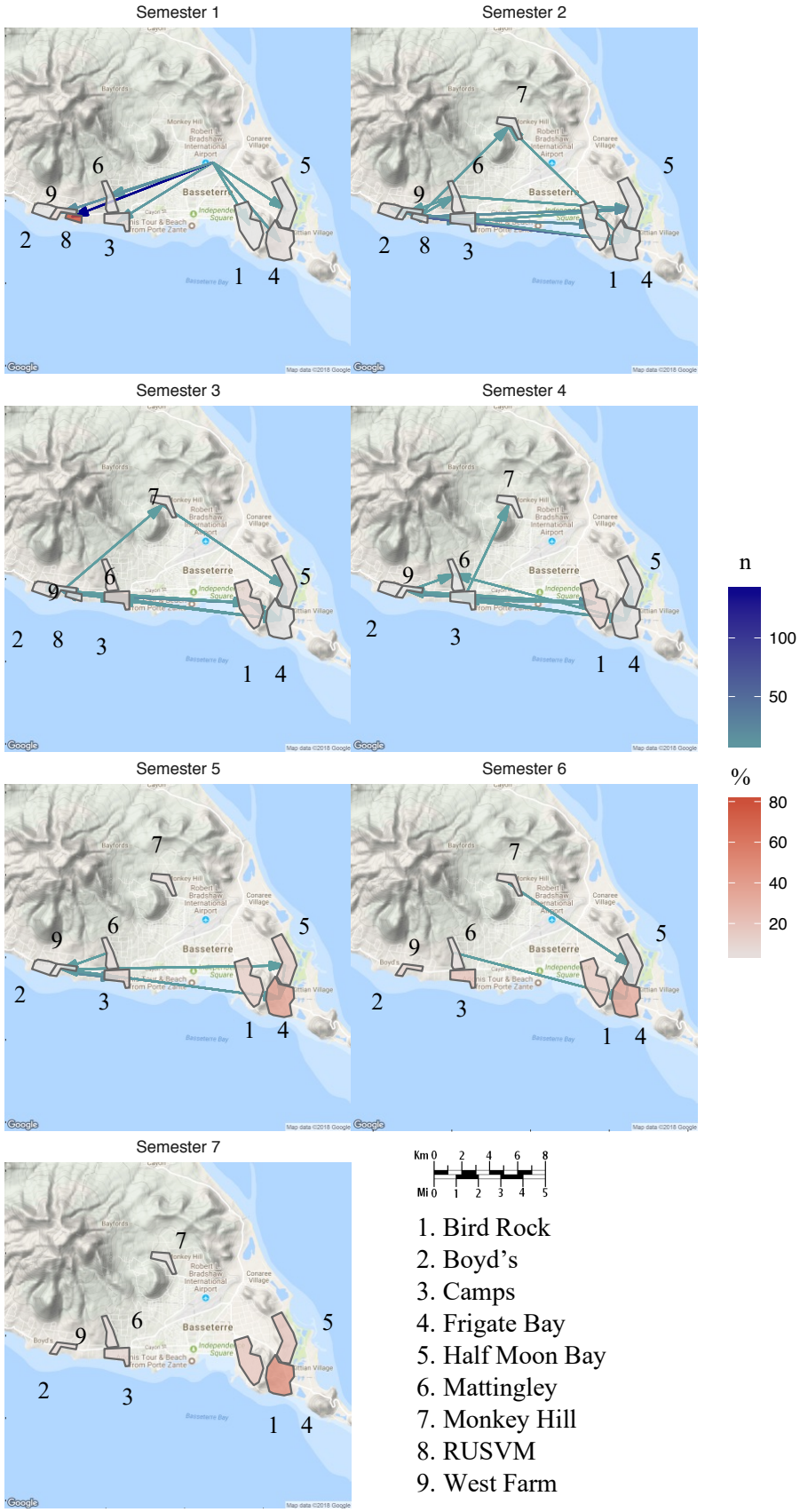


Figure 2.7. Sentinels' residence during the study from semesters 1 to 7 of their arrival. Blue arrows symbolise the number (n) of sentinels moving into each new area. The red polygons symbolise the proportion (%) of sentinels residing in each area and semester (Google Maps, 2018).

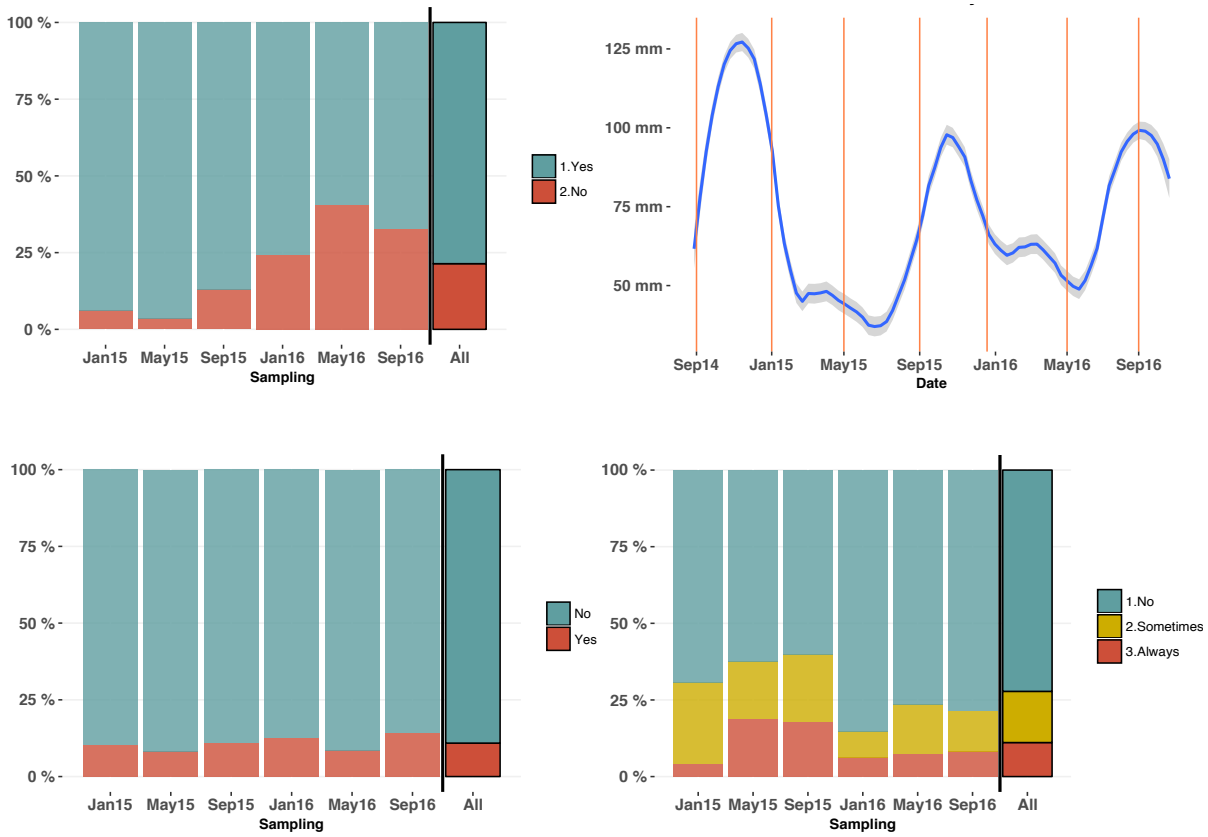


Figure 2.8. Top left: Tap water supply in sentinels' households. Top right: Cumulative 30 days' rainfall with a loess smoothing curve fitted (blue line) and 95% C.I. (grey shaded area). Bottom left: presence of pools of water around houses. Bottom right: Storage of water by sentinels

Presence of larval habitats

Nearly half of the sentinels (45%) reported that the ancillary areas around their house had little or none trash that could act as breeding containers for mosquitoes and only 4% responded that there was 'a lot of trash' around their house. This is not unexpected as domestic refuse is collected regularly in sentinels' neighbourhoods: 94% of the sentinels' litter was collected at least weekly and virtually all of the volunteers had their domestic refuse collected at least fortnightly. The amount of debris around the house was constant over time and no significant differences were seen across sentinel residential areas.

Overall, 20.2% of the sentinels kept plants in pots that could collect rain water and a few of the respondents across all samplings (10.1%) reported that pools of rain water accumulated near their residences (Figure 2.8). Faculty and post-graduate students were 8 times more likely to keep plants in pots than undergraduate students ($p < 0.001$) and, although not statistically significant, undergraduate students in their last semester of studies were 2.8 times more likely to keep plants in pots than the newly

arrived sentinels. The residents in Bladens and Half Moon Bay were significantly more likely to keep plants whereas the residents in Camps and RUSVM were significantly less likely to keep plants in pots ($p < 0.05$) which suggests a positive trend towards keeping more plants the longer the sentinels had lived on the island increasing the risk for mosquito breeding containers. Likewise, nearly a third of the sentinels (27.9%) stored water in the house regularly (Figure 2.8) which increased at the beginning of the restriction announcements during the samplings in May and September 2015 ($p < 0.05$) and there was a highly significant effect correlated with lack of constant water supply that made some sentinels to store water in containers ($p < 10^{-6}$).

2.3.3. Behavioural risk factors and exposure to the *Aedes aegypti* vector.

Behaviour in the house:

The behaviours of the sentinels in the house were generally protective against exposure to mosquitoes. Nearly half of the sentinels (49.6%) reported that they never left unscreened windows or doors open for periods of 30 minutes or more during the day and only a minority (8.7%) left unscreened doors or windows open for 30 minutes or more during the day which decreased with longer periods of stay ($p < 0.001$) (Figure 2.9). Similarly, most of the sentinels spent less than one hour a day outside on the porch, patio or garden (64.2%) and only 19.1% spent longer than one hour a day outside on the porch. The preferred times were equally spread out during the morning, afternoon and the evening and most sentinels were outside at the time of mosquito peak activity in the mornings and evenings and only a small proportion (17.9%) stayed outside only in the afternoon, during the least active periods of mosquito activity. Finally, in households with Air-Conditioning, more than half of the sentinels (54.1%) used it daily or several times per week and around a third (34.9%) used it only a few times a year or never. The use patterns are consistent over time and no differences in sentinel characteristics, neighbourhoods or others have been observed.

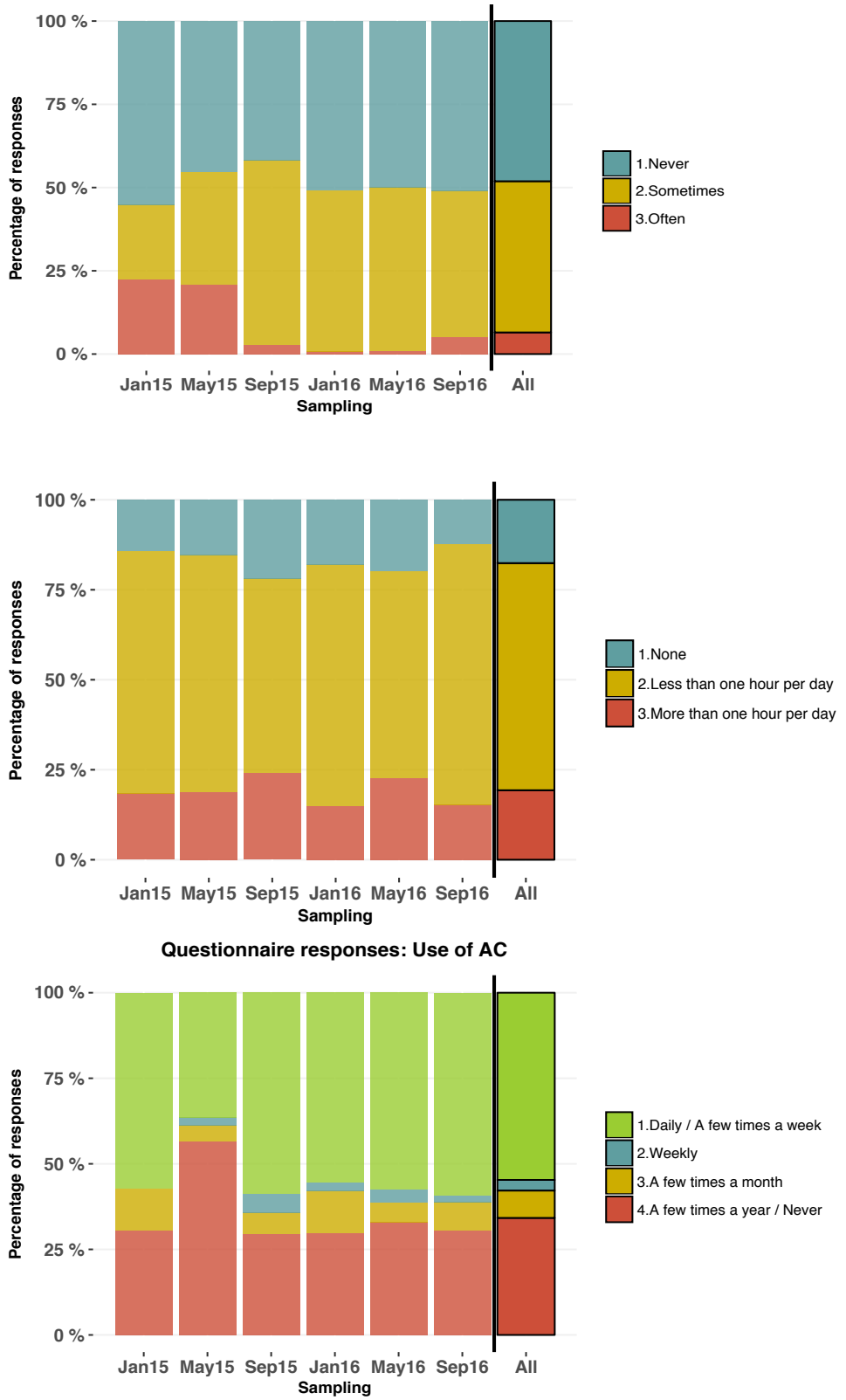
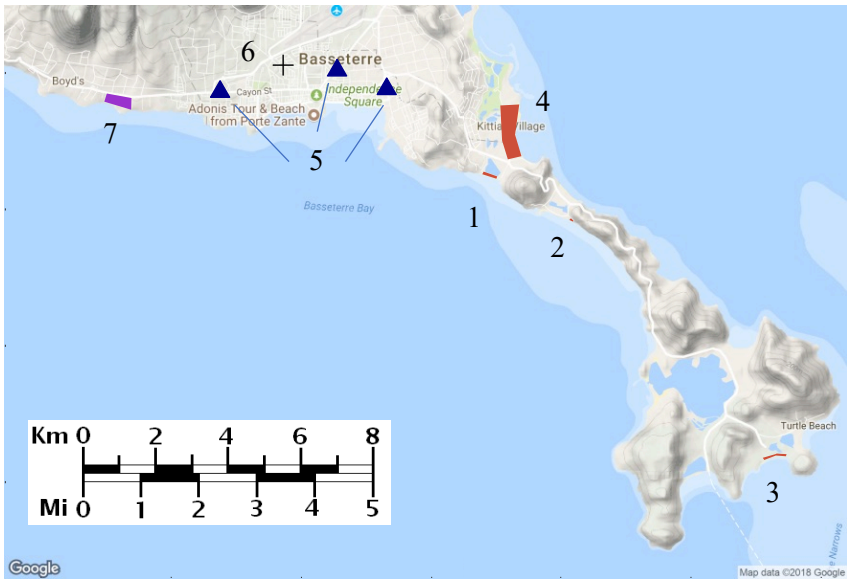


Figure 2.9. Sentinels' Questionnaire responses: Unscreened doors and windows open (top). Time spent in the porch (middle). Use of AC (bottom).

Behaviour outdoors:

The behaviours of the sentinels outdoors varied with their length of residence. The outdoor areas most frequented comprised the resorts at Timothy Beach (The Strip), Shipwreck Beach and Reggae Beach, the restaurants along Zenway Blvd. in Frigate Bay, in addition to the Caribbean Christian Fellowship church and RUSVM (Figure 2.10).



Recreational areas:
1. Timothy Beach
2. Shipwreck Beach
3. Reggae Beach
4. Zenway Blvd.

5. Grocery stores

6. Church

7. RUSVM

Figure 2.10. Outdoor areas frequented by the sentinels (Google Maps, 2018).

Overall, 90% of the sentinels visited a beach at every sampling period and 29.1% frequented one at least weekly, a proportion that decreased steadily with the sentinels' length of stay on the island ($p < 0.05$). On the contrary, the proportion of sentinels that visited a beach monthly or less increased slightly at the same time that the proportion of those who never visited decreased. In other words, more sentinels ventured to a beach with longer times of residence but did so less frequently. Similarly, 85.6% of the sentinels visited a bar or a restaurant during the study and 23.9% frequented a bar or a restaurant at least weekly overall. The length of residence on the island also had a negative effect on the frequency of bars visited ($p < 0.05$), and although not statistically significant ($p = 0.07$) male sentinels were more likely to frequent bars and restaurants during the day or the evening and there was a correlation in the frequency of outdoor activities (Figure 2.11). Conversely, only 30.2% of the volunteers hiked regularly and the proportion of hikers increased slightly over length of stay ($p < 0.01$). Finally, less than one in ten sentinels (9.4%) reported to have visited a church at least once during the sampling intervals with a slight

increase in attendance over time, although any conclusions need to be considered with caution due to the high number of non-respondents.

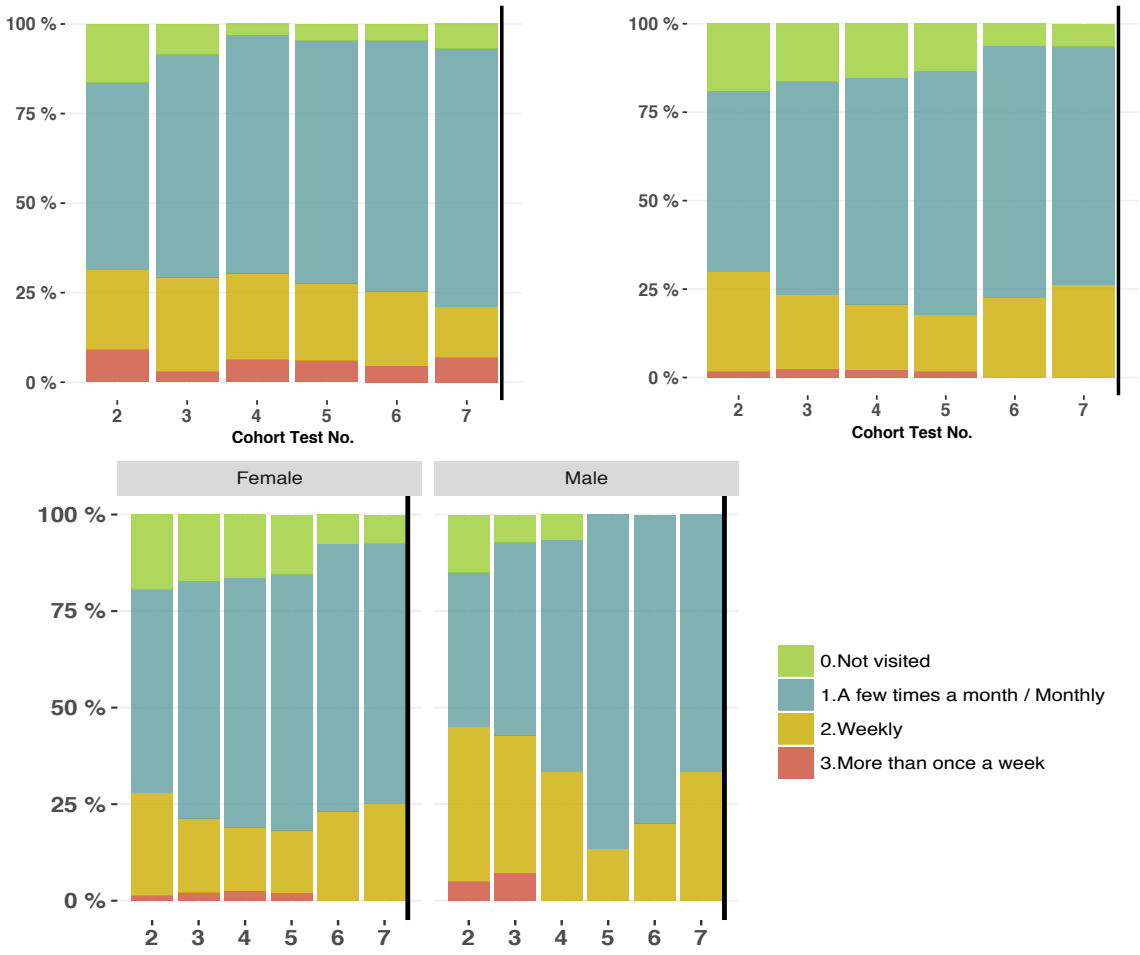


Figure 2.11. Proportion of sentinels frequenting a beach (top left) and a bar or restaurant (top right) according to length of stay in semesters. Proportion of males and females visiting recreational areas (bottom). N.B. observations standardised to time since arrival.

Behaviour protective measures:

The majority of sentinels used some protective measures against mosquitoes depending on the time of the year and the frequency of mosquito bites. The most popular protective method in the house was insecticide spray and 60.5% of sentinels used it at some point. The frequency decreased from 27.7% of the sentinels using insecticide daily or several times per week prior to January 2015 to only 5.7% in May 2016, while a few times a month or never use increased from 34.0% to 47.6% in the same period which correlated weakly but non-significantly with rainfall (Figure 2.12) and likelihood of mosquito bites in the house. The least popular protective method in the house was mosquito coils and only 13.2% of the sentinels reported usage with little variation observed.

The application of individual mosquito repellent varied with cohorts and frequency of mosquito bites. Overall, nearly half of the sentinels (43.9%) used mosquito repellent '*sometimes'* and 29.3% used it '*always or often*' when spending time outdoors. The sentinels from the cohort '*Jan.15*' showed a significantly lower use of repellent than previous cohort '*Sep.14*' ($p < 0.05$), which arrived during the chikungunya outbreak in 2014. It is also worth noting the relationship between the use of repellent and mosquito bites. Figure 2.13 suggests that individuals who reported '*often'* mosquito bites both within the house and when sitting outside the house used repellent more frequently than those who reported no mosquito bites neither in the house nor outside ($p < 10^{-5}$). The correct application of protective measures was not assessed, and it was assumed that the sentinels followed best practices when applying such measures.

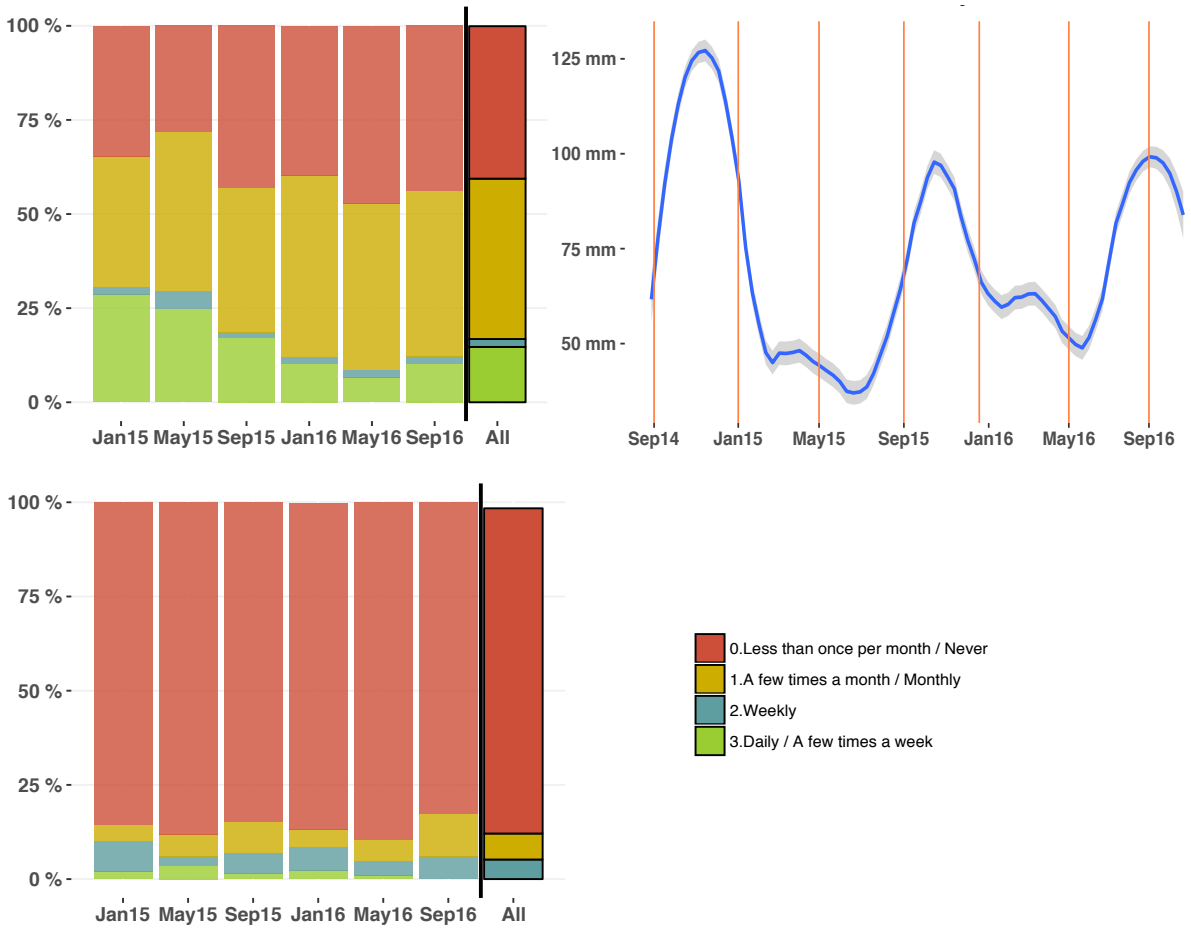


Figure 2.12. Proportion of sentinels' usage of mosquito protective measures: Top left: Use of insecticide spray. Bottom left: Use of burning coils. Right: Daily Rainfall (mm) with a loess smoothing curve fitted (blue line) and 95% C.I. (grey shaded area) obtained from SKN Metereological Office.

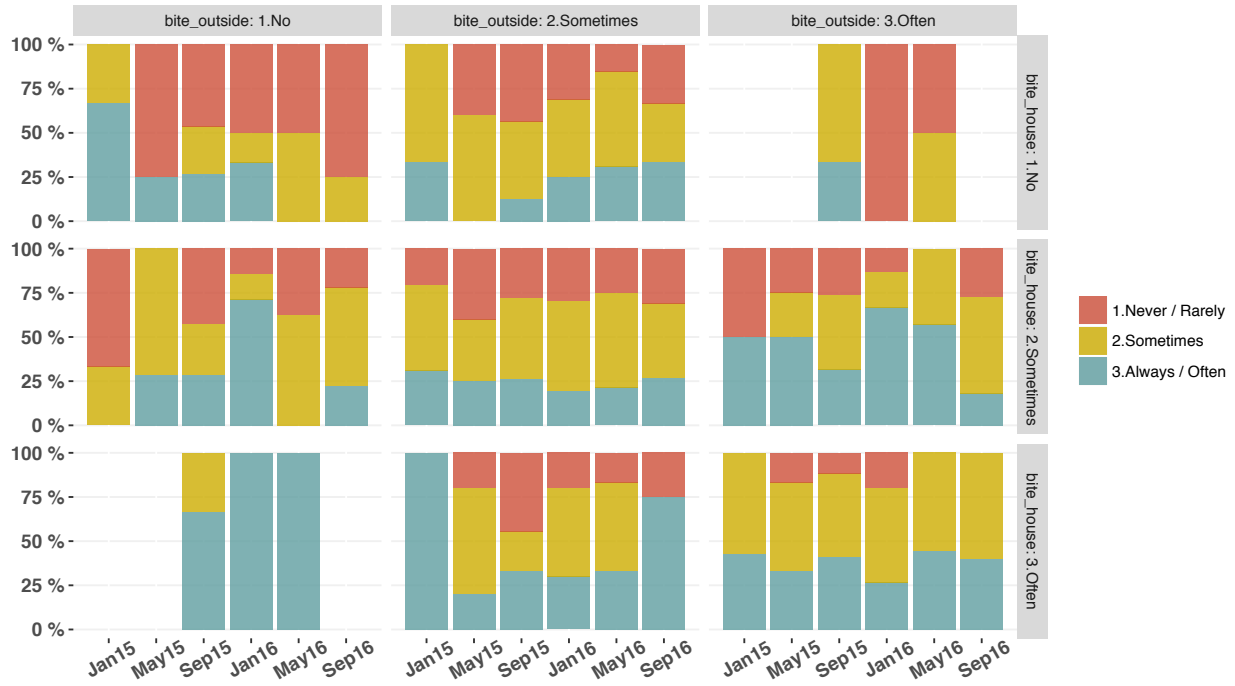


Figure 2.13. Use of individual mosquito repellent by frequency of mosquito bites inside and outside the house.

2.3.4. Frequency of mosquito bites.

The self-reported frequency of mosquito bites varied by the amount of debris on each location, personal characteristics and rainfall. Overall, nearly a fifth (17.4%) of the sentinels reported frequent bites inside the house, 64.8% were bitten '*sometimes'* and 17.9% reported '*no*' mosquito bites. A similar proportion of sentinels (21.3%) reported '*often'* bites outside the house, 64.7% were bitten '*sometimes'* and 14.1% reported '*no*' mosquito bites outside the house. The majority of the bites occurred during the day or the evening (71.5% of the bites inside the house and 84% outside the house) which is consistent with *Aedes* spp. peak feeding activity, and the remaining percentage reported being bitten at night only, which is inconsistent with *Aedes* spp. peak feeding activity. Accumulation of trash around the household (Figure 2.14) and use of repellent outdoors and of coils in the house were significantly correlated with frequency of mosquito bites ($p < 0.005$) but rainfall lacked statistical significance in the frequency of mosquito bites. Of those sentinels who frequented a bar or restaurant, 24.3% reported frequent mosquito bites and 30.7% reported no mosquito bites. A small proportion of those who visited a beach reported frequent mosquito bites at the beach (14.1%) and 43.5% reported no mosquito bites at the beach. Finally, very few sentinels noticed being bitten at the grocery store (3.7% often and 85.6% no bites) or during hiking (9.9% often and 38.6% no bites). Bites were correlated by indoor (within and around the house and in the shops) and outdoor recreational areas.

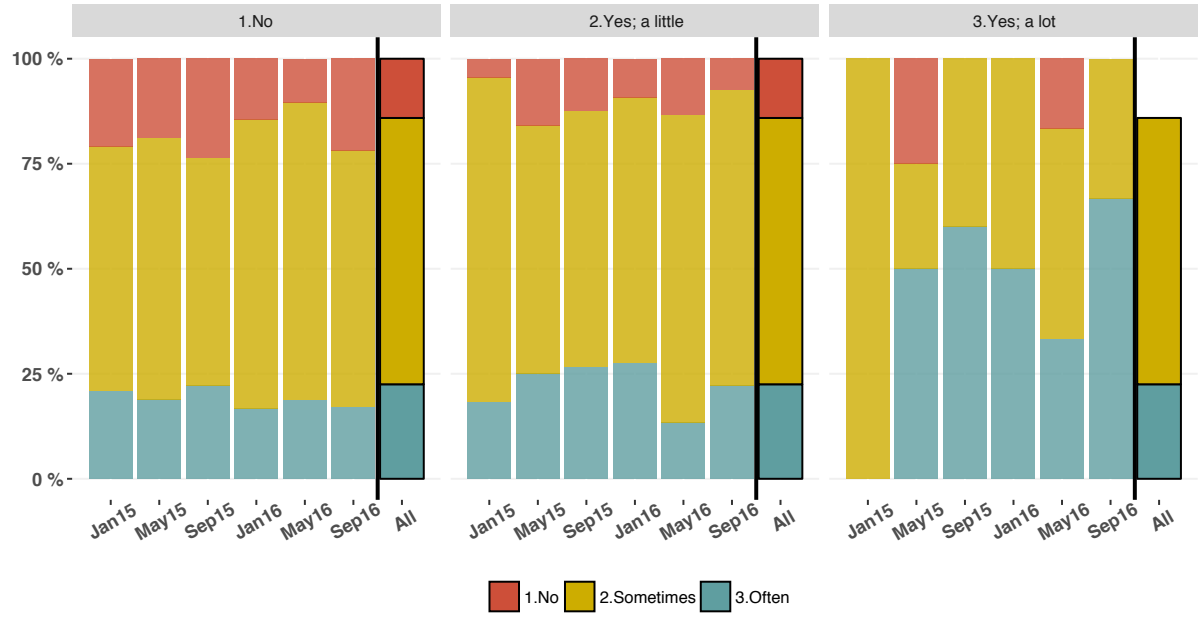


Figure 2.14. Frequency of mosquito bites and presence of debris around the house.

2.3.5. Suspected cases

In addition to the regular sentinel sampling, 90 samples from individuals with arboviral symptoms were submitted by health practitioners between September 2014 and November 2016 of which 78 (86.7%) were collected from non-sentinels, 54 (68.4%) were female, and all the suspected cases ranged between 18.4 and 65.0 years of age. Less than a third of the cases sampled were born in SKN (26.7%) and the rest originated from the US mainland (62.2%), Puerto Rico (3.3%), UK (2.2%), and Barbados, Canada, India, Venezuela and USVI. The majority of the samples (83.5%) were received between September 2014 and January 2015 (33.3%) and between September 2016 and November 2016 (50.5%) which coincided with the peaks of the chikungunya and the Zika outbreaks respectively.

Significant differences in socio-economic factors, environment and behaviours between patients from Kittitian origin (SKN) and foreign residents were observed (Table 2.5). The epidemiological data gathered from suspected cases suggested that SKN patients were at a higher risk of arboviroses as the majority of individuals resided in low socio-economic areas (58.3%) and only 4.2% resided in high socio-economic areas, as opposed to other nationalities who resided mostly in high socio-economic areas (54.5%) and only 3.6% in low socio-economic areas ($p < 0.001$). SKN nationals' households had a higher number of occupants ($\bar{x} = 3.8$) than foreign-born residents ($\bar{x} = 2.6$) and only 8.3% had intact screens in all windows and doors indicating a significantly higher risk ($p < 0.001$) of mosquitoes entering their

household than in other nationalities'. Also, the surroundings of SKN households accumulated more debris that could serve as breeding containers than foreign nationals' ($p < 0.05$), 62.5% of Kittitian patients lacked AC in their house as opposed to 10.3% of overseas ($p < 0.001$) and daily use was significantly lower among SKN (12.5%) than other nationalities (47.3%) ($p < 0.001$). Similarly, suspected cases born in SKN significantly left unscreened windows open for long periods of time (41.7%), spent longer time in the porch (54.2%) than foreign-nationals (23.6%) ($p < 0.005$) and, although frequented less recreational areas (29.1% and 37.5% SKN visited a beach or a bar respectively as opposed to 80.0% and 70.9% of other nationals), attended a church more frequently than other nationals (50.0% vs. 16.3%) ($p < 0.01$). Finally, most Kittitian suspected cases (54.2%) sprayed the house daily or several times a week in contrast to 21.8% of other nationals ($p < 0.001$) but reported mosquito bites more frequently in the house and at church than other nationals ($p < 0.005$).

Table 2.5. Differences in environmental and behavioural factors between SKN and foreign nationals.

		SKN		other		p-value
		n	%	n	%	
Socio-economic status	1.low	14	58.3%	2	3.6%	$2.2x10^{-16}$
	2.medium	9	37.5%	23	41.8%	
	3.high	1	4.2%	30	54.5%	
Mosquito screens	1.All	2	8.3%	17	30.9%	0.0002
	2.Some	7	29.2%	25	45.5%	
	3.None	15	62.5%	13	23.6%	
Trash around the house	1.No	8	33.3%	23	41.8%	0.03
	2.Yes; a little	11	45.8%	25	45.5%	
	3.Yes; a lot	5	20.8%	7	12.7%	
Unscreened door open	1.Never	6	25.0%	18	32.7%	0.003
	2.Sometimes	8	33.3%	28	50.9%	
	3.Often	10	41.7%	9	16.4%	
AC coverage in household	0% coverage	15	62.5%	6	11.5%	1.0E-5
	50% coverage	22	91.8%	17	32.6%	
	100% coverage	2	9.3%	21	40.4%	
Use of AC	1.Daily / A few times a week	3	12.5%	26	47.3%	$6.4x10^{-5}$
	2.Weekly	1	4.2%	7	12.7%	
	3.A few times a month	2	8.3%	5	9.1%	
	4.A few times a year / Never	18	75.0%	17	30.9%	
Time at the porch	1.None	6	25.0%	10	18.2%	0.004
	2.Less than one hour per day	5	20.8%	32	58.2%	
	3.More than one hour per day	13	54.2%	13	23.6%	

(continued)

		SKN		other		p-value
		n	%	n	%	
Frequent a beach	0.Not visited	17	70.8%	11	20.0%	$5.4x10^{-10}$
	1.A few times a month / Monthly	5	20.8%	20	36.4%	
	2.Weekly	2	8.3%	19	34.5%	
	3.More than once a week	-	0.0%	5	9.1%	
Frequent a bar or restaurant	0.Not visited	15	62.5%	16	29.1%	0.002
	1.A few times a month / Monthly	5	20.8%	14	25.5%	
	2.Weekly	3	12.5%	19	34.5%	
	3.More than once a week	1	4.2%	6	10.9%	
Frequent a church	0.Never	12	50.0%	46	83.6%	0.007
	1.A few times a month / Monthly	6	25.0%	5	9.1%	
	2.Weekly	4	16.7%	2	3.6%	
	3.Couple of times a week	2	8.3%	2	3.6%	
Use of insecticide spray	0.Less than monthly / Never	8	33.3%	21	38.2%	0.0003
	1.A few times a month / Monthly	3	12.5%	12	21.8%	
	2.Weekly	-	-	10	18.2%	
	3.Daily / A few times a week	13	54.2%	12	21.8%	
	4.Always / Often	4	16.7%	15	27.3%	
Mosquito bites in the house	1.No	-	0.0%	1	1.8%	0.01
	2.Sometimes	9	37.5%	31	56.4%	
	3.Often	15	62.5%	23	41.8%	

2.4. Discussion.

Longitudinal studies following cohorts of sentinels provide a detailed understanding of processes in arbovirus transmission and epidemiology that are fundamental for more effective, locally adapted disease control programmes including vaccine trials (Morrison *et al.*, 2008, 2010) although a serious impediment to large population-based longitudinal studies is mainly caused by the logistical and technological challenges associated with serological testing (Vorndam and Kuno, 1997). The majority of longitudinal studies have been conducted in children, amongst whom dengue shows high incidence rates (Endy, Chunsuttiwat, *et al.*, 2002; Balmaseda *et al.*, 2006, 2010; Endy *et al.*, 2011; Srikiatkhachorn *et al.*, 2016), and fewer prospective studies have been conducted in adults that mainly reported disease rates and dynamics of different dengue serotypes (Morrison *et al.*, 2010), prevalence of chikungunya symptoms (Schilte *et al.*, 2013) and a few addressed risk factors of infection in adults (Porter *et al.*, 2005; Kuan *et al.*, 2009, 2016). Some authors have advised that reliance on naïve populations for longitudinal studies may underestimate overall dengue activity and that sentinels are inappropriate for early detection as

higher incidence rates have been observed in individuals previously infected by a different dengue serotype than on naïve populations (Morrison *et al*., 2010).

A common limitation of longitudinal studies is missing data arising from subject dropouts and defective questionnaire completion which can cause biased results and incorrect inferences if the mechanism of the missingness is ignored (Little and Rubin, 2002; Gelman and Hill, 2007). Sentinel attendance at regular sampling sessions was generally high. The majority of the sentinels (66.4%) provided at least three samples and 63 sentinels (28.1%) attended all of their sampling calls originating data of high value for subsequent analysis of risk factors of infection performed in section 5.3. Sentinels who provided more than two consecutive samples were significantly more likely to remain in the study at later calls than those who missed intermediate calls ($p < 0.001$) and only a minority of sentinels (19.2%) provided one baseline sample. Analysis of sentinel dropouts was challenging as sentinels enrolled at different times and attendance was irregular with some sentinels missing one or more samplings and returning at later callings. Exploration of dropout patterns initially suggested that the cohort Jan.15 had a statistically significant lower probability of sampling attendance after a positive result from one dengue test in January 2016 ($p = 0.048$) than the rest of cohorts and sampling calls. However, given the closeness of the significance value to the otherwise arbitrary significance threshold (Burnham and Anderson, 2002) and the lack of further evidence from other samplings and cohorts to support the hypothesis that sentinels were less likely to attend a sampling based on their test results, there was little confidence in the overall significance of the statistical result and the sentinels' dropout pattern was regarded as randomly distributed. Also, the level of questionnaire completion was high among sentinels although a few questions were left unanswered. The reasons for the sentinels' non-responses can be attributed mostly to unawareness of some information such as the frequency of pest control at the sentinel's household, or to the nature of the responses, such as attendance at RUSVM which may make some people reluctant to respond (Ackroyd and Hughes, 1981). In order to avoid biased inferences in subsequent analysis due to the high rate of non-responses or by the non-randomness of the missing data, the latter questions were removed from further analysis (Little and Rubin, 2002; Gelman and Hill, 2007).

The great majority of the sentinels recruited were female (87%), which was representative of the student source population at RUSVM and similar to most

veterinary schools (Lofstedt, 2003), around 26 years of age and originated from non-endemic countries in N. America but more than half had travelled to areas 'at risk' of dengue (World Health Organization, 2009) before coming to St. Kitts. Nearly all the sentinels reported being healthy or having conditions unrelated to arboviroses on arrival although a substantial number of sentinels came from areas with WNV and SLEV (Mackenzie, Gubler and Petersen, 2004) circulation, a few reported having been vaccinated against YF or JEV or diagnosed with dengue previously which could interfere with the accuracy of the dengue tests as will be detailed later in chapter 3.

The sentinels' household environments were generally protective against mosquito exposure and mosquito bites, and little differences were observed between cohorts. Sentinels resided typically in medium to upper class neighbourhoods such as Frigate Bay and Half Moon Bay with spread out houses, low occupancy rate, screened windows and virtually all sentinels' households had AC that was used daily or weekly, all of which have been associated with low risk of dengue transmission (Reiter *et al.*, 2003; Sissoko *et al.*, 2008; Kuan *et al.*, 2009, 2016; Wearing, Robert and Christofferson, 2016). Also, the surroundings of most sentinels' households were generally unfavourable to larval development. Domestic refuse was collected weekly and ancillary areas were generally free from debris and containers that could act as mosquito breeding sites (Tun-Lin *et al.*, 1996; Chadee, 2004, 2009) and, although sentinels with longer length of residence tended to keep plants in pots that could accumulate rain water, the overall effect was small. Furthermore, only less than a third of the sentinels stored water in the house regularly that could create additional mosquito breeding sites (Chadee and Rahaman, 2000; Porter *et al.*, 2005), which correlated with the water restrictions in 2015-16 after an exceptionally low rainfall in the region during the ENSO phenomenon (Banu *et al.*, 2015; Muñoz *et al.*, 2016).

Likewise, most sentinels took protective measures against mosquito exposure. The majority of sentinels used insecticide spray in the house and mosquito repellent outdoors which was significantly correlated with the likelihood of suffering mosquito bites and, although not significantly, with rainfall. Sentinels who were bitten often in or around the house reported significantly higher use of repellents and spray than sentinels who experienced little mosquito bites. The likelihood of attracting *Aedes* spp. mosquitoes and being bitten depends greatly on individual characteristics, including movement, body heat, carbon dioxide, lactic acid, octanol, fatty acids, ammonia emanating from their hosts breath or sweat, as well as body odours and

warmth (Bosch, Geier and Boeckh, 2000). The use of mosquito repellent varied between cohorts too. The first cohort arrived during the peak of the chikungunya outbreak in 2014 at a time of high awareness for mosquito bites, whereas the following cohort arrived when the number of acute cases decreased substantially and the awareness for mosquito protection nearly disappeared. This occurrence has also been observed amongst ex-pat NGO workers who showed high awareness for mosquito bites and higher levels of protection against mosquitoes when arriving to dengue endemic areas that the native population (Salyer *et al*., 2014).

Sentinels spent most of their day at RUSVM which had characteristics unfavourable to mosquitoes including effective cleaning and gardening programmes, regular pest control, mosquito screens and heavy use of AC. The most popular outdoor areas visited by the sentinels during the day or the evening included beach resorts, and nearly all sentinels visited a beach with negligible differences in the behaviour between cohorts, gender or academic semesters overall and was correlated with frequenting bars and restaurants. The length of residence had a small negative effect on visiting recreational areas and the overall tendency was for the sentinels to decrease frequency of visits over time, and although non-statistically significant, male sentinels were more likely to frequent bars and restaurants during the day or the evening. In terms of arbovirus epidemiology, frequenting bars may be considered a high-risk behaviour since these are mostly open wooden structures with accumulated debris in surrounding areas that favour larval habitat and concentrate high density of mosquito hosts (Getis *et al.*, 2003; Chadee, 2004; Gómez-Dantés and Willoquet, 2009; Brady *et al.*, 2014). Only a few sentinels reported attending a church or hiked regularly across all parts of the island.

Sentinels living in Bird Rock area reported greater frequency of mosquito bites than sentinels living in Frigate Bay and RUSVM campus, although no significant differences were seen across neighbourhoods. The main significant differences in mosquito biting were correlated with the amount of debris and containers around the property (Tun-Lin, Kay and Barnes, 1995; Chadee, 2004).

In contrast, there were significant differences in the environment and behaviours amongst the suspected cases born in St. Kitts and foreign nationals suggesting that Kittitians may be at a higher risk of arboviral transmission than foreign residents. Although the number of samples collected from suspected cases born in St. Kitts and Nevis was moderate (n = 24; 26.7%) and may not represent accurately the local

population, most Kittitians resided in medium and low-income areas with high occupancy density, increased mosquito larval development sites resulting from poor hygiene, inadequate housing quality and minimal environmental management practices (Brady *et al*., 2014), had less intact mosquito screens and scarce air-conditioning coverage and overall usage, and left windows and doors open for longer periods of time than foreign nationals. Also, Kittitians showed significantly higher use of insecticide around the house than non-Kittitians which can be explained by higher exposure to adult mosquitoes indoors due to the lack of physical barriers and higher poverty index (Mena *et al*., 2011). Also, Kittitians significantly visited less beach resorts and attended a church more frequently than foreign nationals which would increase social contact with other high-risk hosts and exposure to mosquitoes during their peak biting activity (Stoddard *et al*., 2013). Finally, since the different communities observed in St. Kitts showed significant differences in their environment and behaviours and little social interaction in few shared public areas, this could suggest that arbovirus transmission risks are different within communities and sentinels were at a lower risk overall.

2.5. Concluding remarks

The epidemiological data suggested that sentinels were at low risk of arbovirus transmission as most sentinels lived in low risk environments and their behaviours were overall protective against mosquito bites.

Chapter 3. Laboratory tests

3.1. Introduction

The challenges in diagnosing inapparent flaviviral infections with existing methodology are widely recognised, and identifying all lifetime flavivirus infections in areas with concurrent circulation of antigenically related arboviruses has been dubbed as the 'holy grail' of dengue serology (Halstead, 2004). Cross-reactivity amongst flavivirus is one of the primary challenges in arbovirus diagnosis in that previous exposure to one of the Flavivirus will cause a positive result to a different virus and will difficult the diagnosis and epidemiological conclusions (Halstead, 1997, 2004; Gubler, 1998a; Kuno, 2003; Guzmán *et al.*, 2004; Hunsperger, 2012).

In general, during infection with an external agent, an antigen, the host's immune system will recognise the unfamiliar proteins on the antigen and will produce antibodies, proteins able to bind specifically to the antigen (Wagner *et al.*, 2009). Since the presence and amount of specific antibodies is dependent on previous exposure to an infecting agent, the detection and measurement of antibodies will reflect an organism's past antigenic exposure and history, of which IgM and IgG are the most relevant in diagnosis (Crowther, 1995; Guzmán and Kourí, 2004). The first antibody produced in response to a primary exposure of an agent is immunoglobulin IgM followed by the appearance of immunoglobulin IgG (Figure 3.1). Flavivirus-specific IgM antibodies are detectable in 50% of patients by 3 to 5 days after onset of illness, increasing to 80% of the patients by day 5 and 99% by day 10 reaching a peak level about two weeks after the onset of symptoms and then decline to undetectable levels over 2 to 3 months (Gubler, 1998a; Kuno, 2003; Hunsperger, 2012). Anti-dengue IgG is generally detectable in serum at low titres at the end of the first week of infection, increasing slowly thereafter and can be detected in blood after several months and even persist for life (Hunsperger, 2012). If re-exposure occurs in a secondary (or even tertiary) infection by the same virus or by another virus antigenically similar, or by vaccination, a peak of IgM appears at a lower titre than during a primary infection which may be undetectable in some cases (Kuno *et al*., 1991; Kuno, 2003). The presence of anti-dengue IgM is used as a marker of recent infection whereas the presence of anti-dengue IgG is a criterion for past infection with dengue; however, while the presence of a high titre of IgG in a single sample of serum suggests a recent infection, seroconversion or a fourfold increase in the titre of IgG in paired sera from a patient confirms infection and IgG is detectable at high

levels and persists for months or life in some cases (Vorndam and Kuno, 1997; Guzmán and Kourí, 2004; Ngwe Tun *et al.*, 2016). The serological response to CHIKV is similar in that specific IgM antibodies are frequently present within 7 days post-infection and can persist for several months (Powers, 2010). Infection with CHIKV is presumed to result in lifelong immunity as IgG have been documented to persist for years and immunologic memory would provide protection on re-challenge (Powers, 2010; Nitatpattana *et al.*, 2014), although secondary clinical infections have been suggested in Asia (Kosasih *et al.*, 2013).

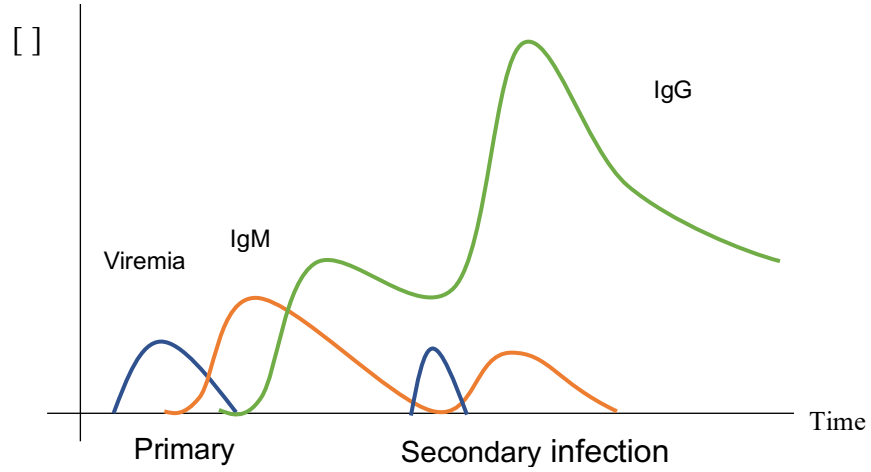


Figure 3.1. Concentration of antibodies IgM and IgG over time during a primary and a secondary infection. Adapted from Handbook for Clinical Management of Dengue 2012 (WHO, 2012)

It is clinically relevant to distinguish primary and secondary dengue infections as serious complications may develop in the latter case and the ratio between IgM / IgG antibodies in standardised procedures and laboratories can be used for this purpose. Several ratios indicative of primary infections have been proposed such as an IgM / IgG ratio greater than 1.2 in sera diluted at 1/100 or greater than 1.4 in sera diluted at 1/20 (World Health Organization, 2009). Alternatively, Guzmán and Kourí (2004) proposed that a ratio smaller of 1.78 would indicate a secondary infection.

Diagnostic tests based in measuring biological components are required to discriminate between healthy and diseased individuals and to do so should provide accurate and precise results (Dohoo, Martin and Stryhn, 2012). The existing assays available in the diagnosis of arboviruses meet most of these requirements but have some limitations. For instance, molecular diagnosis are highly specific and sensitive but are only valid for recent infections and require expensive equipment, in comparison, serologic tests such as rapid detection tests (RDT) allow the detection of past infections within minutes and require minimal equipment and training but some

severely lack specificity (Blacksell *et al.*, 2012; Prat *et al.*, 2014). There are other serologic methods that address the shortfalls of specificity such as plaque reduction neutralization test (PRNT) or Hemagglutination Inhibition (HAI) but these assays require a degree of training, are labour intensive and involve constrains from handling live viruses that are not available in all laboratories (Guzmán *et al.*, 2004). In addition, some of the available ELISA serology have limitations on the validated specimen matrix (Blacksell *et al.*, 2012) or may be not specific enough for the population of study and may need optimization (Vazquez *et al.*, 2007).

3.1.1. Existing diagnostic methods for arboviroses

There is an array of laboratory diagnosis methods available for detecting and confirming arboviruses infection. In general, diagnostics that detect the infecting virus or some of its components such as the virus nucleic acid or its proteins are classified as direct methods. Alternatively, indirect methods are techniques designed to detect proteins produced by the host during the immune response (Figure 3.2).

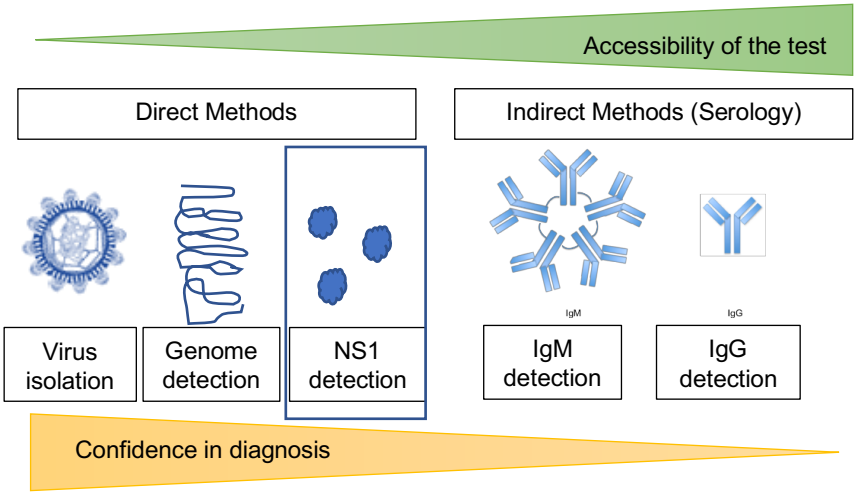


Figure 3.2 Summary of diagnostic methods to detect arbovirus infection in humans. NS1 detection only available for DENV. Adapted from: World Health Organization, 2009, p.92 (Reproduced with permission from the author).

Direct methods

In the early stages of infection, direct methods are the most effective diagnostic tools due to the high sensitivity and specificity but require more complex technologies and technical expertise (World Health Organization, 2009). Virus isolation and amplification in a cell line followed by the observation of cell morphology and cytopathic effects (CPE) is the classic method of confirming a viral infection (Hunsperger, 2012). The cell line most sensitive to infection by arboviruses is C6/36, a clone obtained from *Aedes albopictus* cells although mammalian cell lines such

Vero or LLCMK2 cells are also susceptible (Hunsperger, 2012). Detection of viral genome through amplification by reverse transcriptase polymerase chain reaction (RT- PCR), real-time RT-PCR, and nested RT-PCR are the most commonly used methods for their high sensitivity and specificity and relative ease of use, but their performance will depend on the primer set and / or probe sequences and the method of RNA extraction (Hunsperger, 2012). Additionally, the detection of the viral non-structural protein NS1 secreted during replication, can also be used as diagnostic during the acute stage of dengue although ZIKV have lower viral titres than DENV and its use as diagnostic tool is more unlikely (Hunsperger, 2012). Unfortunately, direct methods are only effective during the phase of viremia when the virus is circulating with the exception of ZIKV which RNA has been detected in semen for up to 125 days after symptom onset (Paz-Bailey *et al.*, 2017).

Indirect methods

At the end of the acute phase and later, serology methods based on the measurement of immunoglobulins IgM or IgG are the method of choice for diagnosis and a number of protocols have been developed to detect immunoglobulins produced by the host after viral infection (World Health Organization, 2009). Some of these methods include hemagglutination inhibition assay (HI), complement fixation (CF), enzyme-linked immunosorbent assay (ELISA) and plaque reduction neutralization test (PRNT) (Gubler, 1998a). ELISA is the most commonly used test for its simplicity of use, time of assay completion and relative confidence with a number of commercial kits and in-house protocols developed including rapid diagnostic tests (RDT). PRNT is considered the gold standard confirmatory test for serologically positive specimens to Flavivirus and other viruses in primary infections (Kuno, 2003), despite indications that PRNT tests may show variability of results depending on the assay conditions (Thomas *et al.*, 2010). Unfortunately, PRNT assays are expensive, laborious, and technically complicated to perform and few laboratories have the capacity and technical expertise to implement this test appropriately (Gubler, 1998a; Roehrig, Hombach and Barrett, 2008). Hence, many studies rely solely on the anti-DENV ELISA results to measure prevalence in a population (Marrero-Santos *et al.*, 2013) although the prevalence of dengue in a population can be overestimated (Radke *et al*., 2012; Mohammed *et al*., 2012).

3.1.2. Challenges in Flavivirus serologic diagnosis

Kuno (2003) indicated that 'when the same specimens are tested by two or more techniques, discordant results have been observed on some specimens, and that the exact cause(s) of the discrepancy in most reports is often highly complex, requiring the consideration of many factors'.

Flavivirus cross-reactivity has been identified as one of the primary challenges in serologic diagnosis (Kuno, 2003) in that when serum specimens are evaluated with antigenically closely related members such as DENV, ZIKV, WNV, SLEV, YFV or JEV, they are found to be cross-reactive, mainly caused by the antibody IgG, even in the early convalescent phase of primary infections (Kuno, 2003). Although subject to debate due to the existence of antibody-dependent enhancement pathogenesis (Halstead, 2014), it has been suggested that infection by a different serotype among related viruses may provide some cross-protective immunity (Hunsperger, 2012; Endy *et al*., 2011). Also, due to original antigenic sin, which is the phenomenon that an individual with a secondary infection with a virus closely related to a previous infection will show higher serological titres to the older virus rather than the newer virus, contributes to the results of a PRNT test for a person with a history of infection by multiple Flavivirus being uninterpretable unless the medical history is known (Halstead, 1983; Hunsperger, 2012).

Moreover, persistence of antibody and viral persistence has been observed in locations where several Flavivirus are active all year round or where repeated introduction is possible. Examples of lengthy persistence of IgM and IgG after flaviviral infections have been reported including occurrence of anti-WNV IgM in serum over 500 days after onset which renders correct etiologic identification more difficult (Kuno, 2003). Also, in rare cases, Flavivirus infection may become persistent (Kuno, 2001; Nalca, Fellows and Whitehouse, 2003). The persistence of both antibodies and virus complicates diagnosis of a recent or acute infection (Hunsperger, 2012). In contrast, a lack of antibody response has also been observed, especially in some vaccine trials that has been partly attributed to the reduced immunogenicity of those vaccines, to immunologic interference, or to individual variation in immune response (Kuno, 2003). For example, during a DENV-1 vaccine trial, some volunteers developed IgG but no IgM, conversely, others developed IgM but no IgG (Edelman *et al.*, 1994). Finally, also the selection of

specimens from cases with mild symptoms or from hospitalized patients has shown a sharp contrast in IgM positivity between 30% and 75%, respectively (Kuno, 2003).

3.1.3. Characteristics of diagnostic tests and determination of test characteristics

The two key characteristics in a diagnostic test that define its ability to detect diseased and healthy individuals correctly are *sensitivity* and *specificity* (Dohoo, Martin and Stryhn, 2012). The sensitivity of a test is the proportion of individuals that have the disease and are correctly identified as positive by the diagnostic test, i.e. the conditional probability that an individual has a positive test given that they have the disease [$p(T^+ \mid D^+)$]. Whereas the specificity of a test is the proportion of individuals that do not have the disease and test negative, i.e. the conditional probability that an individual has a negative test given that they do not have the disease [$p(T^- \mid D^-)$]. These two characteristics are exclusive of each other and establishing a threshold at different levels will affect the number of false positives or false negatives, that is, individuals that are incorrectly identified by the test as 'diseased' individuals but do not have the condition or as 'negative' although they may have the condition (Dohoo, Martin and Stryhn, 2012).

Determination of test characteristics

The typical approach for determining the performance of a test, comprises a 'gold standard' that has a known sensitivity and specificity as close as 100% and drawing a Receiver Operating Characteristic (ROC) graph where the true positive rate (Sensitivity) is plotted against the false positive rate (1-Specificity) to make inference about the optimal cut-off threshold, the test parameters and the true disease status (Greiner, Sohr and Göbel, 1995). In the absence of a gold standard, the simplest method to estimate an appropriate cut-off is to calculate the mean and standard deviation of the index values from a known population of samples and establish a threshold at 2 standard deviations (SD) from its mean index value. An interval obtained by subtracting 2 x SD from the mean and by adding 2 x SD to the mean shows that the chance of a test value coming outside this interval will be less than 5% (Singh, 2006). However, this approach requires the existence of previous training data of known disease status and ignores any skewness of the index values (Dohoo, Martin and Stryhn, 2012). Further approaches involve the creation of a pseudo gold standard by combining imperfect tests as a substitute. A composite reference standard (CRS) is formed by first testing all samples with a reference test and then all

reference test negative samples are tested with a resolver test and the results are interpreted in parallel (Dohoo, Martin and Stryhn, 2012). However, this procedure assumes known sensitivity and specificity and that the reference and resolver test are conditionally independent, i.e. the tests are independent to each other conditional to the true status of disease when they are based in detecting different biological components (Dendukuri and Joseph, 2001).

An alternate methodology involves the use of latent class models (LCM) to estimate the sensitivity and specificity of two tests without any assumption about the true disease status of each individual. LCM involve an unknown (latent) variable that is assumed to be binary (D^+ or D^-) (Dohoo, Martin and Stryhn, 2012). There are two approaches proposed in the literature to resolve latent models: Maximum Likelihood (ML) and Bayesian methods. ML methods require two or more populations with different prevalence and adherence to the assumptions for the Hui and Walter model (Hui and Walter, 1980): that the tests are conditionally independent given the disease status and that the accuracy of the tests remains constant over different populations. Violations of these assumptions may lead to severely biased results (Vacek, 1985; Enøe, Georgiadis and Johnson, 2000). In addition, ML methods will often perform badly in small-sample situations, especially if there are a substantial number of data with very small or zero frequencies (Walter, 2005). A Bayesian approach allows the calculation of the test characteristics and the true prevalence accounting for the dependence between the tests and with less than the four different tests required by frequentist approaches in order to have sufficient degrees of freedom to estimate each of the parameters of interest (Dendukuri and Joseph, 2001). As demonstrated by Joseph, Gyorkos and Coupal (1995), the basic idea behind the Bayesian approach is to eliminate the need for the frequentist constraints by first constructing a prior distribution over all unknown quantities. The data, through the likelihood function, are then combined with the prior distribution to derive posterior distributions using Bayes' theorem, which allows simultaneous inferences to be made on all parameters (Joseph, Gyorkos and Coupal, 1995).

This chapter describes the diagnostic methods available to detect arboviruses in a population and their biological principle alongside the challenges in the diagnosis of Flavivirus and their impact in determining the true level of disease in a population. The laboratory methods used in this study are detailed in this chapter in conjunction with the limitations of the serologic assays and a validation procedure performed for

the specimen matrix collected in the study. An alternative method based on Bayesian inference is proposed to improve the characteristics of some of the serology protocols applied to the study population.

3.2. Methods:

Indirect serologic methods were more appropriate to detect evidence of arbovirus infection in the sentinel samples collected every four months than molecular methods as the virus was unlikely to be circulating in blood at the time of collection, unless infection in the sentinels had occurred on the days before the sample was drawn. Therefore, the tests used in the screening of sentinels relied on the detection of anti-DENV and anti-CHIKV antibodies (IgM and IgG) by ELISA. Alternatively, samples collected from suspected cases by RUSVM health services or the local hospital with acute signs were analysed both by serologic and by molecular methods.

3.2.1. Laboratory tests: Serologic diagnostic methods.

Two serology assays involving an indirect ELISA and a capture ELISA (MAC-ELISA) were used to determine the levels of anti-DENV and anti-CHIKV antibodies in the sentinel population (Table 3.1). The complete protocols for each test are detailed in Appendix 8 to Appendix 13.

Table 3.1. Summary of ELISA methods employed in the testing of sentinels and suspected acute cases

Target virus	Test name	Antibody	Method	Developed by:	Reference:
DENV	InBios DENV *Detect*™ IgM Capture ELISA	IgM	Capture (MAC-ELISA)	InBios Inc, Seattle, Washington, USA	Namekar *et al.* (2013)
	Panbio Dengue IgG indirect ELISA	IgG	Indirect ELISA	Alere (Panbio), Brisbane, Australia	McBride *et al.* (1998)
	In-house protocol	IgG	Multi-layered Capture ELISA	Miagostovich *et al*., 1999	Miagostovich *et al*. (1999)
CHIKV	Anti-Chikungunya Virus ELISA IgM Anti-Chikungunya Virus ELISA IgG	IgM IgG	Capture (MAC-ELISA)	Euroimmun, Lübeck, Germany	Prat, *et al*. (2014)

Capture MAC-ELISA

The IgM and IgG antibody capture DENV ELISA (MAC-ELISA) consists of one enzymatically amplified sandwich-type immunoassay. This test is favoured due to the specificity to capture IgM antibodies using anti-human-IgM antibody fixed to the micro-titre plate which minimizes the interference of the high avidity IgG antibodies from binding to the antigen and it removes non-specific antibody by using specific recombinant antigens (Hunsperger, 2012). In brief, the sample was added to the plate previously coated with anti-human-IgM antibodies, incubated and washed to remove any unbound antibodies. A specific antigen solution was then pipetted to the plate which bound to antibodies selected. Finally, an anti-antibody conjugated with an enzyme was added. The amount of specific antibody binding to the antigen was quantified by spectrophotometer after addition of colour development reagents. After obtaining a measure of the Optical Density (OD) of the sample of interest, and of calibrator controls, a ratio index was calculated.

The InBios DENV *Detect*™ IgM Capture ELISA index threshold was set at 2.84, above which indicated that anti-DENV IgM antibodies were present in the sample at a level considered 'positive', whereas an index lower than 1.65 will indicate a 'negative' sample. When the sample index showed between these figures, the sample was regarded as 'inconclusive'. The in-house protocol developed by Miagostovich *et al.* (1999) varies in that serial dilutions (1:40) for each sample were performed instead of a single-point titration. At the end of the procedure, the negative control values were subtracted from the OD values of each sample. A positive result was considered when the normalized OD was greater than 0.150. The highest end-point titration with an OD greater than 0.150 was the titre value. Titre higher than 1:10,240 were considered as 'high positive' whereas titres lower than 1:10,240 were regarded as 'low positives'. Both the Anti-Chikungunya Virus ELISA IgM and IgG, specified a threshold index of 1.1 above which the sample will be 'positive', and below 0.8 the sample should be considered 'negative' and 'inconclusive' when the sample index occurred between these figures.

Indirect ELISA

The indirect anti-IgG ELISA is often used in epidemiologic studies to determine past exposure because of its improved sensitivity and specificity compared to the direct ELISA method (Hunsperger, 2012; Marrero-Santos *et al.*, 2013). The main difference is that, in the indirect method, the antigen of interest is fixed on a

microplate to which specific target antibodies will bind. The specificity for the assay is directed by the antigen fixed on the micro-titre plate (Crowther, 1995). Briefly, the indirect ELISA method consists of the addition and incubation of the samples containing antibodies and washing of the plates to rid of any unbound antibodies. Then an anti-antibody conjugated with an enzyme such as horseradish peroxidase (HRP) is added. The amount of specific antibody binding to the antigen is quantified after addition of colour development reagents. After obtaining a measure of the Optical Density (OD) of the sample of interest, and of calibrator controls, a ratio index was calculated as above. The Panbio Dengue IgG indirect ELISA index threshold was set at 1.1, above which the sample is considered 'positive', whereas an index lower than 0.9 indicated a 'negative' sample. When the ratio index showed between these figures, the sample was regarded as 'inconclusive'.

3.2.2. Laboratory tests: Molecular diagnostic methods.

Polymerase chain reaction (PCR) assays are faster and easier to implement than virus isolation and their sensitivity varies from 80% to 100% depending on the region of the genome targeted by the primers, the approach used to amplify or detect the PCR products and the method employed for subtyping, and have been widely established as diagnostic techniques in acute cases (World Health Organization, 2009).

RNA extraction

RNA extraction prior to molecular diagnosis was performed using the commercial kit QIAamp® Viral RNA Mini Kit (QIAGEN®). The reagents were prepared and stored according to the manufacturer's instructions (Appendix 14). The specimens collected from acute suspected cases (plasma, serum or urine) stored at -80^{o}C were thawed at room temperature and 140µl of sample was added to previously prepared buffer AVL containing carrier-RNA. After a series of washes and centrifugations (Appendix 14), 40 µl of RNA were obtained. The extraction was performed in duplicate for each individual in order to maximise volume and concentration of RNA resulting in 80µl yielded from each patient. The eluted RNA was kept at -80^{o}C until testing.

RT-PCR protocols

Molecular methods were used to detect the presence of viral RNA in specimens collected from individuals with arboviral symptoms at the health services and the local general hospital described in chapter 2 and in the mosquitoes collected during

the entomologic survey described later in chapter 4. Briefly, the assays employed comprised the CDC DENV1-4 Real-Time RT-PCR Assay (Center for Disease Control, Puerto Rico) for the detection of DENV RNA (Johnson *et al.*, 2005), RealStar Chikungunya RT-PCR Kit (Altona Diagnostics, Germany) (Panning *et al.*, 2009; Gallian *et al.*, 2015) and the protocol described by Kumar, Johnson and Gopal (2007) for the detection of CHIKV RNA, and a modified protocol described by Lanciotti *et al.* (2008) for the detection of ZIKV RNA. The forward and reverse primers selected for each protocol are specified in Table 3.2. The sequential steps and combinations of times and temperatures for each of the RT-PCR protocols are detailed in Appendix 15 to Appendix 18.

Table 3.2. Known oligonucleotide primers used in the DENV, CHIKV and ZIKV molecular assays. (Primers for assay RealStar Chikungunya RT-PCR Kit (Altona Diagnostics, Germany) were not provided)

Primer	Nucleotide sequence	Genome position / target gen	Fluorophore	Reference
DENV1-F	CAAAAGGAAGTCGTGCAATA	8973	FAM/BHQ-1	(Johnson *et al.*, 2005)
DENV1-R	CTGAGTGAATTCTCTCTACTGAACC	9084		
DENV1 probe	CATGTGGTTGGGAGCACGC	8998		
DENV2-F	CAGGTTATGGCACTGTCACGAT	1605	HEX/BHQ-1	
DENV2-R	CCATCTGCAGCAACACCATCTC	1583		
DENV2 probe	CTCTCCGAGAACAGGCCTCGACTTCAA	1008		
DENV3-F	GGACTGGACACACGCACTCA	740	TR/BHQ-2	
DENV3-R	CATGTCTCTACCTTCTCGACTTGTCT	813		
DENV3 probe	ACCTGGATGTCGGCTGAAGGAGCTTG	762		
DENV4-F	TTGTCCTAATGATGCTGGTCG	904	Cy5/BHQ-3	
DENV4-R	TCCACCTGAGACTCCTTCCA	992		
DENV4 probe	TTCCTACTCCTACGCATCGCATTCCG	960		
DVRChk-F	ACCGGCGTCTACCCATTCATGT	10237-10258 (330bp)	N/A	(Kumar, Johnson and Gopal, 2007)
DVRChk-R	GGGCGGGTAGTCCATGTTGTAGA	10544-10566		
ZIKV 1086	CCGCTGCCCAACACAAG	1086-1102	SYBR	(Lanciotti *et al.*, 2008)
ZIKV 1162c	CCACTAACGTTCTTTTGCAGACAT	1162-1139		

Each assay was performed as described elsewhere in the literature (Johnson *et al*., 2005; Kumar, Johnson and Gopa, 2007) or as per the manufacturer's instructions. The protocol described by Lanciotti *et al*. (2008) for Zika was modified for the use of SYBR as the fluorophore instead of a FAM-probe. At the end of the amplification process, reading of the melt curve increased the specificity of the, otherwise, unspecific binding of SYBR green. The melting temperature (T_m) for the ZIKV amplicon was calculated using the formula:

$$T_m = 64.9 + \frac{41\,(G + C - 16.4)}{A + T + G + C}\ (\text{in Celsius}) \qquad (2)$$

where *G, C, A* and *T* are the number of the corresponding nucleotides in the amplicon (Genosys Biotechnologies, 2016). The T_m obtained for the sequence with the primers ZIKV1086 – 1162c was 75.8°C. A peak of the melt curve at the T_m point in addition to positive SYBR fluorescence indicated amplification of the targeted ZIKV genome sequence. The RT-PCR assays were carried out in an Eppendor Mastercycler Nexus Gradient and gel electrophoresis performed in Mupid-One System and the real-time RT-PCR assays were performed in an ABI-7500 Fast Real-Time PCR System amplifier.

3.2.3. Methods: validation of plasma as sample specimen in ELISA protocols.

Manufacturers of serologic assays make specific recommendations for the sample matrix to be used for testing because blood preservatives or anticoagulants may affect assay performance (Blacksell *et al.*, 2012). Blacksell *et al.* (2012) compared the performance of the Panbio Dengue IgG Indirect ELISA using plasma and serum and found no significant difference on the results from either specimen. However, an analogous validation of the performance of the in-house protocol developed by Miagostovich *et al.* (1999) other than for serum was unknown and, therefore, a validation of the in-house Miagostovic protocol for plasma was required.

Paired samples of plasma and serum were taken randomly from the same individuals and assessed simultaneously by the in house ELISA protocol described byMiagostovich *et al*. (1999). Mean OD values for each dilution of paired plasma and serum sample were first compared with the Wilcoxon test. Next, the precision of the ELISA test was assessed for all plasma and serum samples separately to obtain an indication of the intra-specimen variability and then compared with each other for agreement between the specimens by calculating the Coefficient of Variation (CV):

$\mathrm{CV} = \sigma/\mu$, where σ is the standard deviation among test results on the same sample and μ is the average of the test results (Donhoo, Martin and Stryhn, 2012). The level of precision and agreement between the specimens was also compared by the Concordance Correlation Coefficient (*CCC*) (Lin, 1989, 2000) and by observation of limits of agreement plots or Bland-Altman plots which showed the range where 95% of the paired samples would be expected marking the upper and lower limit (Bland and Altman, 1986). Finally, differences in qualitative interpretation of the tests with the different samples were assessed using the McNemar's X^2 test.

3.2.4. Methods: anti-DENV IgG ELISA performance and selection of optimal thresholds.

The performance of the Panbio Dengue IgG indirect ELISA used in the study was uncertain. The technical summary sheet of the Panbio Dengue IgG indirect ELISA provided by the manufacturer claimed a sensitivity of 87% and specificity of 100% compared with HAI assay, although, supplementary characterization of the test performance specified a sensitivity for primary infections as low as 33% (95% CI: 23.4-44.5%) (Table 3.3). While estimates of the sensitivity and specificity are often considered innate characteristics of a test, there is increasing evidence that, for many tests, the sensitivity and specificity may vary across source populations (Greiner and Gardner, 2000; Dohoo, Martin and Stryhn, 2012) or due to the characteristics and source of the dengue antigens used in the tests (Vazquez *et al.*, (2007) citing Porter *et al.* (1999); Sabchareon *et al.*, 2012). Other studies have indicated further discrepancies in the performance of the Panbio Dengue IgG indirect ELISA. McBride *et al*. (1998) reported 99.17% sensitivity and 96.18% specificity compared with HI in a population in North Queensland, Australia (McBride *et al*., 1998), whereas, Shamala (2015) reported a substantially lower sensitivity of 39.8% (95% CI: 4-46%). Since the Panbio Dengue IgG indirect ELISA performance study originated from donors in Malaysia, it was considered that the cut-off values of this test may be suboptimal for the study population from N. America and the Caribbean.

Table 3.3. Panbio DENV IgG Indirect ELISA test performance.

Reference:	Sensitivity (95% CI)		Specificity (95% CI)	
Alere (Panbio)	97.9% (Secondary) 33.3% (Primary)	92.5 – 99.7% 23.4-44.5%	100%	96.6-100 %
McBride *et al*., 1998	99.17%	-	96.18%	-
Shamala, 2015	39.8%	4-46%	95.8%	91-98%

Similarly, the in-house protocol developed by Miagostovich *et al*. (1999) also had unspecified performance characteristics. Based on the results reported by Mohammed *et al.* (2012) using the in-house Miagostovich protocol with a confirmatory PRNT, 100% sensitivity of the ELISA assay and 56% specificity can be inferred. Moreover, Marrero-Santos *et al*. (2013) assessed the performance of a similar indirect IgG ELISA that was originally claimed to have sensitivity of 95%, and specificity of 99% but with PRNT demonstrated 100% sensitivity and 24% specificity for which the authors proposed a new cut-off point that rendered a sensitivity of 85.3% and specificity of 95.7% (Marrero-Santos *et al*., 2013). In addition, in the absence of gold standard tests like PRNT or HAI for reasons outside the scope of the project, the true performance of the test, the disease state and disease prevalence remained uncertain. Therefore an optimal cut-off for the Panbio Dengue IgG indirect ELISA and for the in-house protocol for the study population based on Bayesian methods was pursued.

The methodology proposed by Choi *et al*. (2006) was applied to analyse the ELISA scores from the sentinel samples collected as it has been demonstrated that the inferences from this model for a non-gold standard case are equivalent to the results obtained from a gold standard test (Choi *et al.*, 2006). The index values obtained from the Panbio ELISA were log-transformed to approximate normality and, in the case of the in-house Miagostovich protocol, the normalized OD values of the second dilution (1:160) were log-transformed in order to avoid the high variability of OD results in lower dilutions and deviations from normality were assessed visually by quantile-quantile plots. The model was formulated in terms of conditionals for test 2 (panbio) given test 1 (in-house) values. For cut-off values $c \in (-\infty, \infty)$, ROC curves can be drawn by plotting pairs of 1-specificity and sensitivity. Further assumptions included consideration of individuals classified as ‘positives’ or ‘negatives’ according to their score values as ‘diseased’ (D) or ‘non-diseased’ (D^-) respectively. The

following informative independent prior distributions were used based on the results from the samples tested:

$$\mu_{D1} \sim N(0, 0.147) \qquad \mu_{D2} \sim N(1.1, 0.1)$$

$$\mu_{D^-1} \sim N(-3, 0.001) \qquad \mu_{D^-2} \sim N(-2.75, 0.283)$$

where μ_{D1} to μ_{D2} is the mean of the score test values for each group of diseased and non-diseased obtained from each test. Uninformative gamma(0.001, 0.001) for 1 / σ^2_{Dkk} and 1 / $\sigma^2_{D^-kk}$ where *k* is the test (in-house or Panbio) and uniform(-1,1) for ρ_D and ρ_{D^-} were used. This choice of prior presumes equal probabilities of all possible values of each correlation (Choi *et al.*, 2006). Since prior information about the disease status of the sentinels is unknown, let Z_j be the latent variable that indicates the disease status of the *j*th individual (1 if an individual has been infected to DENV, and 0 otherwise), and assuming bivariate measurements were collected based on the following model: $Z_j \sim$ Bernoulli(π)

$$Sj = \begin{pmatrix} S_{1j} \\ S_{2j} \end{pmatrix} \sim p\,(\cdot\,|\mu_{D1}, \mu_{D2}, \sigma^2_{D11}, \sigma^2_{D12}, \rho_D)^{Z_j} g(\cdot\,|\mu_{D^-1}, \mu_{D^-2}, \sigma^2_{D^-11}, \sigma^2_{D^-22}, \rho_{D^-})^{1-Z_j} \qquad (3)$$

Where S_{1j} and S_{2j} are the test scores for tests 1 and 2 (in-house and panbio respectively) for sentinel *j*; $P(Z_j = 1) = 1 - P(Z_j = 0) = \pi$; p and g are the pdf's for tests 1 and 2 and, where $p(\cdot)$ is the $N_1(\mu_D, \Sigma_D)$ pdf and $g(\cdot)$ is the $N2(\mu_{D^-}, \Sigma_{D^-})$ pdf. For identifiability, $\mu_{D^-k} < \mu_{Dk}$, $k = 1, 2$ was assumed. The Bayesian inferences were calculated using the Gibbs sampler with 'rjags' in R (Plummer, 2016).

3.3. Results

This section presents the results of the validation of plasma specimens and an optimized cut-off for the anti-DENV IgG ELISA tests. The results of the assays for the sentinels and suspected cases, and analysis of the prevalence of dengue, chikungunya and Zika in the study population will be detailed in chapter 5.

3.3.1. Results: analysis of plasma as sample specimen in ELISA protocols.

Paired samples were collected from 102 random sentinels in all the cohorts in December 2015. All samples collected showed an endpoint titration smaller than 4, which is equivalent to a titre equal or smaller than 1:2,560 and were considered 'low positives'.

Intra-specimen test variability:

Overall, plasma OD values showed greater variability than those of serum (overall CV_{plasma} of 0.114, CV_{serum} = 0.062), especially at low dilutions (Table 3.4). This greater variability at low dilutions can be explained by the prozone effect (Figure 3.3) that occurs typically, but not always, when an excess of antibodies inhibits agglutination reactions due to inhibitory substances or to competition by low-avidity antibody that are washed off in subsequent steps (Miagostovich *et al*., 1999). This finding was also supported by the Concordance Correlation Coefficient. While a CCC value of 1 indicates perfect agreement between two sets of samples, the CCC for plasma and for serum specimens alone was 0.440 and 0.937 respectively, suggesting that the precision for plasma specimens was lower than for serum. The coefficients did not vary substantially amongst the different dilutions indicating consistency in the performance of the test (Table 3.4).

Table 3.4. Coefficient of Variation (CV) between plasma and serum specimens.

	CV_{plasma}		CV_{serum}		$CV_{plasma\text{-}serum}$	
	Normalized	*Raw*	*Normalized*	*Raw*	*Normalized*	*Raw*
d1 (1:40)	0.504	0.232	0.207	0.078	0.037	0.037
d2 (1:160)	0.252	0.104	0.178	0.066	0.075	0.037
d3 (1:640)	0.241	0.069	0.239	0.067	0.066	0.037
d4 (1:2560)	0.389	0.054	0.273	0.037	0.103	0.037
Mean	0.346	0.114	0.224	0.062	0.071	0.037

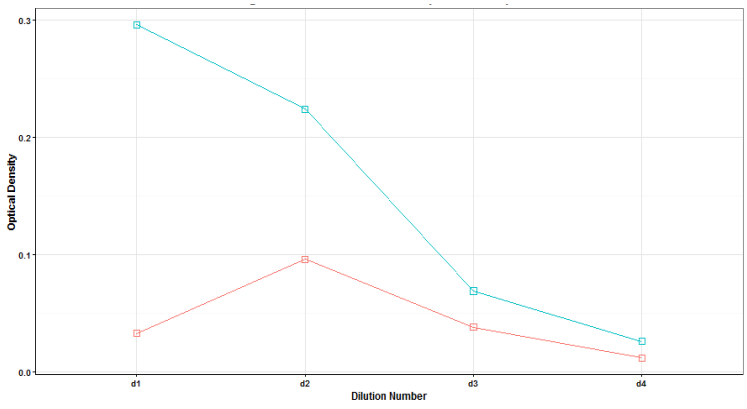


Figure 3.3. IgG ELISA titration curves of two plasma samples indicating the pro-zone effect. The optical density in the red sample is lower at the more concentrated dilution 1 (d1) than at dilution 2 (d2) due to inhibited binding caused by excess antibody.

Table 3.5. CCC estimated by Variance Components of plasma (left) and for serum (right) alone for normalized OD values with 95% CI and SE.

	Plasma specimens				Serum specimens			
Dilution	CCC	95% CI		SE	CCC	95% CI		SE
d1 (1:40)	0.421	0.288	0.538	0.064	0.940	0.913	0.959	0.012
d2 (1:160)	0.467	0.327	0.586	0.066	0.939	0.912	0.959	0.012
d3 (1:640)	0.454	0.321	0.570	0.063	0.928	0.896	0.951	0.014
d4 (1:2560)	0.421	0.272	0.551	0.071	0.941	0.913	0.960	0.012
Mean	0.440				0.937			

Comparison OD plasma vs serum

Overall, there were only 3.5% discordant pairs for the final interpretation of the test (OD mean value > 0.150 at end point of the second dilution) and the standard deviation was just above 7% of the mean of the sample readings. Plasma replicates showed slightly more positive results than sera, however, the difference was non-significant (McNemar's $\chi^2 = 1.785$, $p = 0.18$). No significant differences in mean OD readings for both raw OD (before the OD was subtracted from the corresponding negative control) and normalised OD between specimen types were found (Wilcoxon test, p>0.05, Table 3.6).

Table 3.6. Mean OD values for plasma and serum at dilutions 1 to 4 and Wilcoxon signed-rank test comparing the means of paired samples (n = 102) at each dilution level.

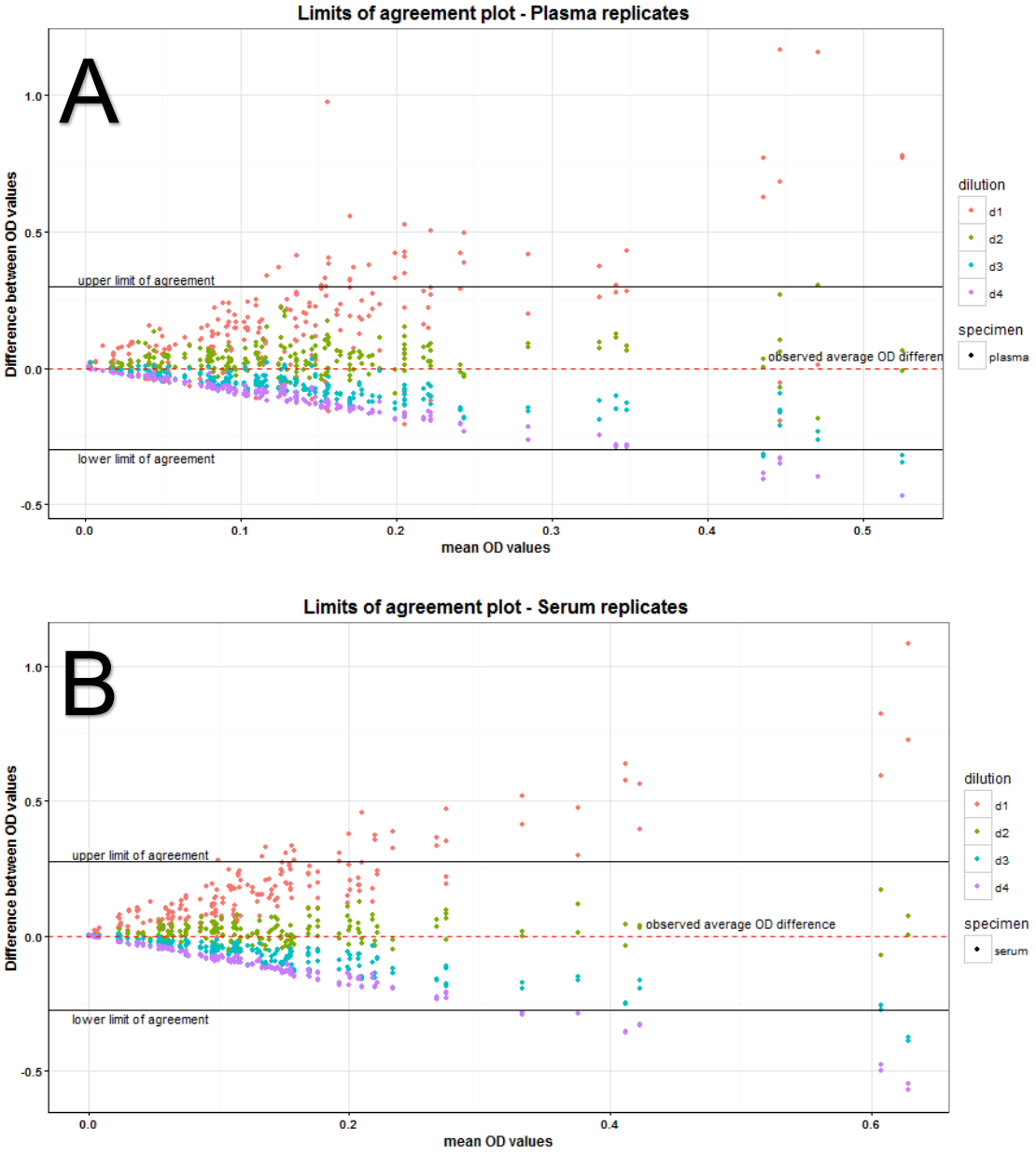
Dilution	OD plasma	OD serum	W	p-value
d1 (1:40)	0.273	0.293	4895	0.467
d2 (1:160)	0.156	0.142	5693	0.254
d3 (1:640)	0.063	0.063	5178.5	0.956
d4 (1:2560)	0.021	0.022	4983	0.604
Mean OD	0.145	0.132		

The concordance correlation coefficient of plasma and serum (Table 3.7) showed substantial agreement between the two specimens (CCC = 0.915, 95% C.I.: 0.877-0.941) indicating an almost perfect agreement between the optical density obtained from plasma and from serum for the same individuals. The Bland and Altman limits of agreement ranged from -0.290 to +0.290 and the majority of values outside this range corresponded to the first dilutions which would be consequent with the prozone

effect at low dilutions (Figure 3.4 A-C). The range and distribution of values between the plasma replicates, serum or the comparison of both was analogous.

Table 3.7. CCC estimated by Variance Components of plasma vs serum for normalized OD values with 95% CI and SE.

	CCC	95% C.I.		SE
d1 (1:40)	0.872	0.817	0.911	0.024
d2 (1:160)	0.935	0.906	0.955	0.012
d3 (1:640)	0.945	0.920	0.962	0.010
d4 (1:2,560)	0.908	0.867	0.937	0.017
Mean	0.915	0.877	0.941	



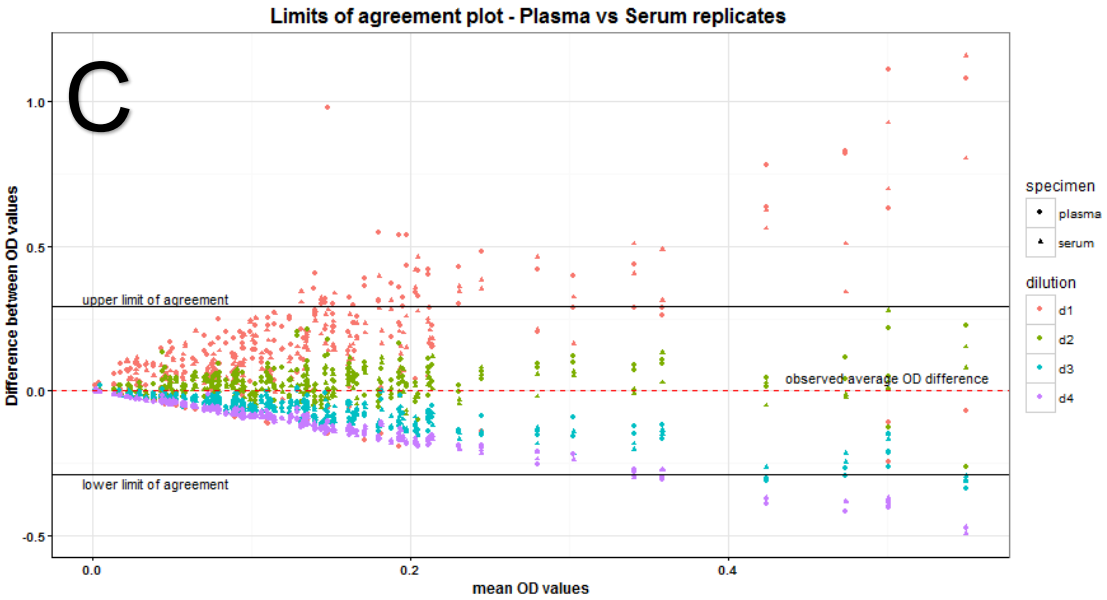


Figure 3.4 Difference against mean plot for plasma and serum. (A) plasma replicates, (B) serum replicates, (C) difference between plasma and serum.

3.3.2. Results: anti-DENV IgG ELISA performance and selection of optimal thresholds.

The Panbio Dengue IgG indirect ELISA performed better in the study population than the in-house protocol, according to the Bayesian-based model (Table 3.8). The AUC for the Panbio test was 0.83 (95% PI: 0.71-0.94) as opposed to the in-house assay with a modest AUC of 0.63 (95% PI: 0.55-0.71) (Figure 3.5). The results indicated a moderately positive correlation in positive cases (0.35) and a non-relevant negative correlation in negative cases (-0.11). The false negative proportion of each test when the specificity is 0.95, as indicated by 'delta1' and 'delta2', is higher for the in-house test (0.39) than for Panbio Indirect test (0.15).

Table 3.8. Posterior means, standard deviation, medians and 95% Probability Intervals for the in-house ELISA and Panbio Indirect ELISA tests for DENV.

	Mean	SD	Median	2.5 %	97.5%
AUC1 (in-house)	0.631	0.042	0.6101	0.5459	0.7108
AUC2 (Panbio)	0.830	0.058	0.8482	0.7142	0.9383
delta1 (in-house)	0.39	0.076	0.4282	0.2409	0.5388
delta2 (Panbio)	0.15	0.057	0.1337	0.0458	0.2611
rho[D]	0.35	0.155	0.4280	0.0295	0.6370
rho[ND]	-0.11	0.077	-0.0888	-0.2518	0.0459

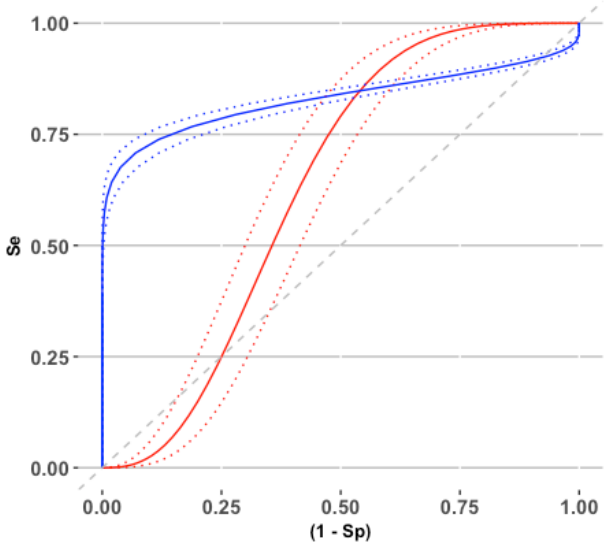


Figure 3.5. ROC Curve DENV in-house IgG ELISA (red) & Panbio Indirect IgG ELISA (blue) with 95% probability intervals. The dotted line (grey) at 45 degrees represents an AUC of 0.5 indicating that the test has no discriminatory ability.

The optimal cut-off index values were approached with a two-graph ROC as demonstrated by Caraguel *et al.* (2011) (Figure 3.6 and Figure 3.7). Several index values were selected based on the maximised outcomes of the tests characteristics, namely sensitivity, specificity, Youden index and Positive and Negative Likelihood ratios (Table 3.9). Two cut-off values are suggested for the in-house Miagostovich protocol at dilutions 1:160 for maximum sensitivity and specificity combined (OD = 0.763) and for a maximum Youden index (OD = 0.272). It is noted that these values were higher than the OD greater of 0.150 proposed by Miagostovich *et al*. (1999) for positivity. For the Panbio ELISA test, the maximum sensitivity and specificity combined was obtained with a cut-off index of 0.448, the maximum Youden Index with a cut-off index of 0.570 and maximum specificity with a cut-off index of 1.069.

Table 3.9. Cut-off figures based on maximisation of several test characteristics. Se: sensitivity; Sp: specificity; Youden: Youden index; LR+: positive likelihood ratio; LR-: negative likelihood ratio.

In-house	Cut-off OD value	Se	Sp	Youden	LR+	LR-
Combination Se + Sp	0.763	0.6054	0.5978	0.2032	1.50	0.66
Max Youden	0.272	0.8946	0.4183	0.3129	1.53	0.25
Panbio	**Cut-off index value**	**Se**	**Sp**	**Youden**	**LR+**	**LR-**
Combination Se + Sp	0.448	0.7881	0.7684	0.547	3.46	0.29
Max Youden	0.570	0.7051	0.9558	0.6609	15.95	0.30
Max Sp	1.069	0.4742	0.9999	0.4741	4742.0	0.52

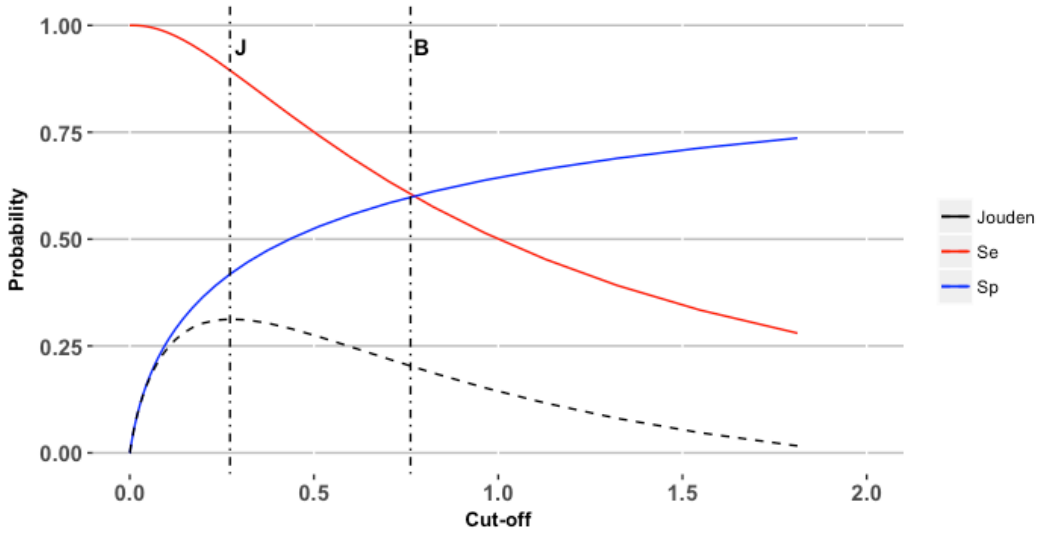


Figure 3.6. Graphical strategies to select threshold index cut-off for an in-house Indirect IgG ELISA: 2-graph receiving operating characteristic (TG-ROC) plot and graphically selection of index cut-off based on the probability of misclassification. Best combination of Sp and Se (line B), best Youden index (line J).

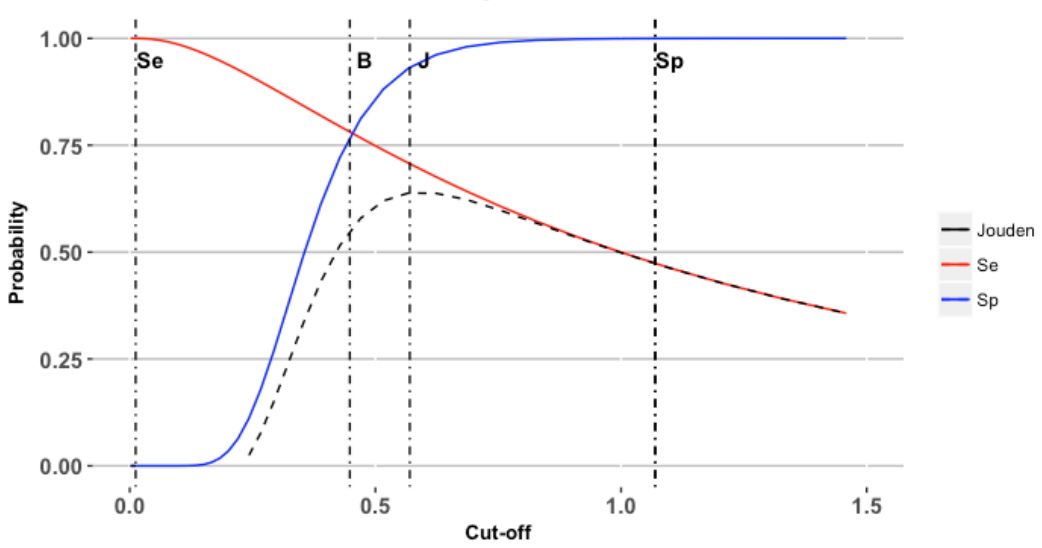


Figure 3.7. Graphical strategies to select threshold index cut-off for the test Panbio. A, hypothesized 2-graph receiving operating characteristic (TG-ROC) plot and graphically selection of index cut-off based on the probability of misclassification. Best diagnostic sensitivity (line Se), best combination of Sp and Se (line B), best Youden index (line J), best diagnostic sensitivity and LR+ (line Sp, LR+).

3.4. Discussion

There are numerous anti-DENV ELISA protocols described in the literature with different degrees of performance (Sang, Cuzzubbo and Devine, 1998; Vaughn *et al.*, 1999; Kit Lam *et al.*, 2000; Vazquez *et al.*, 2007; Marrero-Santos *et al.*, 2013) and validated sample matrix (Blacksell *et al.*, 2012), for which optimization to the population of study has been advised (Vazquez *et al.*, 2007). A validation for the use of plasma as a sample matrix for the in-house DENV IgG ELISA developed by

Miagostovich *et al*. (1999) showed no significant differences in the OD values from paired plasma and serum specimens and, overall, the data suggested that plasma and sera are both suitable samples to use in this DENV IgG ELISA assay. There was higher but non-significant variability between plasma replicates at low dilutions than serum indicating that the variability observed was lower than the variability expected due to individual differences, and testing plasma samples in duplicate and averaging the results will give more consistent results and increase repeatability. Additionally, based on the above results and reports in the literature of similar serology protocols for the detection of Flavivirus (Vaughn *et al.*, 1999; Vazquez *et al.*, 2007; Blacksell *et al.*, 2012; De La Cruz Hernández *et al.*, 2015; Parkash and Shueb, 2015), it was assumed that the performance of the InBios DENV *Detect*™ IgM Capture ELISA with plasma would be equivalent to serum.

Also, although the sensitivity and specificity are considered characteristics of each test, variations due to the source of the dengue antigens used (Porter *et al*., 1999; Vazquez *et al*., 2007) and across source populations have been observed (Greiner and Gardner, 2000; Dohoo, Martin and Stryhn, 2012). Such discrepancies among assays raise concerns of identifiability due to the existence of false positive and false negative results (Gubler, 1998) requiring optimization for the target population (Vazquez *et al*., 2007). The results of the optimization of the cut-off values of the anti-DENV ELISA tests demonstrated that the Panbio Dengue IgG indirect ELISA performed better overall than the in-house Miagostovich protocol (AUC of 0.83, 95% PI: 0.71-0.94 against an AUC of 0.63, 95% PI: 0.55-0.71, respectively). Also, the proportion of false negative was lower in the Panbio Dengue IgG indirect ELISA than the in-house protocol, indicating that the former assay has greater accuracy (Dohoo, Martin and Stryhn, 2012). Furthermore, the performance at the optimal threshold that maximise specificity of the Panbio Dengue IgG indirect ELISA in the sentinel population estimated from the models is consistent with previous reports (Marrero-Santos *et al.*, 2013), and two new cut-off values were estimated that maximised the combination of sensitivity and specificity and the Youden index. New optimal cut-off values proposed for the in-house protocol made little improvement to the modest performance of the assay suggesting a lack of confidence in the results obtained from this assay and the results obtained by the in-house protocol were discarded from further analysis of risk factors of infection in sentinels and suspected cases investigated in section 5.3.1. The test performance characteristics obtained in this

chapter will be used later to establish the status of arboviral seroconversions and the modelling of risk factors of infection in the sentinel population in section 5.3.

3.5. Concluding remarks

In summary, the data suggested that plasma is a suitable specimen sample in the ELISA assays used in the study and is equivalent to serum, and optimal cut-off values have been obtained for the sentinel population that can be used in establishing the prevalence of arboviruses in St. Kitts and models of transmission described in chapter 5.

Chapter 4. Abundance of *Aedes aegypti* in urban and sub-urban areas of St. Kitts

4.1. Introduction: *Aedes aegypti* surveillance and population models.

4.1.1. *Aedes aegypti* surveillance.

Mosquito surveillance is considered an 'essential part in the understanding of dengue epidemiology, the development of dengue surveillance and control strategies' (Getis *et al.*, 2003) and is used routinely to quantify the abundance of *Ae. aegypti* based on the assumption that there is a greater risk of arboviral transmission in areas with higher vector density (Cromwell *et al.*, 2017). Traditionally, entomological indices based on larval and pupal collections have been used to determine the density of *Ae. aegypti* populations (Focks, 2003), namely: the Breteau index (the number of positive containers per 100 houses inspected), the house index (number of positive houses per number of houses inspected), and the container index (number of positive containers per number of containers inspected) (Nathan, 1993). These indices of mosquito breeding are popular due to its relative low cost and simplicity (Codeço *et al.*, 2015), yet larvae and pupal indices disregard the problem that a single female *Ae. aegypti* oviposit in different containers and that each container produces varying numbers of emerging adults resulting in weak correlations between the larval indices and adult abundance, and in unreliable estimations of adult populations (Tidwell *et al.*, 1990; Tun-Lin *et al.*, 1996; Chadee, 2004). Although ovitraps surveillance can be useful to determine the presence of Aedes populations in an area, Getis *et al.* (2003) proposed that the most appropriate measure of entomologic risk was to determine the abundance of adult female mosquitoes as they are in the life stage from which viruses are transmitted.

There are numerous instruments described in the literature for mosquito surveillance (Silver, 2013), of which BG-Sentinel traps with an attractant lure imitating human odours are widely considered a selective tool for capturing adult *Aedes* spp. (Maciel-de-Freitas, Eiras and Lourenço-de-Oliveira, 2006; Williams *et al.*, 2006, 2007; de Ázara *et al.*, 2013; Roiz *et al.*, 2015), although some authors have indicated that the BG-Sentinel traps may have a bias against female mosquitoes seeking a blood-meal (Ball and Ritchie, 2010). Alternatively, battery-powered aspirators can capture mosquitoes of both sexes at different physiological stages directly from their resting sites, allowing better estimations of abundance, sex ratio, age structure, and physiological condition of sampled populations (Vazquez-Prokopec *et al.*, 2009).

The abundance of *Aedes* spp. is strongly affected by weather. Temperature influences the duration of the gonotrophic cycle, the number of eggs laid and mosquito lifespan (Saifur *et al.*, 2012; Carrington *et al.*, 2013; Goindin *et al.*, 2015), and rainfall influences mosquito survival (Scott *et al.*, 2000; Alto and Juliano, 2001; Fouque *et al.*, 2006). The abundance of *Aedes* spp. is also affected by anthropogenic factors (Reiter *et al.*, 2003). *Ae. aegypti* population dynamics are restricted to the household or the adjacent house (Reiter *et al.*, 1995; Honório *et al.*, 2003, 2009; Harrington *et al.*, 2005) and mosquito presence is greatly influenced by socio-economic conditions such as lack of piped water and use of containers to store water, use of air-conditioning, housing quality and the rate of urbanization (Getis *et al.*, 2003; Ramos *et al.*, 2008; Åström *et al.*, 2012; Kraemer, Sinka, Duda, A. Q. N. Mylne, *et al.*, 2015). *Ae. aegypti* have a strong preference for artificial containers such as water drums, basins, brick holes and tyres, also known as 'key containers', to lay their eggs, rather than natural occurring water pockets, resulting in a small proportion of premises that are responsible for the majority of mosquito production, also known as 'hot-spots' or 'key premises' (Tun-Lin, Kay and Barnes, 1995; Chadee, 2004). As an example, less than 2% of inspected premises in Townsville, Australia, accounted for 47% of all positive containers in a study in 1989 and later in 1990, 3% of all premises accounted for 53% of all positive containers in the same location (Tun-Lin, Kay and Barnes, 1995). Similarly, in a separate study in Trinidad, less than 3% of premises contributed to 57.3-64.6% of positive containers in 2002 (Chadee, 2004). 'Key premises' with 'key containers' are more important than other premises in the production and the propagation of mosquitoes to adjacent houses and in the epidemiology of dengue (Chadee, 2004). Consequently, households with a higher degree of tidiness and little shade are less favourable to the presence of larval breeding sites and adult mosquitoes than gardens with highly stratified vegetation and debris (Tun-Lin, Kay and Barnes, 1995; Basker and Ezhil, 2012; Claeys *et al.*, 2016). Also, 'key premises' can be temporary (Chadee, 2004; LaCon *et al.*, 2014) as mosquito populations are affected by human activities such as clearing of vegetation, buildings or existing vector control activities (Iyaloo *et al.*, 2014) that induce changes in the location of mosquito clusters through time (Getis *et al.*, 2003).

4.1.2. *Aedes aegypti* population modelling.

There are a number of mathematical models described recently in the literature to explain *Ae. aegypti* abundance and population dynamics at different levels. Kraemer

et al. (2015) modelled the relative contribution of environmental covariates to predict the global distribution of *Ae. aegypti* and *Ae. albopictus* and reported that temperature had the strongest influence followed by rain and vegetation cover but land use had little influence. Similarly, Chaves *et al.* (2012) concluded that the abundance of mosquitoes were highly variable and depended on environmental conditions and were mostly influenced by rainfall in Puerto Rico, and temperature in Thailand. At a finer scale of detail, Ezanno *et al*., (2015) created a generic weather-driven model of mosquito population dynamics that was applied to *Aedes caspius* in southern France and predicted mosquito abundance based on local environmental factors. Additionally, Lana *et al*., (2014) reported that neighbourhoods in Rio de Janeiro differed in the strength of seasonality and their model reflected the weak influence of seasonality observed. However, these agent-based models are difficult to apply to small geographic areas (Chaves *et al.*, 2012; Kraemer *et al*., 2015), addressed temporal variation in the mosquito population from ovitraps rather than adult abundance (Lana *et al.*, 2014), or ignored human and household variables (Ezanno *et al.*, 2015).

An alternative modelling approach that included analysis of local meteorological and environmental data with entomological data in a generalized linear mixed model (GLMM) framework was implemented by Yoo (2014) who developed a site-specific prediction model of the abundance of a WNV vector, *Cx. pipiens-restuans*, in Toronto, Canada, and Manica *et al*., (2016) and Flacio *et al.* (2016), who described the abundance of *Ae. albopictus* at small scale in Italy and in Switzerland, respectively. These approaches controlled for a wider range of interacting factors including rainfall, temperature and vegetation cover and reported an association between highly anthropized habitats and high adult abundance with a peak of abundance due to heavy rains and mild temperatures (Yoo, 2014; Flacio *et al.*, 2016; Manica *et al.*, 2016).

4.1.3. Previous entomological surveys in St. Kitts.

Little data are available on the dynamics and density of the *Aedes aegypti* population in the Caribbean (Chadee, Mahabir and Sutherland, 2012). Exceptions include studies in Trinidad (Chadee, 2009; Chadee, Mahabir and Sutherland, 2012), Puerto Rico (Scott *et al*., 2000; Harrington *et al*., 2001; Reiter *et al*., 2007), French Guiana (Fouque *et al*., 2006), in addition to Jamaica (Chadee *et al*., 2009), the Dominican Republic (Tidwell *et al.*, 1990), and USVI (Kenney *et al.*, 2017). The heterogeneous

entomological indices reported ranged between 0.66 to 1.51 female adults per person in the dry season and 0.23 to 1.12 in the wet season in Jamaica (Chadee *et al*., 2009), 8.69 adult mosquitoes per house (although it could vary between 0 and 397) in French Guiana (Fouque *et al.*, 2006), and as much as 21 mosquitoes per trap and day on St. John's island in the USVI (Kenney *et al*., 2017).

St. Kitts adult mosquito population has been studied on only two previous occasions resulting in 14 species of mosquitoes identified (Table 4.1). The first known mosquito survey in St. Kitts was published by Belkin and Heinemann (1976) who captured mosquitoes during resting, by human landing capture and with live bait on different locations in 1966 (Appendix 19). The total number of mosquitoes collected was unspecified but the two species most commonly found associated to domestic areas were *Ae. aegypti* and *Cx. quinquefasciatus*. The next known mosquito survey in St. Kitts was published by Smith *et al.* (2011) and Mohammed *et al*. (2015) who captured mosquitoes using BG-Sentinel traps in urban and non-urban ecosystems during the wet and the dry season in 2010 (Appendix 20). Similarly, *Ae. aegypti* and *Cx. quinquefasciatus* were the species most abundantly found in urban and semi-urban areas, particularly the latter, but the total number of mosquitoes captured was lower in the rainy season, which was attributed to 'a flushing effect from torrential downpours' (Mohammed *et al*., 2015). Other published data from St. Kitts include a brief report of larval indices that showed a decreasing House Index of 58.0%, 26.6% and 3% in the years 1982-83, 1984-85 and 1990, respectively (Nathan, 1993).

Table 4.1. Mosquito species recorded in St. Kitts. (*) species captured in urban and semi-urban areas.

	Belkin and Heinemann, 1976	Mohammed *et al*., 2015
Ae. busckii	√	√
*Ae. aegypti**	√	√
*Ae. taeniorhynchus**	√	√
Ae. tortilis		√
An. albimanus		√
*Cx. bahamensis**	√	√
Cx. bisulcatus	√	
Cx. declarator	√	
Cx. madininensis	√	
Cx. nigripalpus		√
*Cx. quinquefasciatus**	√	√
Deinocerites magnus	√	√
Psorophora pygmaea	√	√
Toxorhynchites guadeloupensis	√	

Existing mosquito data in St. Kitts was considered insufficient for quantifying the risk of arboviral infection in the study population described in chapter 2. Therefore, a mosquito survey was conducted in outdoors and indoors locations in urban and semi-urban areas associated with the sentinels. This survey aimed to achieve a greater understanding of *Aedes aegypti* population at a fine scale and the prevalence of arboviruses in mosquitoes to assist in predicting accurate projections of mosquito population sizes and local chains of transmission.

4.2. Methods: Mosquito trapping and testing methods.

4.2.1. Trapping and identification methods

Adult mosquitoes were captured over two trapping seasons between July and December of 2015 and between July and December of 2016 from 18 different urban and semi-urban areas of St. Kitts which, according to the land use, 13 were residential, 2 were recreational and 3 were areas of study and other uses (Figure 4.1).

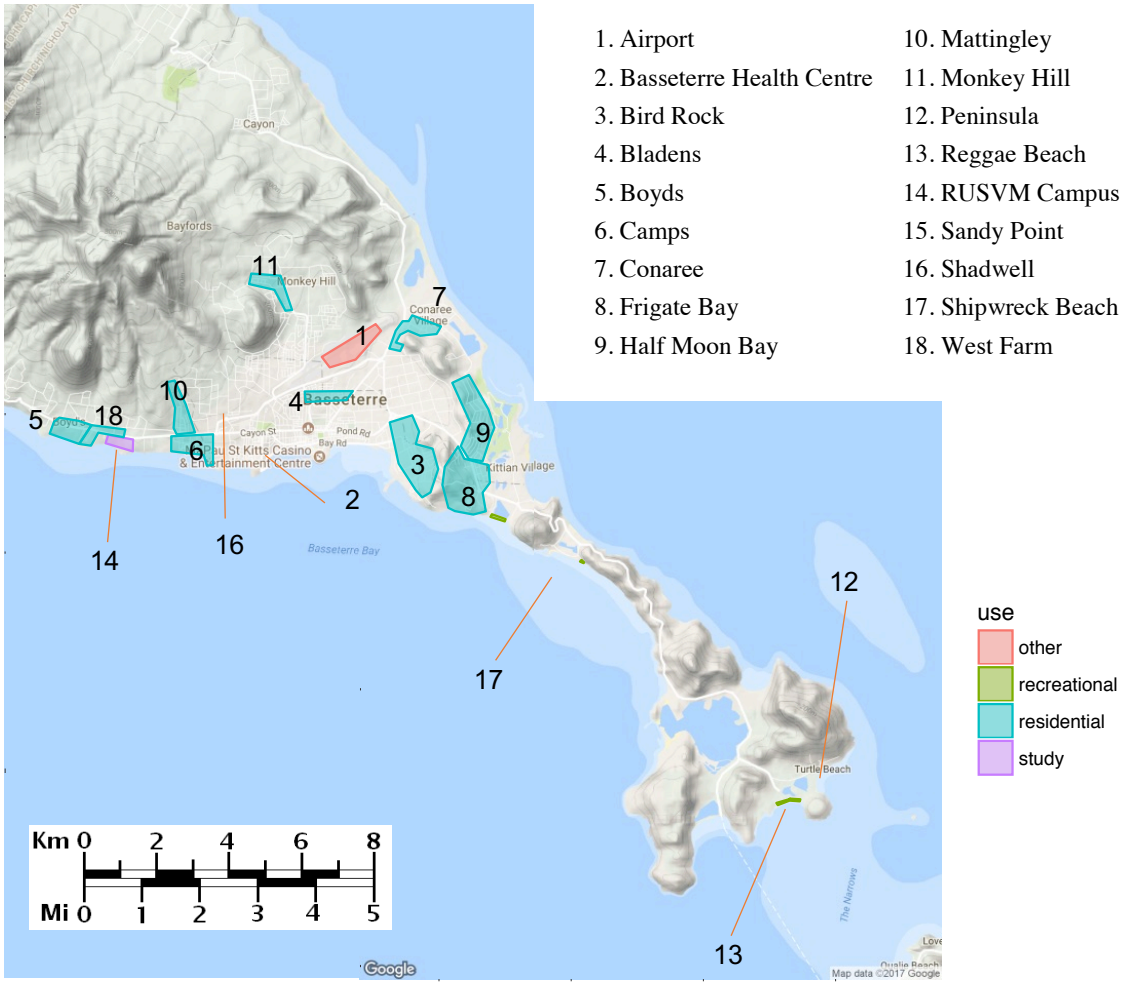


Figure 4.1. Trapping areas according to the land use. Area no. 15. Sandy Point (not shown in map) was surveyed and classified as residential although no sentinels resided in this area (Google maps, 2017).

Collections were made using BG-Sentinel traps with the attractant BG Lure (BioGents, Regensburg, Germany), a long-lasting synthetic lure that consists of lactic acid, ammonia and caproic acid and imitates human odours (Roiz *et al*., 2015). The traps were placed at the time of mosquito peak activity in the morning (Figure 1.6) (Chadee, 2009), protected from the rain under canopies and similar structures and connected to a battery (12 V) for periods of 24 hours (Johnson, Spitzauer and Ritchie, 2012). The traps were located outside of properties where the sentinels lived, worked and spent their leisure time. In addition, individual houses were aspirated inside and in the immediate surroundings with the backpack aspirator Prokopack Model #1419 (John W. Hock Company, Gainesville, Florida, USA) (Vazquez-Prokopec *et al*., 2009) following methodology suggested by Focks *et al*., (1993) and applied later by Getis *et al*. (2003) and by Morrison *et al*. (2004). Aspirations were made in randomly selected houses, after permission from the owner was obtained, by a team of at least 2 people including officers from the SKN Vector Control office. When permission to enter was not granted or the house was empty, the adjacent house was inspected. All permitted rooms were aspirated including under the furniture, above and inside wardrobes and other resting sites for 15 minutes per house, and outside the households, exterior walls, vegetation, under the eaves and other outdoor stored materials were also sampled (Focks *et al*., 1993; Getis *et al*, 2003; Morrison *et al*., 2004). Captured mosquitoes were transported live in their collection bags to RUSVM research laboratory in an insulated thermal box filled with ice packs and stored at -80^{o}C on arrival.

Key features of the trapping site such as GIS coordinates, altitude, land use and relative position of the household from the dominant wind were recorded. In addition, a Premise Condition Index (PCI), a scoring system developed by (Tun-Lin, Kay and Barnes, 1995) and used later by Basker and Ezhil (2012), was applied to determine household features. The PCI involves the scoring of three characteristics of the household, namely, house condition, yard condition and degree of shade into three categories as detailed in Table 4.2 (Tun-Lin, Kay and Barnes, 1995; Basker and Ezhil, 2012). Examples of households and their PCI scores can be seen in Figure 4.2.

Table 4.2. Definition of PCI scores. Adapted from Tun-Lin et al., (1996) and Basker and Ezhil, (2012).

Definition		1	2	3
House	Human living place. Condition is determined by its periodical maintenance	Well maintained (e.g. newly painted, or new house)	Moderately well, a maintained house	Not a well-maintained house (e.g. paint peeling, broken items visible, dilapidated)
Yard	Space available either in front of the house or at the back of the house	Tidy yard (e.g. well-maintained gardens and debris and litter free)	Moderately tidy yard	Untidy yard, trash abundant, and overgrown vegetation.
Shade	Determined on the vegetation or cover around the house	Very little or no shade (< 25%)	Some shade with major trees and vegetation (50%)	Major trees and shrubs (75%) (e.g. large trees, shrubbery, or shade cloth)

Figure 4.2. Examples of households according to their PCI classification.

As per Basker and Ezhil (2012) methodology, categories 1 and 3 were scored first and category 2 was selected by elimination of the others. In order to overcome the potential degree of subjectivity highlighted by the authors, the scoring was conducted by the same person and previously scored premises were reviewed every 10 new sites added. Additional information was collected during the aspirations from the household owner and included the number of occupants and rooms, presence of intact mosquito screens, and behaviours such as unscreened doors or windows left opened frequently, usage of AC, water storage patterns and use of insecticide spray and coils.

Mosquito identification was performed with a stereoscopic microscope using keys and species descriptions by Belkin, Heinemann and Page (1970), Rueda (2004) and Darsie and Ward (2005). Specimens missing exclusive identification features or belonging to genera other than *Aedes* spp. were recorded down to their genus only. Identified *Aedes* spp. from each season were grouped by species, sex and trapping area in pools of up to 60 mosquitoes, and stored at -80ºC.

The number of mosquitoes trapped were used to calculate entomological indices and compare mosquito abundance in different areas. The Female Trap Index (FTI) represented the percentage of traps positive to female *Ae. aegypti*, the Female Aedes Density Index (FADI), represented the number of female *Ae. aegypti* caught from selected sites in each area, and the number of female *Ae. aegypti* per house and per person, represented the density of mosquitoes per household.

4.2.2. Laboratory methods

Mosquito specimens were prepared following the protocol described by Huang *et al.* (2001) and modified by Parreira (R. Parreira 2015, personal communication, 4 April). First, each mosquito pool was triturated on a vortex-Genie 2 with sterile glass beads in a 15ml conical tube with 1.5ml of phosphate-buffered saline (PBS) containing 4% bovine plasma albumin for one minute and was subsequently held in ice for at least another minute. This process was repeated three times. The tubes were then centrifuged at 2,500g for 10 minutes at +4ºC and the supernatant was transferred into a 1.5ml tube for a second centrifugation at 14,000g for 10 minutes at +4ºC. Finally, the supernatant was extracted and stored at -80ºC.

Following supernatant preparation, RNA was extracted from each mosquito pool using the QIAGEN Mini Viral Kit protocol as described in section 3.2.2. RT-PCR was performed sequentially or, alternatively, the RNA yielded was stored at -80ºC until

molecular testing was completed following methodology described in section 3.2.2 with the primers summarised in Table 3.2 for amplification of viral DENV, CHIKV and ZIKV RNA sequence in mosquito supernatant. Housekeeping control gene primers for the 18S gene were used to indicate successful RNA extraction and amplification (Appendix 15 to Appendix 18).

4.2.3. Mosquito models

The abundance of *Ae. aegypti* was modelled separately for each collection method (i.e. BGS and aspirator) with the explanatory variable being the number of specimens captured as a function of household characteristics, climatic conditions and human density and behaviours. The modelling of viral infection in mosquitoes was also attempted.

Abundance of *Ae. aegypti* outdoors

The abundance of *Ae. aegypti* captured with BG-sentinel traps placed outside households was modelled within a generalised linear mixed effect (GLMM) framework that can accommodate hierarchically structured data and account for dependencies within hierarchical groups by the introduction of random-effects (Pinheiro and Bates, 2000; Bolker *et al.*, 2012) following the methodology described by Zuur *et al*. (2009). Observations were considered to be non-independent as sites were trapped on different occasions and some traps were placed within theoretical mosquito flying range (Appendix 2), therefore the variable 'trapping site' was included in the models as a random effect. Patterns of autocorrelation from consecutive trapping days on the same site were assessed by the auto-correlation function (ACF) (Zuur *et al*., 2009). The number of mosquitoes captured was assumed to follow a negative binomial (NB) distribution rather than a Poisson distribution to account for the potential over-dispersion in the model due to the focal clustering of mosquitoes (LaCon *et al*., 2014). Additionally, zero-inflation was investigated originally due to the potential 'excess' of zero values in the model (Zuur *et al*., 2009), as a result of nil observations due to mosquitoes being present in other parts of the house and not trapped by the BGS (Johnson, Spitzauer & Ritchie, 2012) either because of lack of attraction to the lure (e.g. mosquitoes that had had a blood meal recently or gravid mosquitoes), inaccessibility to the trap (e.g. trap was placed outdoors and some mosquitoes may be resting indoors), or to adverse weather at time of peak activity rather than 'true' zeros (i.e. zero mosquitoes captured due to their absence). However, the results detailed later in section 4.3.1 indicated that the number of zero

collections was within an expected negative binomial distribution and zero inflated models fitted to the data showed little advantage over non-zero inflated models, therefore, subsequent models were assumed to follow a NB distribution without zero inflation.

Model description

Let NAed_i be the number of *Ae. aegypti* mosquitoes (either female only or both male and female) captured per site *i*, assuming a negative binomial distribution:

$$\begin{aligned} \mathrm{NAed}_i &\sim \mathrm{NB}(\mu_i, k) \\ \log(\mu_i) &= \alpha + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_n X_n + a_{siteID} \end{aligned} \quad (4)$$

where $X_{1 \ldots} X_n$ = fixed effects and a_{siteID} = random effect for trapping site, assumed to be normally distributed with a mean zero and variance σ^2, i.e., $a_i \sim N(0, \sigma_I^2)$.

Abundance of *Ae. aegypti* indoors

The abundance of *Ae. aegypti* captured with a backpack aspirator inside households was modelled within a generalised linear model (GLM) assuming a negative binomial distribution. Houses were aspirated on a single occasion only, the observations were considered to be independent from each other and no risk of autocorrelation was anticipated in these models. The model distribution is analogous to the previously described for outdoors abundance without a random effect.

Viral infection of *Ae. aegypti*

Arboviral infection of *Ae. aegypti* mosquitoes captured in urban areas of St Kitts was modelled by a GLMM assuming a binomial distribution in a similar stepwise manner. Let P_{vir} be the probability of virus infection in *Ae. aegypti* captured:

$P_{vir} \sim Bin(1, \pi_i)$

$$\mathrm{logit}(\pi_i) = \alpha + \mathrm{offset}(\log(n_i)) + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_n X_n + Z_i\, a_i \quad (5)$$

where $X_{1 \ldots} X_n$ = fixed effects, a_i = random effect for trapping site *i*, and including an offset for the log number of mosquitoes captured on each site (n_i).

Variables selection

The variables included in the original models related to factors that are known to affect mosquito abundance and contained the household characteristics detailed in section 4.2.1, in addition to climatic conditions and human density. Climatic data was provided by the Saint Kitts and Nevis Meteorological Service and consisted of

maximum, minimum and mean daily temperature (Celsius), daily rainfall (mm), hours of sunshine, relative humidity, atmospheric pressure (mb), wind speed (knots) and wind direction recorded at the meteorological station located in the Robert L. Bradshaw International Airport. Human density was depicted by the number of buildings and the estimated number of persons within 10 and within 30 meters from the site (Getis *et al*., 2003; LaCon *et al*., 2014). The number of buildings per trapping site were calculated by analysing the latest satellite imagery available for St. Kitts (Google Maps, 2017) and revised during trap deployment by comparing the images with the number of houses around the trap. The number of persons in residential areas was calculated using the latest census that estimated an average of 2.7 persons per household (Statistics Department, 2012; Vassell, 2014), and the number of persons at recreational sites was estimated by averaging observations of attendance at different times during different days.

Numerical variables were standardised by subtracting their means and dividing by their standard deviation so that they had a mean of zero and standard deviation of one in order to improve convergence of the fitting algorithm and to allow better comparison of effect sizes by having the estimated coefficients on the same scale (Zuur *et al*., 2009). Collinearity among variables was assessed by Spearman rank correlation coefficient rather than Pearson correlation coefficient as the former makes no assumptions about linearity in the relationship between the two variables (Zuur *et al*., 2009), in addition to visual observation of pairwise plots and assessment of the Variance Inflation Factor (VIF) (Zuur *et al*., 2009). A correlation coefficient greater than ±0.6 was considered an indication of high collinearity (Dormann *et al.* (2013) citing Booth (1994)). Variables showing high collinearity such as rainfall in consecutive days, maximum, minimum and average daily temperatures, and the number of houses and population within 10 meters and within 30 meters of the trapping site were removed from the model and assessed separately from each other. VIF of the remaining variables were calculated by fitting a negative binomial model with the number of mosquitoes captured to all explanatory variables (Hosmer and Lemeshow, 2000) and VIFs calculated for each variable from the resulting model. VIF values above 10 suggested high collinearity (Zuur *et al*., 2009) and the variables socio-economic status, land use, PCI score of the house and the yard, were included separately in the initial model.

Model selection

An information-theoretic approach was adopted to identify the most parsimonious model (Burnham and Anderson, 2002) and consisted on formulating a series of presumptive models followed by a selection of those models that were nearer to reality than any of the rest (Rushton, Ormerod and Kerby, 2004). First, all non-collinear variables were introduced in the original model and removed one at a time in a backward stepwise manner until the model indicated the lowest Akaike Information Criteria (AIC) (Akaike, 1973). This process was repeated with different combinations of household PCI scores, cumulative rainfall for the previous 1 to 30 days before trapping and human density within 10 and 30 meters of the trap. A series of presumptive models were obtained and ranked by ascending AIC values. Next, the Akaike weights for each model ($\mathrm{AIC}\, w_i$), which are the weights of evidence in favour of each model being the nearest to reality (Rushton, Ormerod and Kerby, 2004), were calculated and used to identify a 95% confidence set of models, the relative probability of each model being the best and the relative importance of each variable (Zuur *et al*., 2009 citing Burnham and Anderson, 2002). Diagnostic models were assessed visually by quantile-quantile plots to investigate error distribution of the models and departures from normality, and by plots of residuals against fitted values to assess departures from linearity and homoscedasticity assumptions (Zuur *et al*., 2009).

4.3. Results: Mosquitoes captured

A total of 5,552 mosquitoes were captured in 231 trappings and only two genera were found: *Culex* spp., comprising 3,583 specimens (64.5%) and *Aedes* spp. comprising 1,969 specimens (35.5%). In 2015, 2,150 mosquitoes (38.7%) were collected from 99 trappings with a mean of 21.7 mosquitoes per trapping, of which, more than half (55.0%) were *Culex* spp. (n = 1,182) and 968 were *Aedes* spp. (n = 968), half were female (49.3%, n = 491). In 2016, 3,402 mosquitoes were collected from 132 trappings resulting in 25.8 mosquitoes per trapping, of which, 2,401 (70.6%) were *Culex* spp. and less than a third (29.4%) were *Aedes* spp. (n = 1,001), of which, 488 (48.7%) were female (Appendix 21). The traps were placed at altitudes between sea level and 203 meters. The minimum distance between trapping sites other than within RUSVM campus was 29 meters with an average of 86 meters of separation between trapping sites (Appendix 22)

Trappings were mostly done in residential areas (80.9%), which provided 75.0% of the mosquitoes captured with an average of 22.3 adults per collection overall (Table 4.3). The recreational sites selected for this survey accounted for only 6.1% of the collections and 17.3% of the total number of mosquitoes, however, the number of adults per collection in recreational areas was remarkably higher than in the other areas with an average of 68.6 adults. *Aedes* spp. were captured in 78.3% of all the collections and 67.4% of the sites with an average number of mosquitoes per collection ranging between 8.9 and 9.7, although recreational areas yielded 28.3 adult *Aedes* spp. per collection. The traps located at the recreational area of Timothy Beach / The Strip showed evidence of alterations during their deployment and collections from this site were removed from the analysis.

Table 4.3. No. of mosquitoes trapped per use of area. (Top: all mosquitoes captured. Middle: Aedes spp. only. Bottom: Culex spp. only)

Use	No. collections	% collections	No. mosq.	%	No. areas	No. Sites	No. mosq. / collection
Residential	187	80.9%	4,165	75.0%	12	77	22.3
Recreational	14	6.1%	960	17.3%	2	3	68.6
Work / Study	22	9.5%	340	6.1%	1	8	15.5
Other	8	3.5%	87	1.6%	3	7	10.9
Total	*231*		*5,552*		*18*	*95*	$\bar{x}$ = *29.3*
Aedes spp.							
Residential	147	81.2%	1,393	70.7	12	64	9.5
Recreational	14	7.7%	396	20.1	2	3	28.3
Work / Study	17	9.4%	151	7.7	1	7	8.9
Other	3	1.7%	29	1.5	1	3	9.7
Total	*181*		*1,969*		*16*	*77*	$\bar{x}$ = *14.1*
Culex spp.							
Residential	155	81.6%	2,772	77.4	11	63	17.9
Recreational	12	6.3%	564	15.7	2	3	47.0
Work / Study	18	9.5%	189	5.3	1	6	10.5
Other	5	2.6%	58	1.6	3	5	11.6
Total	*190*		*3,583*		*17*	*77*	$\bar{x}$ = *21.7*

4.3.1. Results: Entomological Indices

Aedes spp. were found in all the areas surveyed except at Robert L. Bradshaw International Airport and Basseterre Health Centre. The areas where female *Ae.*

aegypti were captured more abundantly over both trapping seasons by any trapping method were the recreational Shipwreck Beach and Reggae Beach, and the residential Bird Rock which gathered the highest number of mosquitoes per trapping and area (FADI: 11.0, 10.0 and 9.16, respectively) and the highest number per person (0.69, 0.62 and 0.26). The areas where *Ae. aegypti* was captured less abundantly overall were Half Moon Bay and the Peninsula with a FADI of 0.84 and 1.0 respectively and an abundance of less than 0.04 female mosquitoes per household (Table 4.4). Half Moon Bay also showed the lowest probability of capturing female *Ae. aegypti* (42.1%) whereas the overall mean probability of capturing female *Ae. aegypti* in all the trapping areas was 94.7%.

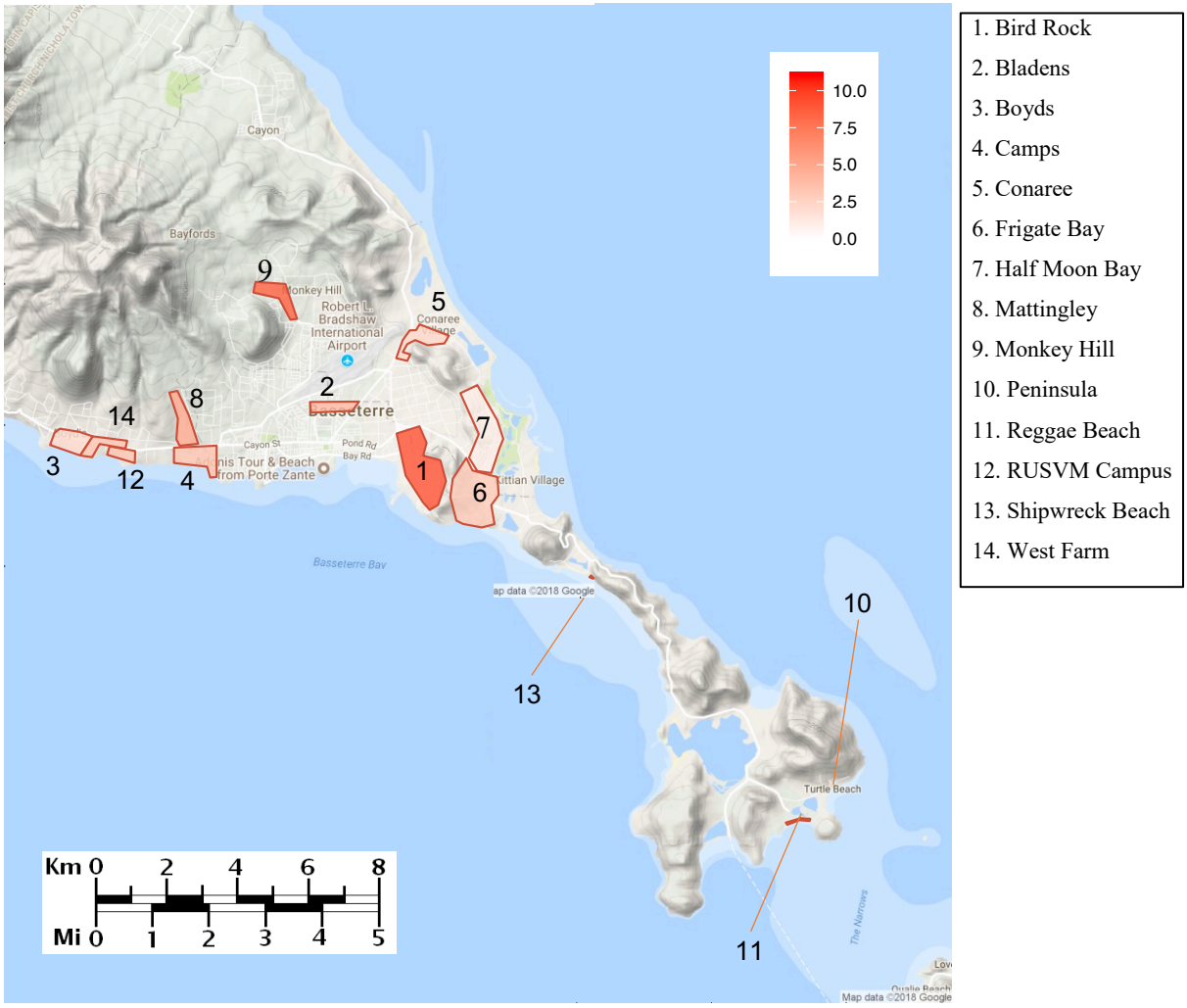


Figure 4.3. Female Ae. aegypti Density Index (FADI) in different trapping areas (Google Maps, 2017).

The entomological indices in Reggae Beach and Bird Rock were consistently high and in Half Moon Bay were consistently low between trapping seasons (Table 4.4). Indices in RUSVM campus where most sentinels spent much of their day, were also

mostly consistent and slightly below average in each trapping season. In contrast, FADI indices in Conaree, Mattingley and West Farm were high in 2015 (9.0, 6.27 and 7.5 respectively) but substantially lower in 2016 (1.5, 2.43 and 2.1) and the probability of success in capturing *Ae. aegypti* represented by the FTI varied between 54.5% and 100%, respectively, which is consistent with the changing nature of mosquito clusters (LaCon *et al.,* 2014). Other examples of varying indices included Frigate Bay, which showed a higher probability of trapping mosquitoes in 2015 than in 2016 (FTI = 84.6 vs 45.5) and in greater quantity (FADI = 4.54 vs. 1.09), and Monkey Hill with a FADI that increased from 5.75 to 11.25. In addition to these figures, the complete list of indices is detailed in Appendix 24.

Table 4.4. Female Ae. aegypti entomological indices overall (left) and for the trapping seasons of 2015 (middle) and 2016 (right).

Area	overall		2015			2016		
	FTI	FADI	FTI	Fem. / house	FADI	FTI	Fem. / house	FADI
Bird Rock	89.5	9.16	84.6	0.62	8.08	100.0	0.82	11.50
Bladens	83.3	4.00	83.3	0.33	4.00	-	-	-
Boyds	57.7	2.00	83.3	0.33	3.50	50.0	0.10	1.55
Camps	90.0	3.35	90.9	0.27	4.18	88.9	0.15	2.33
Conaree	66.7	2.33	100.0	1.12	9.00	62.5	0.16	1.50
Frigate Bay	66.7	2.96	84.6	0.25	4.54	45.5	0.04	1.09
Half Moon Bay	42.1	0.84	20.0	0.03	0.70	66.7	0.04	1.00
Mattingley	61.1	4.78	54.5	0.78	6.27	71.4	0.30	2.43
Monkey Hill	93.8	8.50	87.5	0.44	5.75	100.0	0.70	11.25
Peninsula	-	1.0	100.0	0.25	1.00	-	-	-
Reggae Beach	100	10.0	100.0	3.00	12.00	100.0	2.38	9.50
RUSVM Campus	68.2	3.27	77.8	0.19	3.00	61.5	0.29	3.46
Shipwreck Beach	75.0	11.0	-	-	-	75.0	2.75	11.00
West Farm	74.1	3.30	100.0	1.88	7.50	66.7	0.14	2.10

Capture method and gonotrophic cycle

The majority of mosquitoes captured were unfed (68.2%) and only a minority were gravid (15.6%) or had had a blood meal recently (8.6%). The BGS trapped a higher proportion of unfed *Aedes* spp. females (82.6%) than of unfed *Culex* spp. females (60.1%) and only a handful that had taken a recent meal (1.5%) or were half-gravid (2.6%) whereas the aspirator captured a similar proportion of female *Aedes* spp. in each different stage (Figure 4.4).

	Aedes spp.			
	Aspirator		BGS	
stage	**N**	**%**	**N**	**%**
unfed	20	27.8%	749	82.6%
gravid	13	18.1%	120	13.2%
blood-fed	18	25.0%	14	1.5%
half gravid	21	29.2%	24	2.6%
Total:	72		907	

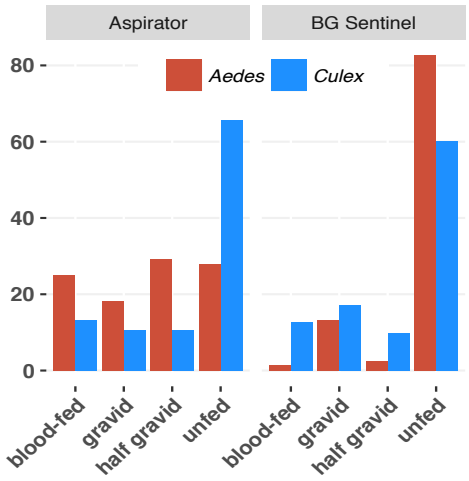


Figure 4.4. Proportion of female mosquitoes captured by different methods

Premise Condition Index

A greater number of *Ae. aegypti* were captured in households with high PCI scores than in households with low PCI scores (Figure 4.5). Households with the highest scores resulted in 10.21 female mosquitoes captured per collection whereas households with the lowest score resulted in 2.23 female mosquitoes captured per collection (Appendix 28). Also, the scores for the house maintenance, yard tidiness and amount of shade were highly correlated.

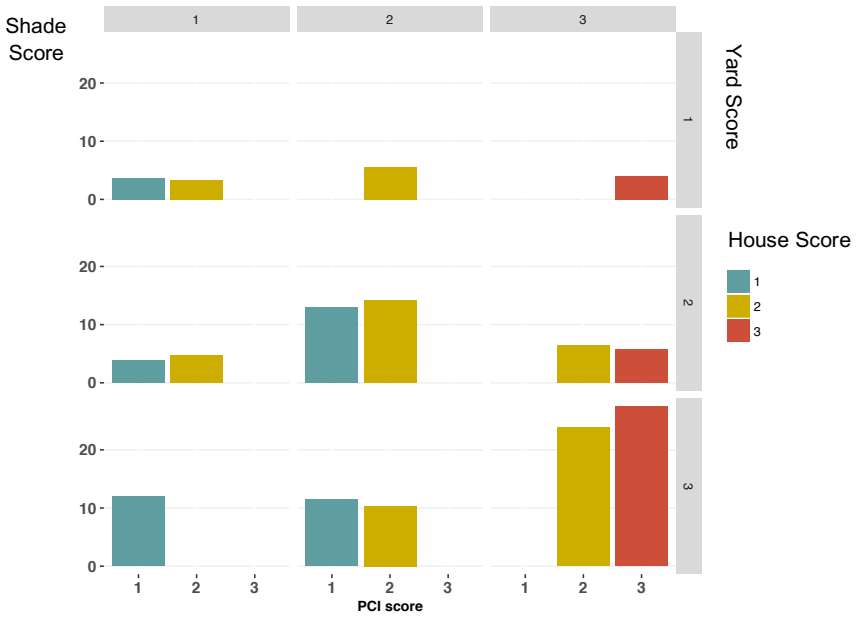


Figure 4.5. Number of adult Ae. aegypti per PCI score (Rows: Shade PCI score; Columns: Yard PCI score; Scale Colours: House PCI score)

Climatic conditions

Exploratory analysis of climatic conditions suggested that the number of female *Ae. aegypti* captured were influenced by humidity and temperature (Figure 4.7). Rainfall

showed a dual effect in that less mosquitoes were captured after torrential downpours on the day of collection, suggesting a flushing effect, whereas increased rainfall on the previous 14 to 30 days before trapping had a positive effect on the number of mosquitoes trapped. Also, the relative humidity showed a similar positive effect in the number of female *Ae. aegypti* captured until an optimal was reached. Likewise, the number of mosquitoes trapped were greater when the mean temperatures ranged between 27°C and 28°C after which the number of specimens captured declined especially at temperatures higher than 30°C.

Exploratory analysis of climatic conditions also suggested a decreasing effect in the number of mosquitoes captured during high winds. St. Kitts receives the 'trade winds' from N and NE direction that are generally stronger between December and April (Appendix 29). Some of the areas in the lowest quantile of entomological indices (Q1) such as Half Moon Bay and Frigate Bay, are situated exposed to the dominant winds and wind paths with the exception of the neighbourhood Boyds. On the contrary, some of the areas in the highest quantile of entomological indices (Q4) such as Bird Rock and Monkey Hill are located behind higher terrain (Figure 4.6) with the exception of the neighbourhood Conaree which would suggest that wind speed and direction, in addition to other factors, affected the number of mosquitoes captured.

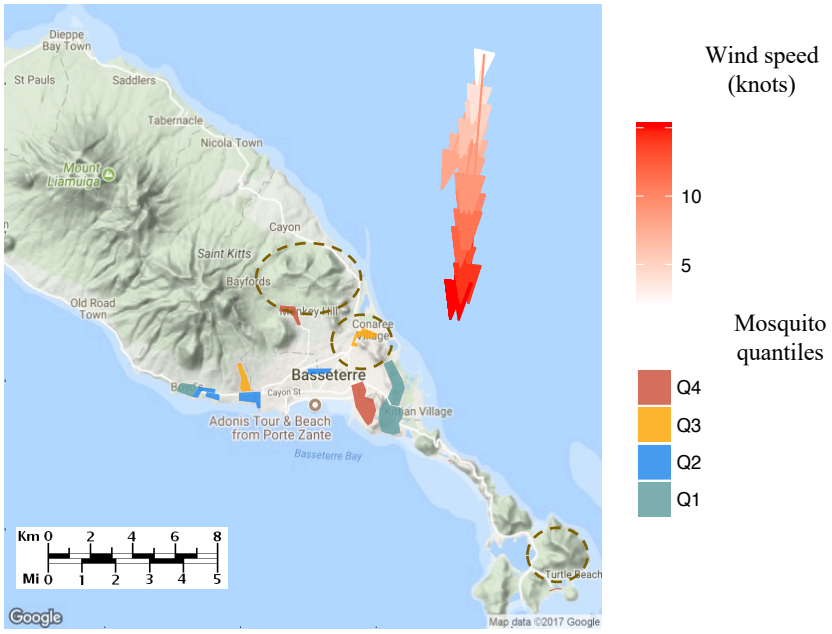


Figure 4.6. Wind direction and trapping areas. The length and colour intensity of the arrows indicate the monthly wind speed in knots during trapping periods. The trapping areas (bottom) are classified by quantiles (Q1-Q4) of mosquitoes captured. The dashed circles show higher ground terrain.

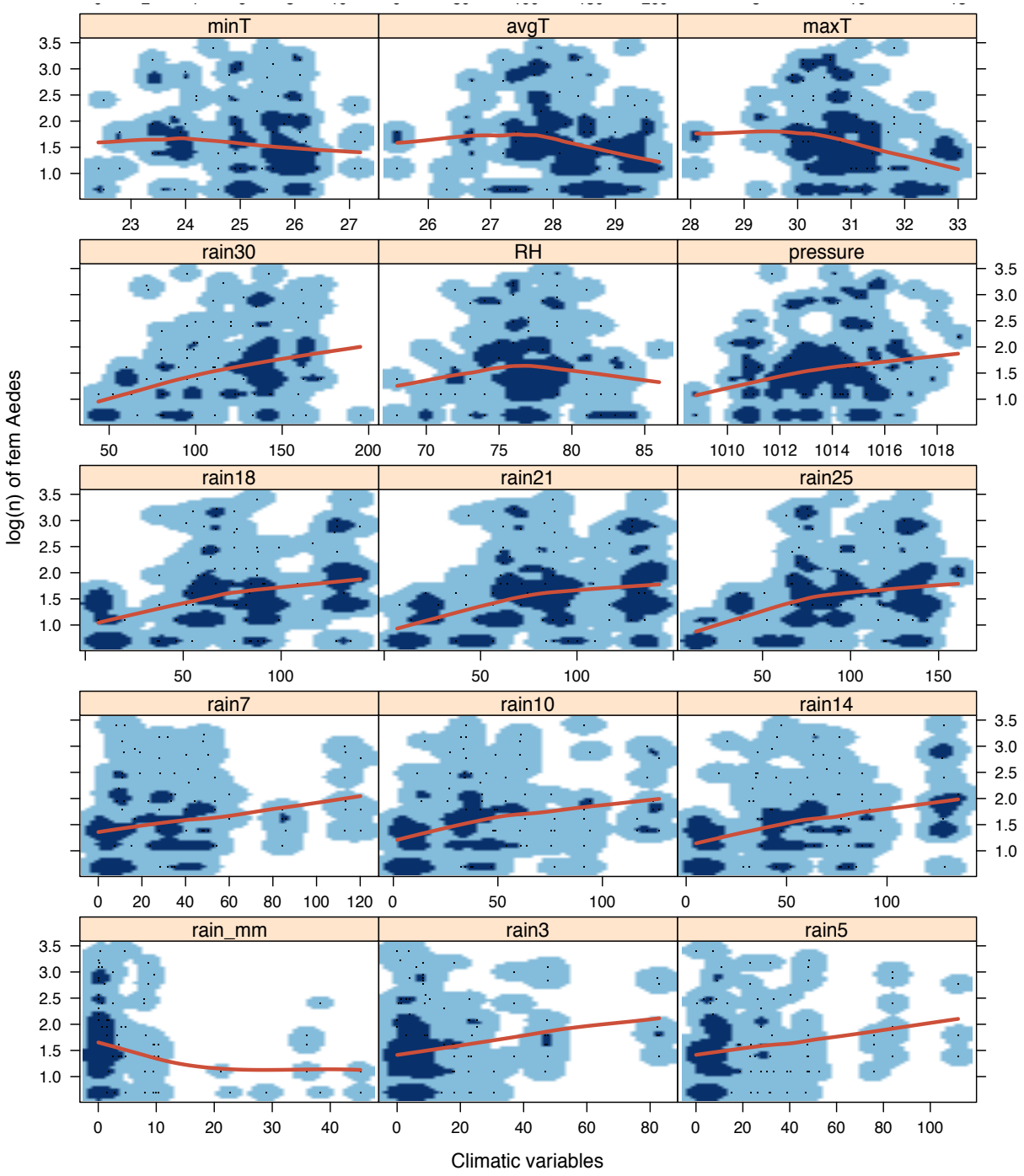


Figure 4.7. Relationship of climatic variables and log(n) of female Ae. aegypti captured with a loess curve fitted to each variable (red line). From bottom left to top right: rainfall in mm on the day of trapping (rain_mm), cumulative daily rainfall on the previous 3, 5, 7, 10, 14, 18, 21, 25 and 30 days (rain3, rain5, rain7, rain10, rain14, rain18, rain21, rain25, rain30, respectively), relative humidity (RH), atmospheric pressure in mbar (pressure), daily minimum, mean and maximum temperature in Celsius (minT, avgT maxT, respectively).

4.3.2. Results: Mosquito arboviral infection

Molecular analysis revealed evidence of ZIKV infection in mosquitoes during the time of the active outbreak in St. Kitts in 2016 but evidence of further arboviral infection in mosquitoes was elusive at other times. In 2015, 491 female *Aedes* spp. mosquitoes were tested for DENV and CHIKV, the only arbovirus described on St. Kitts until date,

in 18 pools of up to 50 mosquitoes but no indication of arbovirus genome could be found in any of the pools. In 2016, a total of 738 *Aedes* spp. mosquitoes were tested for DENV, CHIKV and ZIKV in 42 pools of up to 58 mosquitoes, of which 29 pools corresponded to female mosquitoes (n = 488) and 13 to male mosquitoes (n = 250). A total of 7 pools of mosquitoes captured from 6 different sites in five areas between the end of October and beginning of December 2016 (Figure 4.8) showed evidence of ZIKV genomic material. Five of the positive pools were female *Ae. aegypti* and two pools were male *Ae. aegypti*. Since only female mosquitoes bite infected hosts and so acquire the virus, the most plausible explanation for detecting viral RNA in male mosquitoes would be the possibility of vertical transmission from an infected female to their progeny. The minimum infection rate in mosquitoes was 9.1 per 1,000 mosquitoes (assuming 1 infected mosquito per pool).

Exploration of the factors associated with infectivity in mosquitoes was attempted, however, analysis of the data failed to produce a satisfactory model for the infection of virus in the mosquitoes captured. No variables were found to be statistically significant and the variability of the coefficients was excessively large to produce meaningful inferences, and diagnostic plots indicated very weak normality of the residuals.

Area	Area No.	No. mosq. per pool	sex
Bird Rock	3	14	Female
Boyds	5	7	Female
Camps	6	9	Female
Half Moon Bay	7	11	Female
Monkey Hill	11	47	Female
Camps	6	10	Male
Half Moon Bay	11	2	Male

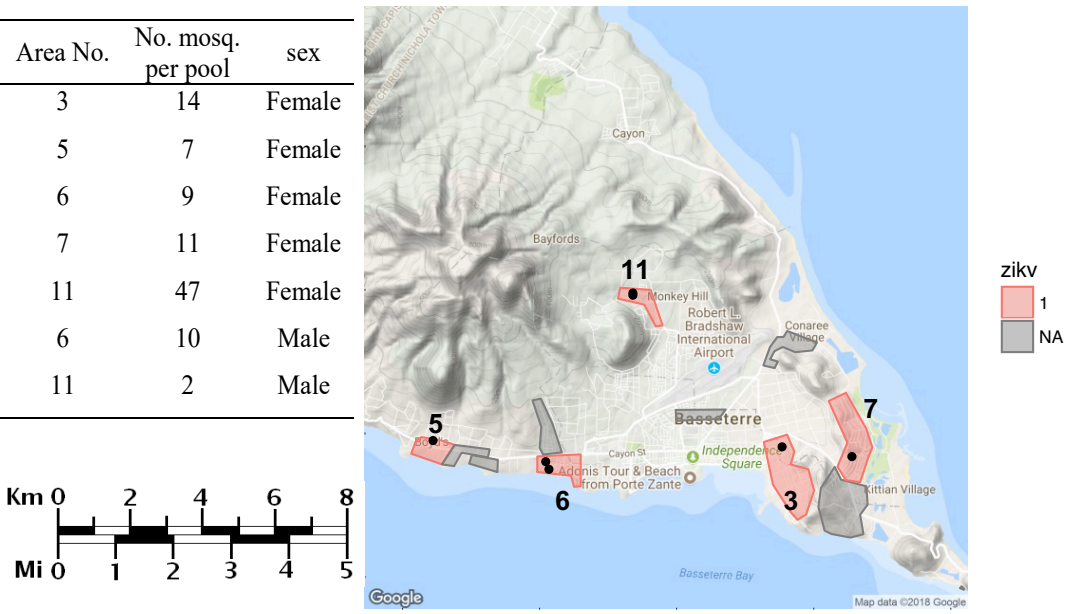


Figure 4.8. Trapping sites (black dots) and areas (red polygons) with evidence of ZIKV infection in mosquitoes (Google Maps, 2018).

4.4. Results: Mosquito models

Abundance of outdoor female *Ae. aegypti* in urban areas.

The range of valid mosquito abundance models within a 95% confidence set are shown in Table 4.5. It is worth noting the large number of models within the confidence range, suggesting that there is substantial uncertainty from model inference. The AIC weights indicated that the model with the lowest AIC value is only 1.6 times more likely to be the best model than the next one. All of the models within the confidence range included household and climatic variables such as the position of the house in relation to the dominant winds and the condition of the yard, daily mean temperature and wind speed. The first 7 best models also included cumulative rainfall ranging from 14 to 30 days. The relative importance of human density in outdoor mosquito abundance models was very small and none models within the confidence range included it as a significant variable.

Table 4.5. The 95% confidence set of models and significant variables of mosquito abundance in urban and semi-urban areas.

rank	name	Household		Climatic				Inter	Human	AIC	AIC
		Location: L/W	PCI_y	Rain < 7d	Rain > 7d	Temp	Wind speed	Rain:t	density		weight
1	fit_rain18	√	√	-	√	√	√	-	-	933.1	0.27
2	fit_rain21	√	√	-	√	√	√	-	-	934.1	0.166
3	fit_rain18b	√	√	-	√	√	√	√	-	934.7	0.118
4	fit_rain14	√	√	-	√	√	√	-	-	935.6	0.077
5	fit_rain21b	√	√	-	√	√	√	√	-	935.9	0.068
6	fit_rain14b	√	√	-	√	√	√	√	-	936.7	0.044
7	fit_rain30	√	√	-	√	√	√	-	-	936.9	0.04
8	fit_rain_mm	√	√	√	-	√	√	-	-	937.0	0.037
9	fit_rain3b	√	√	√	-	√	√	√	-	937.2	0.035
10	model1a.1	√	√	-	-	√	√	-	-	937.6	0.028
11	fit_rain10	√	√	-	√	√	√	-	-	938.0	0.023
12	fit_rain1	√	√	√	-	√	√	-	-	938.1	0.022
13	fit_rain1b	√	√	√	-	√	√	√	-	938.8	0.015
14	fit_rain3	√	√	√	-	√	√	-	-	939.3	0.012
	Relative importance	0.955	0.955	0.121	0.806	0.955	0.955	0.28			

The model with the lowest AIC (*fit_rain18*) is summarised in Table 4.6. According to this model the number of outdoor female *Ae. aegypti* in urban households of St. Kitts is mostly affected by the condition of the yard ($p < 0.001$) increasing 2.7 times with

higher PCI scores and by the daily mean temperature ($p < 0.005$). Temperature showed a dual effect. Whereas mean temperature increased the number of mosquitoes by 1.49 ($p < 0.001$), high temperature decreased the number of mosquitoes by 0.59 ($p < 0.001$). The cumulative rainfall for the previous 18 days, although highly significant ($p < 0.005$), showed a small effect that increased the number of mosquitoes by a very small factor (1.01). In contrast, high winds ($p < 0.05$) and a leeward location exposed to the wind ($p < 0.001$) had a significant negative effect in mosquito abundance. Households situated in the direction of the dominant winds showed a smaller number of captured mosquitoes by a factor of 0.46 in respect to households situated in the lee side of the wind.

*Table 4.6. Summary of 'best' model as indicated by AIC. Signif. codes: 0 '***' 0.001 '**' 0.01 '*'; No. of observations: 194; siteID = 65; Log-likelihood: -456.55; AIC: 933. NB parameter: 2.3824 (sd: 0.56314); random effect sd: 0.3174*

	e^{β}	Estimate	Std. Error	z value	Pr(>|z|)
PCI yard: 2	2.75	1.011	0.18461	5.48	$4.4x10^{-08}$ ***
PCI yard: 3	2.73	1.005	0.23144	4.34	$1.4x10^{-05}$ ***
Avge. Temp.	1.49	0.401	0.13622	2.94	0.003 **
Rain (18 d.)	1.01	0.005	0.00200	2.64	0.008 **
Wind speed	0.94	-0.059	0.02990	-1.97	0.049 *
Max. Temp.	0.59	-0.518	0.13007	-3.98	$6.8x10^{-05}$ ***
Leeward: Wind	0.46	-0.783	0.20483	-3.82	0.0001 ***

The next best models showed similar estimated coefficients (Figure 4.9), including a positive effect of rainfall on the previous 21 days of trapping ($p < 0.005$). The most notable difference was the positive effect of the interaction between rainfall and mean temperature ($p < 0.005$) and the negative effect on the number of mosquitoes trapped of shorter rainfall (previous day and previous 3 days) consistent with reports of downpours disturbing mosquito populations. Negligible autocorrelation ($\rho = 0.2$) on consecutive day of trapping on the same site and lower on successive days auto-correlation was discarded, model diagnostics showed minor deviations of the residuals from normality although within acceptable limits and diagnostic plots of the assumption of linearity and homoscedasticity were satisfactory.

The inclusion of male mosquitoes in the models changed very little the models outcome. The main difference observed between the models for female mosquitoes only and the models for all adult mosquitoes arose on the size of the effect of the

yard condition (PCI) that is greater in the adult model than the female only model with each higher score increasing the number of total mosquitoes by a factor of 3.4 instead of 2.7 (Figure 4.10).

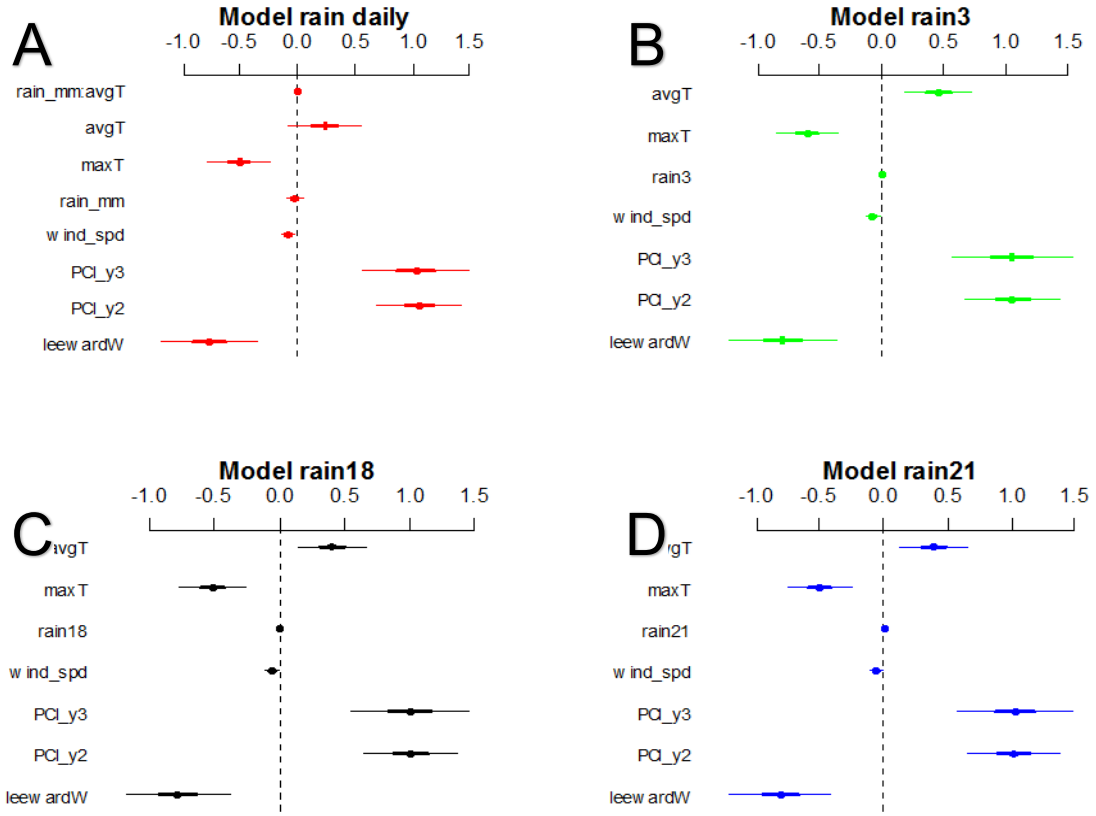


Figure 4.9. Regression estimates (x-axis indicates the estimated coefficient β) for models including different combinations of rainfall: A: rainfall on previous trapping day; B: rainfall previous 3 days; C: previous 18 days; D: previous 21 days.

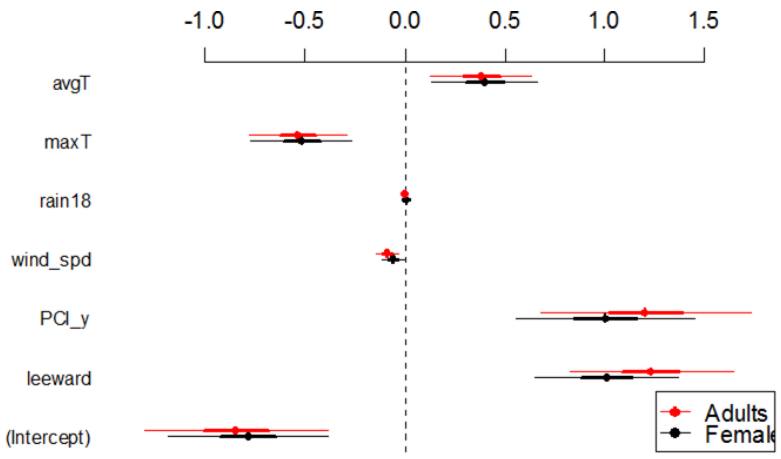


Figure 4.10. Comparison of estimated coefficients β (x-axis) for Females only and for Adults models.

Abundance of indoor female *Ae. aegypti* in urban areas.

Likewise, two analogous models were obtained to describe the abundance of indoor female *Ae. aegypti* which suggested that rainfall in the previous 7 days ($p < 0.005$) and the condition of the yard household significantly increased the number of female mosquitoes captured indoors ($p < 0.005$), whereas high temperatures had a negative effect ($p < 0.05$) in the captured number of mosquitoes in surveyed houses (Table 4.7). A similar model incorporated the amount of shade in the house and yard condition as the sum of both PCI scores (PCI2.y.sh, $p < 0.05$) and the rainfall in the previous 7 days ($p < 0.005$) (Table 4.8). A model describing the abundance of indoor adult *Ae. aegypti* was equivalent to the model of abundance of female mosquitoes only already described and no new parameters were identified.

Table 4.7. Summary of model asp_0_rain7.1b. AIC: 118.81; Null deviance: 59.4 on 36df; Residual deviance: 31.31 on 32 df; Theta: 0.781, std. err: 0.367.

	e^{β}	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	$3.4x10^{-45}$	-102.38	46.1284	-2.220	0.02645 *
PCI yard: 3	5.94	1.7817	0.62501	2.851	0.00436 **
Rain (7 d.)	1.15	0.1394	0.04916	2.837	0.00456 **
Max. Temp.	0.18	-1.7176	0.80273	-2.140	0.03237 *

Table 4.8. Summary of model asp_0_rain7.2.y.sh2. AIC: 118.04; Null deviance: 64.4 on 36df. Residual deviance: 30.72 on 31 df; Theta: 0.91, std. err: 0.447

	e^{β}	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	$1.3x10^{-3}$	-6.589	1.90910	-3.451	0.000558 ***
Rain (7 d.)	1.17	0.161	0.05075	3.187	0.001438 **
PCI2.y.sh3	9.52	2.253	0.88811	2.537	0.011171 *
PCI2.y.sh4	12.4	2.519	1.16263	2.167	0.030215 *
PCI2.y.sh5	13.7	2.619	0.94287	2.779	0.005457 **
PCI2.y.sh6	78.7	4.366	1.03283	4.228	$2.4x10^{-05}$ ***

Diagnostic plots showed some mixed results although still within acceptable limits for the current dataset. The quantile-quantile plot indicated satisfactory normality distribution of the residuals on both models. The plots of the assumption of linearity and homoscedasticity appeared to be reasonably closely approximated for the data although the distribution of the residuals against the predictor PCI yard and shade is slightly weaker than the rest of variables showing some clustered residuals. The

modelling of the abundance of indoor *Ae. aegypti* was attempted despite that the number of household aspirations was small resulting in 37 observations and very few sites were possible to be sampled using both aspiration and BG sentinel traps placed outdoors methods making any comparison between the outdoor and indoor models challenging.

4.5. Discussion

The results from the mosquito survey conducted have enhanced the understanding of the mosquito population in urban and semi-urban areas of St. Kitts associated with the sentinels described in chapter 2 and the prevalence of arboviruses in mosquitoes. Only two genera of mosquitoes were captured: *Culex* spp., which comprised 64.5% of the specimens trapped and *Aedes* spp., which comprised 35.5% of the specimens trapped. These results were consistent with previous surveys performed in the island (Belkin and Heinemann, 1976; Mohammed *et al.,* 2015) and with mosquito ecology (Gubler and Kuno 1997; Harrington *et al.* 2001). Overall, the highest density of female *Aedes* spp. was recorded in 2016 with 5.4 specimens per collection which was lower than reports in French Guiana (Fouque *et al.*, 2006) and in USVI (Kenney *et al.,* 2017), and the lowest density was 1.4 specimens per person which was similar to reports from Jamaica (Chadee *et al.*, 2009). A greater number of mosquitoes were trapped in 2016 than in the previous year after the passing of the ENSO phenomenon that made 2015 the warmest and the driest year in the Atlantic region (Banu *et al*., 2015; Muñoz *et al.,* 2016).

The entomological indices varied also by trapping area and human activity. Overall, the areas with the highest *Aedes* spp. indices were the recreational sites at Reggae Beach and Shipwreck Beach and Bird Rock neighbourhood in contrast to the residential areas of the Peninsula and Half Moon Bay where less than half of the trappings captured any *Aedes* spp. The consistently higher mosquito density observed in recreational areas than in other areas may represent a higher risk of arbovirus transmission (Chadee, 2009) and can be explained by the poor condition of the yard in these premises, with numerous water holding containers and debris suitable to act as breeding sites (Tun-Lin, Kay and Barnes, 1995; Chadee, 2004), the amount of shade (Tun-Lin *et al*., 1996) and the isolated location in respect of other buildings further than 30 meters (Getis *et al.,* 2003; LaCon *et al.,* 2014) plus the readily abundance of human hosts for feeding (Gómez-Dantés and Willoquet, 2009). Interestingly, the neighbourhoods Conaree, Mattingley and West Farm showed

varying indices between trapping seasons, which may be explained by changes in the location of mosquito clusters affected by human activity such as the clearing of breeding sites (Getis *et al.,* 2003; Iyaloo *et al*., 2014). Also, some residential areas including Frigate Bay were subject to outdoor fogging by the Vector Control office as part of the Zika outbreak control in late 2016 during or shortly before the traps were placed, and although these practices are generally regarded as ineffective in controlling mosquito populations (Gubler, 1997; Focks *et al.*, 2000), it may have underestimated the actual number of mosquitoes in specific sites and also explain partly the differences in the indices observed. Additionally, most of the areas with the lowest entomological indices (Half Moon Bay and Frigate Bay) are located exposed to the dominant wind while most areas with the highest entomological indices (Bird Rock and Monkey Hill) are located protected from the dominant winds behind higher ground. The exceptions of Conaree area which is exposed to the wind but a great number of mosquitoes were collected on one season and Boyd's that is located leeward but the average number of mosquito was below average suggest that other factors associated to the specific sites affected mosquito numbers.

The only arbovirus identified in mosquitoes was ZIKV and no evidence of DENV or CHIKV was found on either trapping period. Positive pools originated from mosquitoes captured between October and December 2016 during the Zika outbreak in five different areas. The neighbourhoods Camps and Half Moon, which had the lowest abundance of mosquitoes, produced two positive pools suggesting that viral infection in mosquitoes was independent of mosquito abundance (Focks, 2003; Bowman, Runge-Ranzinger and McCall, 2014). The minimum infection rate in the mosquitoes captured was 9.1 per 1,000 mosquitoes, smaller than reported rates of DENV infection in mosquitoes of 1.8% in Mexico (Garcia-rejon *et al.*, 2008) and 1.3% in Thailand (Yoon *et al.*, 2012) but greater than 0.51 per 1,000 mosquitoes (Kuno, 1995) and 1 per 2,755 mosquitoes in French Guiana (Fouque, Garinci and Gaborit, 2004), although it has been highlighted that the minimum infective ratio is known to suffer from errors caused by variation in the size of the pool (Kuno, 1995). A model to describe the probability of arbovirus infection was attempted, however, the amount of variation was excessively high and model performance was rather weak failing to identify significant factors of infection in mosquitoes and is consistent with reports indicating little evidence of association between the number of adult mosquitoes and dengue transmission (Focks *et al.,* 2003; Wu *et al.,* 2007; Bowman, Runge-Ranzinger and McCall, 2014).

Aedes spp. specimens were found in all the areas surveyed except at Robert L. Bradshaw International Airport, Basseterre Health Centre and Timothy Beach the Strip which was unforeseen as the habitats surrounding these premises were favourable for the development of larval *Aedes* spp. (Tun-Lin *et al.,* 1996; Gubler, 1998; Chadee, 2004) and adults have been spotted at other times. The unsuccessful captures may have been caused by trapping constrains and restricted access to parts of the official premises, and also by tampering of the traps deployed at one recreational site, and illustrates the difficulties in estimating actual population size by mosquito surveillance (Ritchie, Montgomery and Hoffmann, 2013).

The method of trapping determined differences in the number and physiological stage of the mosquitoes captured. BG-Sentinel traps located outside of households captured primarily unfed mosquitoes, and only a handful were gravid or had taken a recent blood meal while the aspirations inside houses captured a uniform proportion of female *Aedes* spp. in different stages. These findings are consistent with mosquito ecology as female *Ae. aegypti* tend to rest indoors after a blood meal and feel less attracted to the lures mimicking human odour than unfed ones (Scott *et al.,* 2000; Maciel-de-Freitas, Eiras and Lourenço-de-Oliveira, 2006; Reiter, 2007; Chadee, 2013) and agree with previous reports in the literature that indicated a bias of BG trapping against nulliparous female mosquitoes (Ball and Ritchie, 2010) while indoor aspirations collect different mosquitoes of both sexes and all physiological stages (Vazquez-Prokopec *et al.,* 2009; Silver, 2013). Also, whereas some authors have indicated that the number of *Ae. aegypti* mosquitoes caught in BG traps showed non-significant variation regarding the place where the trap was installed (Maciel-de-Freitas, Eiras and Lourenço-de-Oliveira, 2006), others have reported that BG traps may be unable to capture mosquitoes present in other areas of the house and under-represent the total number of mosquitoes at a residence (Johnson, Spitzauer and Ritchie, 2012).

The main factors affecting the number of mosquitoes captured outdoors were the condition of the yard followed by temperature and wind speed. Although there were a number of similar models that were equally suitable to describe mosquito abundance, the most likely models indicated a greater impact of climatic and household factors in the number of mosquitoes captured than human density. Household yards with debris and containers that could act as breeding sites harboured 2.7 times more female *Ae. aegypti* outdoors than tidier yards in agreement

with Tun-Lin, Kay and Barnes (1995) and Basker and Ezhil (2012). Additionally, temperature and rainfall showed a dual effect in that daily mean temperatures had a significantly positive effect on the number of female *Aedes* spp. captured, however, the number of mosquitoes captured decreased at temperatures higher than 30°C which is consistent with previous reports indicating that temperatures between 24°-27°C were favourable to mosquito daily survival, fecundity and parity rate, however, temperatures above 30°C decreased mosquito survival in French Guiana (Goindin *et al.*, 2015). Also, rainfall on the few previous days of trapping had a negative effect on the number of mosquitoes collected as heavy rainfalls were unfavourable to female survival (Fouque *et al.*, 2006), contrary to reports from Kuala Lumpur that indicated greater number of mosquitoes associated with rainfall during the previous week of trapping (Rohani *et al.*, 2011). However, cumulative rainfall on the previous to 14 to 30 days had a small positive effect in the number of mosquitoes trapped (Manica *et al.*, 2016), and the interaction of rain and average daily temperature indicated a minimal but positive effect on the number of mosquitoes, which is consistent with previous findings indicating that temperature has a greater effect than rainfall in mosquito abundance (Codeço *et al.*, 2015; Kraemer *et al*., 2015). In contrast, significantly less female *Ae. aegypti* were captured at sites in a windward location than at sites protected from the wind. These findings demonstrate that although climatic conditions at a high scale affect mosquito abundance (Kraemer, Sinka, Duda, A. Mylne, *et al.*, 2015; Wearing, Robert and Christofferson, 2016), local weather and microclimate conditions also have an impact on trap captures (Johnson, Spitzauer and Ritchie, 2012; Ezanno *et al*., 2015).

Finally, models describing indoor mosquitoes agreed overall with the models describing outdoor mosquitoes and untidy yards with higher amount of shade had a positive influence in the numbers of mosquitoes captured as described previously (Basker and Ezhil, 2012), and suggested that the use of air-conditioning or mosquito coils, or socio-economic status of the neighbourhood had little effect on *Aedes* spp. abundance contrary to previous findings (Reiter *et al.*, 2003; Bloch *et al.*, 2016), although any conclusions need to be taken with caution due to the small number of indoor observations and the weak performance of models.

4.6. Concluding remarks

Household characteristics such as accumulating debris and amount of shade, and climatic conditions, especially temperature, wind speed, and rainfall to a lesser

extent, affected *Ae. aegypti* abundance around sentinels' houses. Human density and other factors such as unscreened windows and storing water had a small impact in the number of mosquitoes found. The models developed will assist with accurate projections of *Aedes* spp. exposure analysed in chapter 5.

Chapter 5. Epidemiology and models of arbovirus infection in St. Kitts.

5.1. Introduction

Dengue is generally considered to be hyper-endemic in the Caribbean and cases are notified regularly by national health services (World Health Organization, 2017d) although the epidemiology of dengue in the region is heterogeneous (Kumar, Gittens-St Hilaire and Nielsen, 2013) and the dynamics of different serotypes, the incidence rate and symptomatic to inapparent infections ratio vary greatly from year to year (Balmaseda *et al.*, 2006, 2010; Biswas *et al.,* 2012). In some Caribbean islands, such as Barbados and Trinidad, low incidence rates have been reported between 0.29-2.92 and 0.49-5.05 cases per 1,000 population, respectively, with lower incidence during inter-epidemic periods and most cases occurring in the rainy season between October and January (Kumar, Gittens-St Hilaire and Nielsen, 2003; Chadee, 2007). Whereas in other Caribbean islands, such as St. Barthelemy and St. Martin, high incidence rates reaching 47.6 and 36.3 cases per 1,000 population, were reported by their respective national health services in 2015 (Pan American Health Organization, 2018). Incidence rates of dengue are generally high in immunologically naïve children with rates of seroconversion of up to 25.6% per year described in Maracay, Venezuela (Comach *et al*., 2009). High incidence rates in children leads to high seroprevalence in adults reaching nearly the totality of the population: 90.7% in Martinique and 96.2% in Guadeloupe in the French Caribbean (L'Azou *et al*., 2015), 95-100% in Nicaragua, Dominican Republic, Jamaica and Trinidad (Balmaseda *et al.,* 2010; Yamashiro *et al*., 2004; Brown *et al.,* 2009; Campbell *et al*., 2007). In the adjacent island of St. Eustatius a dengue seroprevalence of 83.3% was recorded in 2011, of which 62% presented antibodies to all four serotypes and 15% presented antibodies to serotype DENV2 only, which corresponded to the youngest age group, and the only factor associated with seroconversion was the length of residence on the island (Leslie *et al*., 2014). This finding was similar to studies in the French Caribbean where the only significant variables found were age and birth on the islands as opposed to birth in continental France (L'Azou *et al.*, 2015). In contrast, seroprevalence of chikungunya and Zika has been reported to be lower than dengue ranging between 13.1% in Managua, Nicaragua, (Kuan et al., 2016) to 22-76% in French Polynesia (Aubry et al., 2018), and 49% seroprevalence of Zika in French Polynesia (Aubry et al., 2017).

One of the aims of this chapter is to determine the burden and persistence of

arboviruses in the study population. Measuring of disease frequency and of exposure is the basis for epidemiological studies and contributes to inferring causation by associating exposure and frequency of disease (Dohoo, Martin and Stryhn, 2012). Establishing the incidence and prevalence of arboviroses is challenging because of the short viremia (Weaver and Lecuit, 2015), in addition to the high rate asymptomatic infections (Grange *et al*., 2014) and the diagnostic shortfalls of flavivirus (Hunsperger, 2012) discussed in section 3.1.2. The most common method to ascertain the presence of arbovirus in an asymptomatic population is through confirmation of serologic samples by a laborious and skilled-demanding gold standard test (Kuno, 2003). However, such tests can be unavailable on many instances (Gubler, 1998a; Roehrig, Hombach and Barrett, 2008) and relying solely on ELISA assays may cause unreliable estimations of the prevalence in a population (Radke *et al.*, 2012; Marrero-Santos *et al.*, 2013). An alternative approach to estimate the prevalence of disease when the tests performance parameters are uncertain involves the use of latent class models (Dendukuri and Joseph, 2001) described in section 3.1.3, which have also been applied to estimate the accuracy of dengue diagnostic tests (Pan-ngum *et al.*, 2013) and the drivers of transmission of chikungunya and Zika epidemics in the French Polynesia and the French West Indies (Riou, Poletto, and Boëlle, 2017).

In an endemic environment, the risk of seroconversion in the residents will generally increase with time and following cohorts of sentinels provides an opportunity to quantify the relationship between human factors, mosquito abundance, weather and infections and the time until such infections occur. However, untangling the risk factors associated with arboviral infections is also demanding. A common approach consists on cross-sectional studies and the use of simple statistical methods that require assumptions such as normality and homoscedasticity that are often unlikely in disease transmissions. The benefits of prospective studies were described in section 2.1, however, when repeated samples are collected from the same individuals, observations will be strongly correlated, violating the principle of independence required in generalised linear models (GLM). Generalised Estimating Equations (GEE), an extension of GLM for analysing repeated measures, can account for correlation of responses within subjects and produce efficient and unbiased regression estimates (Ballinger, 2004; Zuur *et al.*, 2009). GEE models were introduced by Zeger and Liang (1986), and later developed by Hardin and Hilbe (2002) and Fitzmaurice, Laird and Ware (2004) and have also been used to

determine early symptoms of dengue in children in Nicaragua (Biswas *et al.,* 2012). A GEE approach is appealing because the estimator is very robust, yielding a consistent estimate of coefficients even if the within-subject associations among the repeated measures have been misspecified and inferences are still valid without requiring the same number of observations for all individuals or that are taken at the same time interval (Fitzmaurice, Laird and Ware, 2004; Zuur *et al.*, 2009).

A further aim of this chapter is the determination of time until an arboviral infection occurred in the study population. However, in the periodic sampling of asymptomatic sentinels, any observed seroconversions were only known to have occurred within an interval of several months and some sentinels or suspected cases may show evidence of past infection but in either case seroconversions happened at an unspecified time, i.e. the observations were interval or left censored. Also, some of the sentinels dropped out of the study or the study finished before they became infected, i.e. these observations were right censored (Therneau and Grambsch, 2000). Survival analysis is a set of tools where the outcome variable of interest to predict is time until an event occurs, for instance arboviral infection, and can accommodate censored observations, therefore keeping valuable information that would be otherwise excluded from logistic regression analysis (Kleinbaum and Klein, 2012). Instead of quantifying the risk of infection, survival analysis focuses on the distribution of survival times and examines the relationship between time until an event and a number of explanatory variables (Therneau and Grambsch, 2000). There are numerous models of survival analysis described in the wider literature (Kaplan and Meier, 1958; Cox and Oakes, 1984; Wang, Chen and Yan, 2013), of which the semi-parametric Cox proportional hazards (PH) model (Cox, 1972) is the most popular because no assumptions need to be made about the distribution of the survival time while the inferences are still robust and will approximate the correct parametric model. The only fundamental assumption in Cox PH analysis is that the hazards are proportional for all observations, that is, the relative risk for two observations with time independent covariates is unchanged over time (Therneau and Grambsch, 2000; Kleinbaum and Klein, 2012).

In summary, this chapter aims to determine the burden and persistence of arboviral diseases in the study population, to measure the relationship between arboviral infection and human factors discussed in section 2.4, the mosquito abundance investigated in section **Error! Reference source not found.**, and climatic variables,

and to determine the time to arboviral infection and the factors associated with the time to infection.

5.2. Methods:

5.2.1. Prevalence of arboviruses in the study population

The prevalence of dengue and chikungunya in the sentinel population was estimated by detection of positive cases when IgM and IgG ELISA antibodies were above the thresholds established in section 3.3.2. Dengue seroconversion in the sentinels was defined as a four-fold rise in antibody against any serotype between two sequential samples obtained during the surveillance months (Gubler, 1998; Endy *et al*., 2011). The prevalence of Zika was estimated by detection of viral RNA by RT-PCR as described in section 3.2.2. Since a confirmatory PRNT test was unavailable, the ELISA assays were conditionally dependent as both tests measured the same biological component and their performance was uncertain, the traditional frequentist approach was inappropriate and would render unreliable estimated prevalence rates (Georgiadis *et al.*, 2003, 2010). Instead, the two-test, one-population Bayesian model developed by Dendukuri and Joseph (2001) and adapted by Branscum, Gardner and Johnson (2005) was applied. The parameters of the model included the latent prevalence of the virus, and the sensitivity and specificity of each test for the corresponding antibody, which were assumed to follow a beta prior distribution. The conditional dependence between the tests was estimated by the covariance between tests with the feasible range of covariances determined by the tests sensitivities among the infected individuals and the specificities among the non-infected individuals (Dendukuri and Joseph, 2001). Informative priors (Table 5.1) were specified for at least four of the seven parameters because of the non-identifiability of the model otherwise (Branscum, Gardner and Johnson, 2005).

Table 5.1. Informative independent priors for DENV and CHIKV prevalence model. Beta parameters (α, β) were obtained as described by Joseph and Belisle (2017).

DENV	*Median*	*95% C.I.*	*Prior*	*Reference:*
Prevalence	*0.063*	*0.024-0.132*	dbeta(5.4, 74.4)	*Mohammed et al. (2012)*
Se1 (*IgM*)	*0.40*	*0.281-0.532*	dbeta(23.4, 34.7)	*Granger et al. (2017)*
Sp1 (*IgM*)	*0.978*	*0.874-1.00*	dbeta(17.7, 0.44)	
Se2 (*IgG*)	*0.398*	*0.04-0.46*	dbeta(98.1, 148.3)	*Shamala (2015)*
Sp2 (*IgG*)	*0.958*	*0.91-0.98*	dbeta(134.8, 6.95)	

CHIKV	*Median*	*95% C.I.*	*Prior*	*Reference:*
Prevalence	*0.29*	*0.203-0.393*	dbeta(5.4, 74.4)	*CARPHA (2015)*
Se1 (*IgM*)	*0.85*	*0.71-0.93*	dbeta(35.4, 7.1)	*Prat et al. (2014)*
Sp1 (*IgM*)	*0.82*	*0.63-0.94*	dbeta(18.6, 4.3)	
Se2 (*IgG*)	*0.88*	*0.76-0.96*	dbeta(25.3, 50.1)	
Sp2 (*IgG*)	*0.95*	*0.75-0.99*	dbeta(11.7, 0.84)	

Following methodology of Branscum, Gardner and Johnson (2005), the response data $y = (p_{11}, p_{12}, p_{21}, p_{22})$ comprised the cross-classified positive test results for the n number of sentinels, as:

$$y = multinomial(n, (p_{11}, p_{12}, p_{21}, p_{22})), \quad (6)$$

$$p_{11} = P(T_1^+, T_2^+) = \pi[Se_1 Se_2 + cov_D^+] + (1 - \pi)[(1 - Sp_1)(1 - Sp_2) + cov_D^-],$$

$$p_{12} = P(T_1^+, T_2^-) = \pi[Se_1(1 - Se_2) - cov_D^+] + (1 - \pi)[(1 - Sp_1)Sp_2 + cov_D^-],$$

$$p_{21} = P(T_1^-, T_2^+) = \pi[(1 - Se_1)Se_2 - cov_D^+] + (1 - \pi)[Sp_1(1 - Sp_2) - cov_D^-],$$

$$p_{22} = P(T_1^-, T_2^-) = \pi[(1 - Se_1)(1 - Se_2) + cov_D^+] + (1 - \pi)[Sp_1 Sp_2 + cov_D^-].$$

Where T_j^+ and T_j^- are the sentinels detected as positive and negative, respectively, by test *j*. This model is strongly influenced by the choice of priors (Branscum, Gardner and Johnson, 2005) and simulations were performed to assess the validity of the inferred prevalence. Data sets were generated for a population of the same size as the sentinels' (n = 224) by using combinations of known test parameters with varying population prevalence. Simulations were performed with misspecified priors for a range of prevalence and the rest of the parameters were correctly specified (Georgiadis *et al.*, 2003).

5.2.2. Methods: Modelling risk factors of infection and time to event

The variables included in the models were obtained from the sentinels and suspected cases' questionnaires described in section 2.2.3 and comprised individual factors, environmental factors, individual behaviours and mosquito bites frequency as indicated in Table 5.2. The number of variables gathered from the questionnaires was considered excessively high (Babyak, 2004) and principal component analysis (PCA) to reduce the dimensionality of the data while retaining as much of the variation was applied (Jolliffe, 2002). Subsets of information were arranged in

relation to environmental factors within the household, mosquito breeding sites, individual behaviours indoors, outdoors and protective measures, and mosquito bites frequency, and transformed into their principal components (PC's). The first two PC's extracted for each of the subsets that explained broadly 50% of the variance (Appendix 30 and Appendix 31) were included in the models described in the following sections. Correlations between variables and test results were assessed by visualization of correlation circles also known as variable correlation plots where positively correlated variables are grouped together, negatively correlated variables are positioned on opposite quadrants of the plot origin and the distance between variables and the origin measures the quality of the variables on the factor map (Kassambara, 2017b).

Other variables included in the models of suspected cases comprised climatic data obtained from SKN Meteorological Office such as cumulative rainfall in days 1 to 21 before the sample collection and daily mean temperature lagged day 1 to 18 previous to the collection date, in addition to relative humidity, hours of sunshine, and wind speed for the previous 3, 5, 7 and 10 days. Estimated mosquito abundance was assessed by the predicted outdoor Female Aedes Density Index (FADI) in residential areas obtained from the models defined in section **Error! Reference source not found.**. The climatic variables in the sentinels' models were averaged for the duration of the previous sampling interval. Seasonality was modelled by introducing an artificially constructed variable (s) following the equation: $s = sin(2\pi t/365.25) + cos(2\pi t/365.25)$ where t is the time since the beginning of the study on 1st September 2014 and positive values of the coefficient obtained coincided roughly with the rainy season and negative values coincided roughly with the dry season.

Factors associated with risk of infection were investigated within two separate approaches based on the nature of the data collected. The suspected cases of arboviral infection were analysed by univariate analysis to identify the most relevant factors to be considered later into a multivariable GLM model, whereas the sequential samples collected from the asymptomatic sentinels were examined with a multivariable GEE model (Fitzmaurice, Laird and Ware*.,* 2004). Additionally, the complete dataset was also analysed for time to infection by a Cox proportional hazards survival analysis (Cox, 1972).

Table 5.2. Summary of variables analysed.

Subset of data		Covariate
Individual factors		Gender Age Length of residence
Environmental factors	I. Household	Intact mosquito screens Water supply Air-Conditioning Construction materials No. occupants in the house No. rooms in the house Socio-economic area
	II. Mosquito larval habitat	Debris around house Trash collection Plant pots Storage water Pools water
	III. Climatic and mosquito	Rainfall Temperature Wind Mosquito abundance
Behaviours	I. Indoors	Door open for > 30 min. Use of AC Length time in the porch Part day in the porch: morning / afternoon / evening
	II. Outdoors	Visit to bars / restaurants Visit to beach Hiking Visit to church
	III. Protective measures	Use of house spray Use of coils Use of repellent
Mosquito biting		Bites inside the house Bites outside / around the house Bites at bar / restaurants Bites at beach Bites hiking Bites at grocery store Bites in church

Univariate analysis of suspected cases

Risk factors of infection were assessed individually for each separate virus identified from suspected cases. Infections detected by serology were modelled by the log-transformed index level of anti-IgM ELISA antibody (y_M) assuming a normal distribution. Additionally, infections were also modelled by the joint diagnosis of positive cases discriminated by either anti-IgM and by detection of viral RNA by RT-PCR in parallel testing with the response variable being the probability of infection (p_v) assuming a binomial distribution. Infection with ZIKV was modelled as the probability of detecting viral RNA by RT-PCR assuming a binomial distribution with probability of infection (p_{zikv}) on samples collected after the first case of ZIKV was discovered in May 2016. Furthermore, risk of infection to an arbovirus was modelled as the probability of detecting a positive case to either virus (p_{arb}) by joint diagnosis to any test.

Multivariable analysis of suspected cases

Covariates that showed a significant effect individually were included in a multivariable GLM model in a forward stepwise manner until the model showed the lowest residual deviance and AIC value for each virus. Serologic assays indexes were log-transformed and assumed to follow a normal distribution and probability of detection of viral genome was assumed to follow a binomial distribution. Model internal validation and calibration was performed by bootstrapping with 200 repetitions following methodology of Harrell, Lee and Mark (1996).

Multivariate analysis of sentinels: GEE models

Infection in the asymptomatic sentinels was modelled by the levels of anti-IgM and anti-IgG antibody indexes measured by sequential ELISA assays. Following methodology described by Fitzmaurice, Laird and Ware (2004), the continuous response Y_{ij} was the log-transformed ELISA index for the sentinel i, on the sampling j, and the aim to relate changes in the mean response (μ_{ij}) over time to the covariates Xi'_{ij} by an identity link function:

$$g(\mu_{ij}) = \mu_{ij} = Xi'_{ij}\beta \qquad (7)$$

The within-subject association among the vector of repeated measurements for sentinel i, on the sampling j was assumed to follow an autoregressive correlation of order 1 (AR1) structure (Hardin and Hilbe, 2002) that depended only on the

correlation parameter (α), $0 \leq \alpha \leq 1$, modelled as a function of the distance between the observations (Zuur *et al.,* 2009). Models were obtained with the R package geepack version 1.2-1 (Højsgaard, Halekoh and Yan, 2016). The model with the lowest quasi-likelihood under the independence model criterion (QIC), an analogue to AIC developed by Pan (2001) for non-likelihood based models for which the likelihood-ratio is inappropriate, was accepted as the better model (Hardin and Hilbe, 2002). Goodness of fit was evaluated by plotting the deviance residuals against fitted values and assessing the lack of clustering of the residuals which indicated a uniform distribution and by quantile-quantile plots to judge the normality of residuals (Hardin and Hilbe, 2002; Zuur *et al.,* 2009).

Multivariable analysis: Time to event models

Cox Proportional Hazards (PH) analysis was used to investigate potential impacts of different factors on time to infection for each virus. Chikungunya virus was circulating on St. Kitts before any of the cohorts of sentinels arrived and Zika virus was introduced on St. Kitts after all the cohorts of sentinels had arrived, therefore, all the sentinels and suspected cases were at risk of arboviral infection since their arrival to the island. Since event was effectively a threshold on a continuum, models were analysed at different thresholds that included the highest and the lowest cut-off level of the ELISA tests obtained in section 3.3.2. Single observations were left or right censored and multiple observations from the same sentinel were interval censored. Interval censoring has computational challenges and a common practice involves the simplification of interval censoring structure of the data into a standard right censoring situation by imputing the midpoint of the interval (Gómez *et al.*, 2009). Events in left or interval censored observations were assumed to have occurred within the first quarter of the observed time when infection was established by the long-term antibody IgG. Alternatively, events were assumed to have occurred at the time of collection when infection was established by the short-term antibody IgM or by presence of viral RNA. This was done to reduce underestimating the time since infection from samples that only showed evidence from past infection. Censoring assumptions of randomness, independence and non-informative were investigated by logistic regression (Kleinbaum and Klein, 2012). Covariates were introduced in the full model one at a time in a forward stepwise manner until the model showed the lowest Akaike Information Criterion (AIC) and models were fitted with the R package 'survival' version 2.41-3 (Therneau, 2017).

The proportional hazards assumption of the models was evaluated by testing of independence between scaled Schoenfeld residuals and time for each variable and by observation of the scaled Schoenfeld residuals plotted against time (Harrell, Lee and Mark, 1996; Kleinbaum and Klein, 2012). When time-dependent variables were identified, i.e.: the values of a variable for a given sentinel differed over time, the violation of the proportional hazard assumption was addressed by adding time interactions with the variable 'seasonality' (Therneau and Grambsch, 2000). Outliers and influential observations were assessed by plotting Martingale and dfbeta residuals against time. Evaluation of goodness of fit was performed by analysing the estimated cumulative hazard function for Cox-Snell residuals and validation and calibration of the models for overfitting and optimistic evaluation by bootstrapping with 200 repetitions (Harrell, Lee and Mark, 1996; Therneau and Grambsch, 2000; Kleinbaum and Klein, 2012). Validation of models was done with the R package rms version 5.1-1 (Harrell Jr, 2017). Power was calculated using the methodology of Hsieh and Lavori (2000) with the R package 'powerSurvEpi' version 0.0.9 developed by Qiu *et al*. (2015) where given the type I error rate $\alpha = 0.05$ for a two-sided test, the power required to detect a hazard ratio as small as $\exp(\beta_1) = \theta$ is:

$$power = \Phi(-z_{1-\alpha/2} + \sqrt{n[log\theta]^2\sigma^2\psi(1 - \rho^2)}) \quad (8)$$

where $\sigma^2 = \mathrm{var}(x_1)$, ψ is the proportion of subjects who had the event, and ρ^2 is the multiple correlation coefficient of the linear regression: $x_1 = b0 + b^T x_2$, $\rho^2 = R^2$, and R^2 is the proportion of variance explained by the regression of x_1 on the vector of covariates x_2 (Qiu *et al.*, 2015).

5.3. Results:

5.3.1. Prevalence and incidence of arboviroses in the target population

DENV

Overall, 43 samples (14.8%) showed evidence of dengue infection, 34 (79.1%) were collected from suspected cases and 9 (20.9%) from asymptomatic sentinels (Appendix 32). Of the suspected cases, 23 (67.6%) were from individuals born in SKN and 11 (32.3%) from foreign residents. All samples (100.0%) analysed from individuals born in St. Kitts had higher antibody titres than samples from foreign-born individuals but only anti-DENV IgG were detected indicating past infection in the suspected cases with no evidence of recent dengue infection identified neither by

molecular nor serological methods. One sentinel, no. 77, presented at RUSVM health services with high fever and flu-like unspecific symptoms in October 2016 during the Zika outbreak. A sample was collected as a suspected case and showed evidence of anti-DENV IgG ELISA but no IgM or viral RNA was found and the cause of infection was unclear. Likewise, within the 9 asymptomatic sentinels that showed antibodies against DENV, five were anti-IgG positive on the first sampling of which, four had travelled to endemic dengue areas, another was from the US state of Maryland, an area endemic to WNV, and one sentinel had been vaccinated against yellow fever. Since the time between arrival to St. Kitts and the collection of four of those samples was less than one week and only showed evidence of IgG antibodies it is rather likely that the high titres observed were due to a Flavivirus infection before arriving to the island. The remaining four ELISA positive sentinels (1.8%) showed anti-IgM antibodies between 4.5 to 8.5 months of residence on the island (Figure 5.1). Applying the lower threshold to the Panbio Indirect IgG ELISA that enhanced the sensitivity of the test discussed in section 3.3.2, increased the number of cases detected on the initial and subsequent sampling but only increased the number of presumptive seroconversions on St. Kitts by an additional 2 sentinels, one of which tested positive shortly after a yellow fever vaccination, and the other was diagnosed an unrelated bacterial infection.

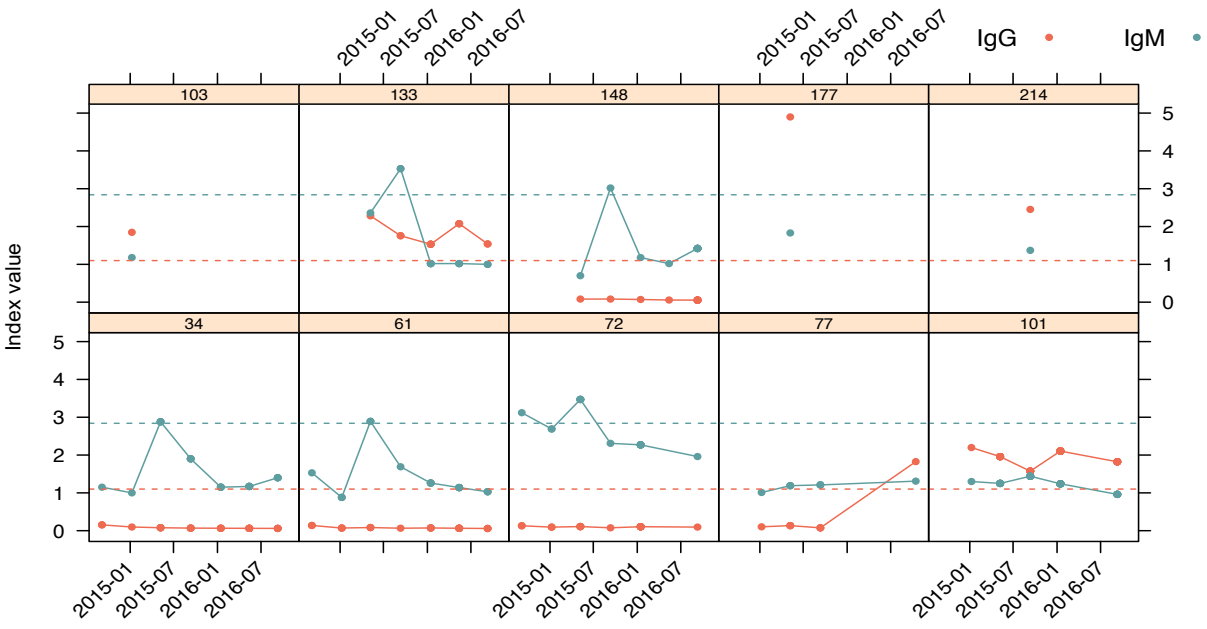


Figure 5.1. Summary of DENV ELISA results in sentinels with evidence of DENV infection (including nine asymptomatics and sentinel no. 77 showing unespecific symptoms). Each dotted line represents the threshold for each antibody: (red) Panbio Dengue IgG Indirect ≥ 1.1; (blue) InBios DENV Detect IgM ≥ 2.84.

Additionally, the mean levels of the index value obtained from the ELISA tests were used as a proxy of the amount of anti-DENV antibodies in the sentinel population. The index value corresponding to the anti-DENV IgM antibody in the sentinel population remained uniform throughout time with a slight decrease at longer times of residence (Figure 5.2, left). Moreover, the mean level of the index value corresponding to anti-DENV IgG antibodies measured in the sentinels against the time of exposure on St. Kitts showed a descending trend (Figure 5.2, right) indicating that the antibody levels in the population were higher on arrival and decreased over time and suggesting that few or none dengue infections happened on the island.

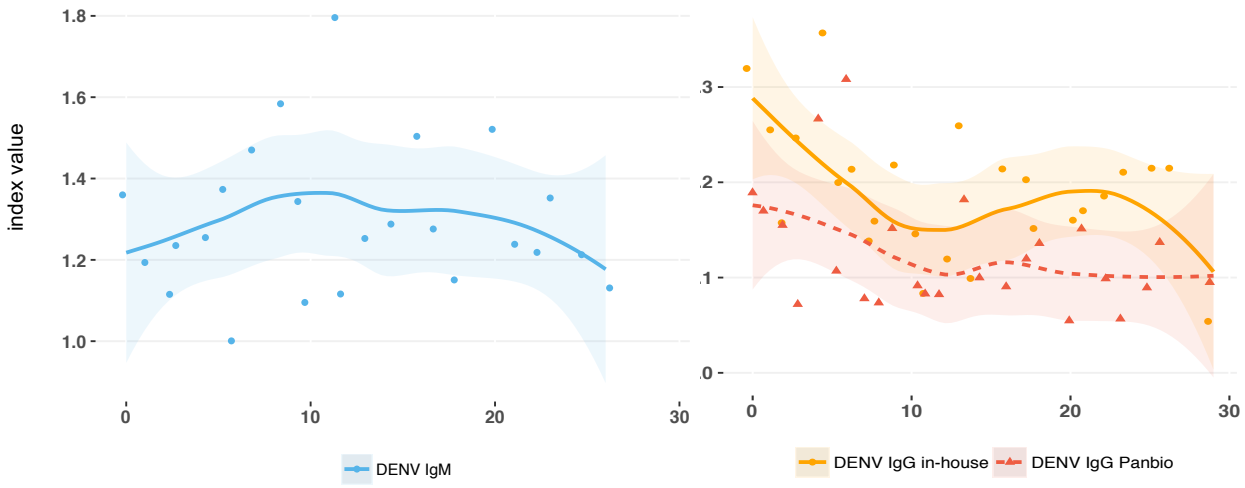


Figure 5.2. Mean index value of DENV absorbance ratio (solid lines) with 95% C.I. (shaded areas) in the sentinel population over the time of residence standardised to time since arrival (in months). Left: IgM antibody index. Right: IgG antibody index.

Accordingly, the estimated posterior dengue median prevalence in the sentinel population obtained from serology was 3.5% with a 95% probability interval of 0.1-7.2% assuming that all the infections discovered in the sentinels occurred in St. Kitts and a prior prevalence in RUSVM population of 6% (Mohammed *et al.*, 2012). However, the estimated posterior dengue median prevalence dropped to 2.1% (0.01-6.5%) assuming uninformative prevalence priors. Selecting a lower positivity threshold made minor difference to the estimated prevalence. Simulations performed with several estimated prevalence scenarios based on previous reports (Mohammed *et al.*, 2012) indicated that the observed data was significantly lower ($p < 0.05$) than the expected number of positive samples and the observed data was only contained within the 95% probability interval of the lowest prevalence scenario (Table 5.3), supporting the hypothesis that the true dengue prevalence in the study population

was very low. Besides, the predictive performance of assays is influenced by the true prevalence of disease in a population and, in consequence, the negative predictive values (NPV) of the DENV ELISA tests were very reliable in the sentinel population but the positive predictive values (PPV) were lower than 50% for both the InBios DENV Detect IgM ELISA and Panbio Dengue IgG indirect ELISA tests (Figure 5.3) suggesting the possibility that some or all of the positive results may be due to false positives.

Table 5.3. Estimated expected number of samples to test positive given different prevalence within 95% P.I. Figures marked with (*) are observed data

Simulated Prevalence	Reference:	N	Expected no. of samples positive to IgM & IgG	Expected no. of samples positive to IgM only	Expected no. of samples positive to IgG only	Expected no. of negative samples
2.5%	-	224	1-6	3-12	4-12	199-214
6%	Mohammed *et al.*, 2012	224	1-8	4-14	5-14	193-210
30%		224	5-16	17-33	15-30	156-181
44%		224	7-21	23-41	22-39	135-162
Observed data:		224	1*	3*	6*	215*

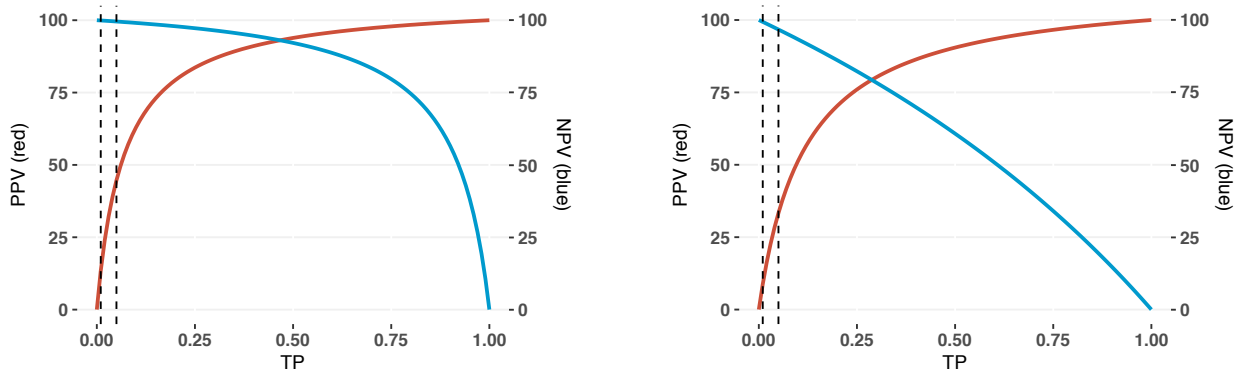


Figure 5.3. Estimated PPV and NPV curves for anti-DENV ELISA. Left: InBios DENV Detect IgM; Right: Panbio Dengue IgG indirect ELISA. The dashed vertical lines correspond to the estimated 95% P.I. posterior prevalence in the sentinel population. TP: True Prevalence.

CHIKV

Overall, 33 (11.3%) samples showed evidence of chikungunya infection, of these, 24 (72.7%) were collected from suspected cases and 9 (27.3%) from sentinels. Since CHIKV was not in circulation in the Americas before 2014 (Cassadou *et al.*, 2014), it was assumed that infections to chikungunya in the study population occurred on St.

Kitts. Evidence of recent infection were observed only between September 2014 and January 2015. Thirty samples were collected from suspected cases during this interval, of which 20 (66.7%) were positive, 15 samples (75.0%) were positive to both RT-PCR and IgM ELISA, 3 (15.0%) to IgM and IgG and 2 (10.0%) to IgG ELISA only, suggesting an incidence rate of 4.9 cases per 10 persons per year. The samples received from the JHF General Hospital showed a higher proportion of seropositivity (68.4%, C.I.: 43.4-87.4%) than the samples received from RUSVM Health Services (46.2%, C.I. 19.2-74.9%). Fourteen of the positive suspected cases were female (70.0%), between 18.4 and 56.7 years of age, mean of 34.5 years, 14 were born in SKN (70.0%). The lowest proportion of cases originated from the most affluent residential areas (20.0%), and 65.0% of the cases used the AC rarely or never and reported frequent mosquito bites (70.0%). Four further positive samples were observed after February 2015 until the end of the study although these samples only showed evidence of the long-term anti-CHIKV IgG antibody.

Similarly, the mean levels of the index value obtained from the ELISA tests were used as a proxy of the amount of anti-CHIKV antibodies in the sentinel population. The mean levels of the index values corresponding to anti-CHIKV IgM antibodies in the sentinel population standardised to the time since arrival decreased after the first 10 months of residence on St. Kitts and the index values corresponding to anti-CHIKV IgG antibodies peaked shortly after 20 months of residence suggesting that the virus still circulated actively in the earlier stages of the study since viral introduction in February 2014 and decreased its activity in later stages (Figure 5.4). Four sentinels were positive to IgM only, 5 to IgG only, and no samples were positive to both IgM and IgG. Three sentinels showed seroconversion after 12 days of arriving to St. Kitts and all positive samples were detected before the sampling call in May 2015 and at the last sampling call in September 2016 which corresponded to sentinels that had missed previous samplings and their time of infection is unclear (Table 5.4 and Figure 5.5).

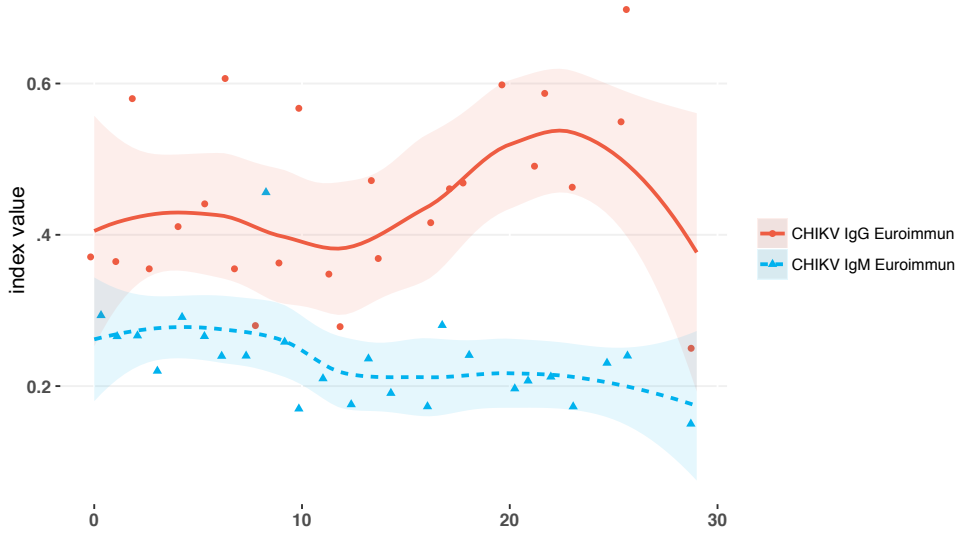


Figure 5.4. Mean index of CHIKV antibodies in the sentinel population over the time of residence standardised to time since arrival (in months). Shaded areas correspond to 95% C.I.

Table 5.4. CHIKV positive cases. N: number of samples; n: number of positive cases; AP: Apparent Prevalence; I: Incidence

Sampling	Sample date	N	n	AP	New cases	I	n	AP	New cases	I
			Index 0.8				Index 1.1			
1	Sep. 2014	69	0	-	0	-	0	-	0	-
2	Jan. 2015	104	5	4.8%	5	4.8%	3	2.9%	3	2.9%
3	May 2015	147	7	4.7%	3	2.0%	6	4.1%	4	2.7%
4	Sep. 2015	146	7	4.8%	2	1.4%	4	2.7%	0	-
5	Jan. 2016	128	4	3.1%	1	1.5%	2	1.6%	0	-
6	May 2016	106	10	9.4%	5	4.7%	2	1.9%	0	-
7	Sep. 2016	98	13	13.3%	9	9.2%	4	4.1%	2	2.0%
	Total	224			25	11.2%	9	4.0%		

The estimated apparent prevalence in the sentinel population was 4.0% (95% C.I.: 1.9-7.5%) applying the most stringent threshold for positivity. Alternatively, applying the least restrictive threshold, 25 sentinels tested positive to chikungunya, and the estimated apparent prevalence in the sentinel population was 11.2% (95% C.I.: 7.4-16.0%). Five of those samples were positive to IgM only, 21 to IgG only, and one sentinel was positive to both IgM and IgG. The chikungunya posterior median prevalence in the sentinel population estimated by Bayesian analysis was 10.2% with a 95% probability interval of 6.9-14.3% considering the prior prevalence reported by CARPHA (2015) and the most stringent threshold for positivity. Selecting a less

stringent threshold, the estimated median prevalence only increased slightly (12.8%, 95% PI: 8.2-18.4%) which shows agreement with the frequentist approach of least stringent threshold of positivity. Estimating a parallel tests performance of 99.0% sensitivity and 97.5% specificity (Mayo Clinic, 2016), the diagnostic PPV for the combined tests was 82.04% and the NPV was 99.9% raising high confidence in the ELISA results. The estimated prevalence of chikungunya in the population born in St. Kitts was 69.6% (95% CI: 47.1-86.8%).

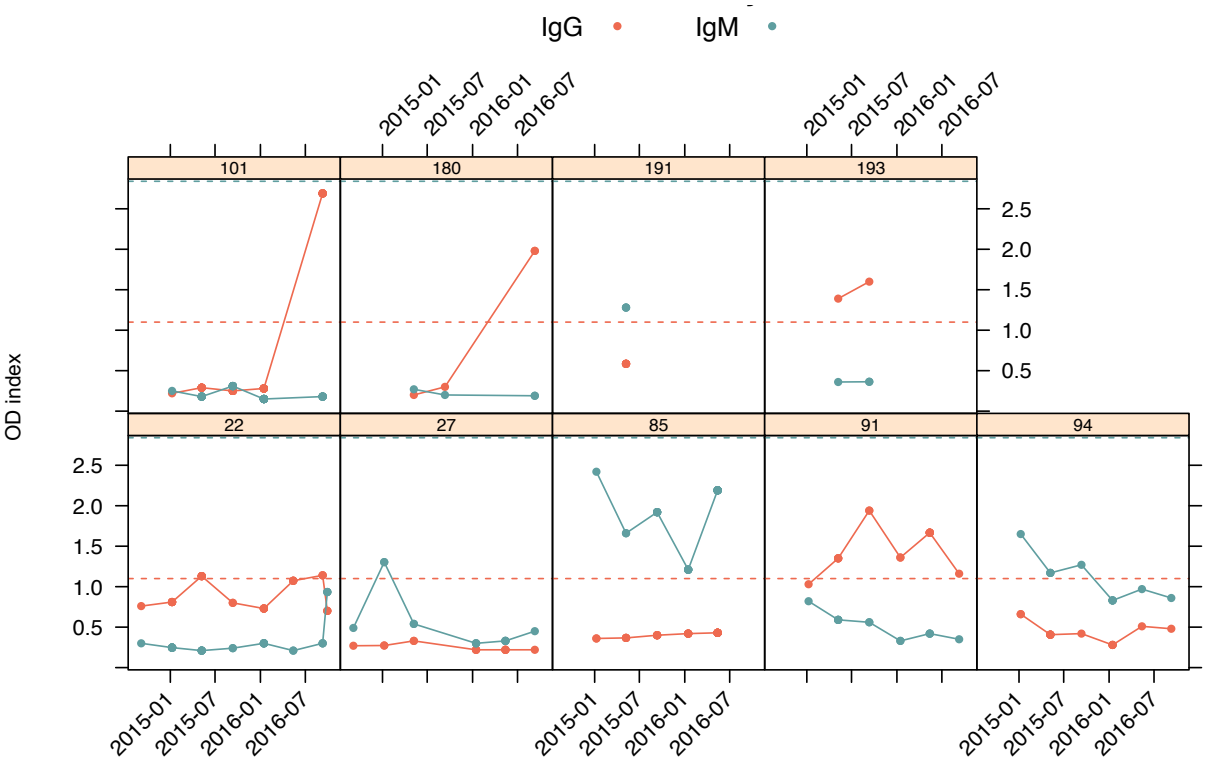


Figure 5.5. Summary of CHIKV ELISA results in sentinels with signs of infection. The dotted line represents the threshold for each antibody: EUROIMMUN Anti-Chikungunya virus ELISA IgM (blue) & IgG (red).

ZIKV

Overall, 18 (39.1%) samples showed evidence of Zika infection by molecular analysis, suggesting a prevalence proportion between 25.1-54.6% with 95% confidence interval from 46 samples collected from suspected cases between May and December 2016. The first sample with indications of ZIKV RNA was collected in May 2016, four months earlier than the official outbreak notification from the health authorities and the incidence rate was 3.66 cases per person and year after the declaration of the outbreak in September 2016 until December 2016. Nearly all the samples (93.5%) were collected from non-Kittitians at RUSVM health services, over half were female (55.6%), between 19.9 and 53.2 years of age, (mean: 29.2 years). Most of the suspected cases used the AC daily (50.0%), and a few lived in low socio-

economic areas (11.1%), however, all of these tested positive to ZIKV RNA (100.0%) and the majority were bitten often (50.0%), although there were no significant differences with other groups.

5.3.2. Univariate analysis of risk factors of infection

Exploratory analysis

Exploratory analysis of correlation plots in sentinels and suspected cases showed positive correlation between the level of anti-CHIKV antibodies, damaged mosquito screens and lack of constant water supply and strong negative correlation with residence in affluent areas (Figure 5.6 A). The presence of debris and containers around the household was also strongly positive correlated with anti-CHIKV antibodies (Figure 5.6 B) alongside individual behaviours such as spending time in the porch and weak with use of AC although may have a smaller effect (Figure 5.6, C). Frequenting recreational areas was negatively correlated with the level of CHIKV antibodies (Figure 5.6, D), contrary to use of sprays and coils that showed positive correlation (Figure 5.6, E). Mosquito bites indoors in places such as at church, stores or house were highly correlated with antibody levels but weakly with bites outdoors recreational areas (Figure 5.6, F). Analysis of anti-DENV antibodies and ZIKV RT-PCR results showed unclear correlations overall between viral infection and risk factors analysed.

Suspected cases: infection by CHIKV

Univariate analysis of IgM and RT-PCR results from samples collected from chikungunya suspected cases between September 2014 and February 2015, indicated that frequent mosquito bites in the house and individuals born in SKN had the greatest positive effect associated with recent infection of all the variables analysed (OR: 13.0 and 10.5 respectively, $p < 0.01$) and the amount of unexplained variability indicated by the deviance was amongst the lowest (Table 5.5). Environmental conditions in the house such as greater number of rooms and occupants, behaviours as rare use of AC or leaving doors open for long periods of time and mosquito abundance were significantly associated with increased risk of chikungunya infection. In contrast, heavy rainfall between the days 3 and 21 prior to sample collection (OR: 0.03; $p < 0.01$) and residence in an affluent area (OR: 0.16) indicated a negative effect on infection although the latter was non-significant.

CHIKV

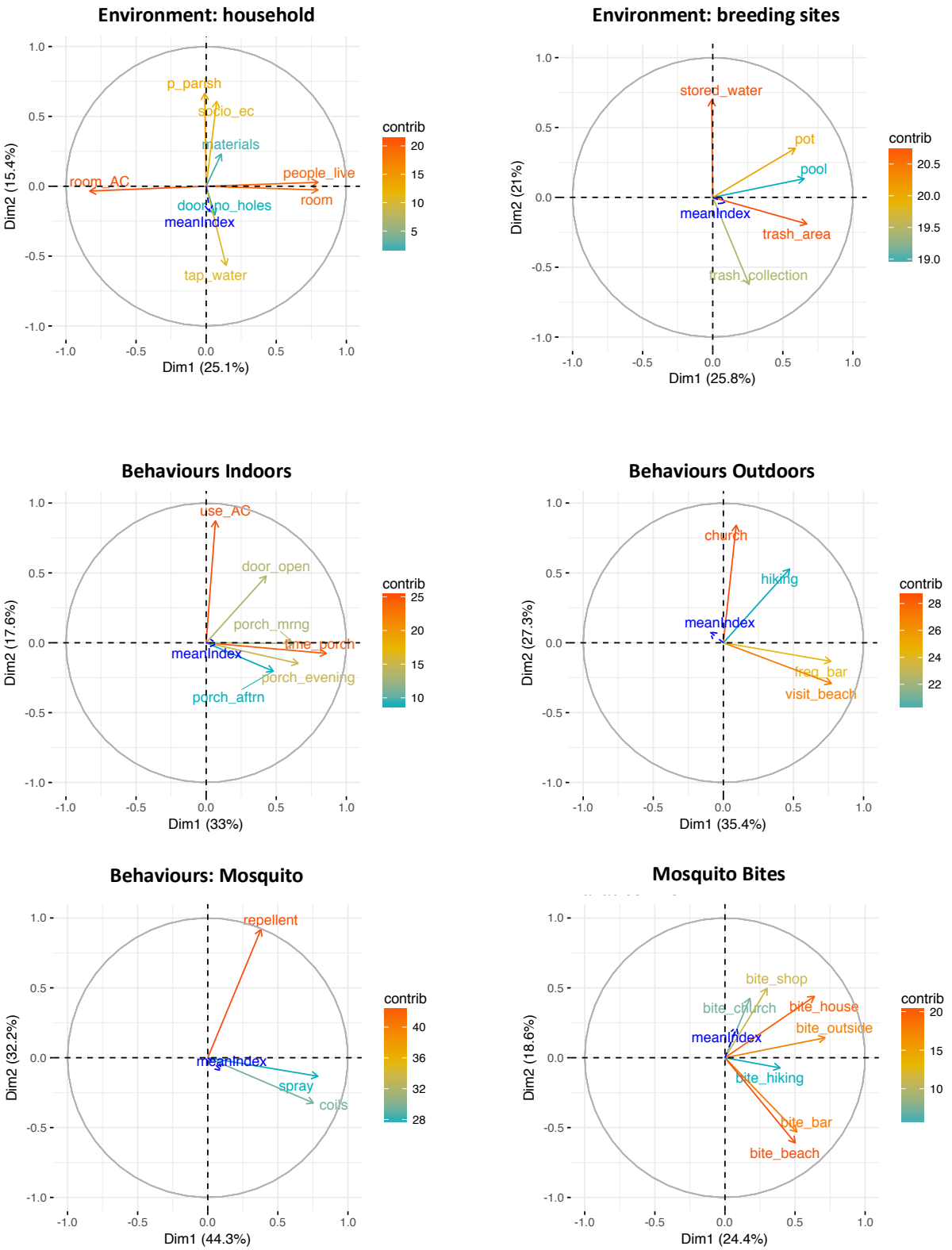


Figure 5.6. Exploratory analysis: Component loadings for subset of covariates. The above correlation plots show positively correlated variables grouped together, negative correlated variables are positioned on opposite quadrants of the plot origin and the distance between variables and the plot origin measures the quality of the variables on the factor map (Kassambara, 2017b)

Table 5.5. Univariate analysis of CHIKV suspected cases (IgM & RT-PCR). Null deviance: 40.4 on 28 d.f.

	OR	95% C.I.	Estimate	Pr(>|z|)	AIC	Deviance
Mosquito bites in house	13.0	2.41-107.4	2.56	0.0061 **	34.8	30.8
Birth in SKN	10.5	2.09-68.8	2.35	0.0072 **	35.9	31.9
Household (No. rooms, no. occupants)	1.91	1.13-3.87	0.64	0.0354 *	37.9	33.9
Behav. Indoors (doors open, use AC)	1.88	1.04-3.72	0.63	0.0457 *	39.8	35.8
FADI	1.25	1.03-1.58	0.22	0.030 *	38.7	34.7
Socio_economic (high)	0.16	0.18-1.10	-1.79	0.079	40.3	34.2
Rain (3-21 days)	0.03	10^{-3}-0.26	-3.58	0.0076**	32.7	28.7

Further analysis of the samples including the results from the long-term marker of infection IgG in chikungunya suspected cases confirmed the significant effect of frequent mosquito bites inside the house (OR: 21.0; $p < 0.005$) and birth in St. Kitts (OR: 5.45; $p < 0.05$) (Table 5.6).

Table 5.6. Univariate analysis of CHIKV suspected cases (IgG). Null deviance: 33.8 on 28 d.f.

	OR	95% C.I.	Estimate	Pr(>|z|)	AIC	Deviance
Mosquito bites in house (often)	21.0	1.11-32.8	3.04	0.0087 **	31.5	27.5
Birth in SKN	5.45	1.11-32.8	1.69	0.045 *	37.8	33.8

Suspected cases: infection by ZIKV

Univariate analysis of samples from Zika suspected cases collected between May and November 2016, showed little evidence of association with climatic, household, behavioural factors or mosquito bites. Only high temperatures on the prior 4th and 17th days from the collection day showed a negative effect on the risk of infection (OR: 0.42 and 0.18, respectively) but neither were significant.

Suspected cases: infection by any arbovirus

Univariate analysis of the suspected cases collected during a period of an arbovirus outbreak (n = 84) that included 36 positive cases detected of CHIKV and ZIKV by

either IgM ELISA or RT-PCR suggested that country of origin had a significant effect (OR = 4.18; $p < 0.01$), followed by mosquito biting in outdoor areas (OR = 1.40; $p < 0.05$), mosquito density (OR = 1.18; $p < 0.05$), and relative humidity lagged by 7 and 5 days (OR = 1.18 & 1.11; $p < 0.05$ & $p < 0.001$, respectively) (Table 5.7). In contrast, residence in affluent areas reduced the odds of infection by 81.0% ($p < 0.05$) followed by behaviour outdoors (OR = 0.74; $p < 0.05$), recent rainfall had a small negative effect (OR = 0.94; $p < 0.05$) and length of residence with the odds of infection decreasing by 0.3% per day, indicating that higher socio-economic status, frequenting less bars or beaches in the evening and longer length of residence implied a lesser risk of contracting an arbovirus infection. Models exploring rainfall, in addition to mosquito abundance and birth on St. Kitts vs. other countries showed the lowest deviance suggesting that these variables gathered a substantial part of the total variability.

Table 5.7. Summary of univariate analysis of suspected cases to CHIKV and ZIKV. No. observations: 86; No. of cases: 36; Null deviance: 114.7 on 82 d.f.

	OR	95% C.I.	Estimate	Std. Error	Pr(>|z|)	AIC	Deviance
Rain (2 days) Lag7	0.94	0.70-0.94	-0.166	0.076	0.029*	104	100.5
FADI	1.18	1.04-1.36	0.166	0.067	0.015*	110	106.5
Birth in SKN	4.18	1.52-12.47	1.43	0.530	0.007**	111	106.9
Rain (4 days) Lag7	0.95	0.90-0.99	-0.04	0.022	0.028*	111	107.5
Wind speed (lagged 10 days)	0.82	0.70-0.95	-0.19	0.075	0.011*	111	107.5
Mosquito bites in outdoor areas	1.40	1.08-1.86	0.33	0.138	0.015*	112	108
Rain (3 days) Lag7	0.95	0.90-0.99	-0.046	0.022	0.039*	112	108.3
Socio-economic (High)	0.19	0.04-0.70	-1.65	0.668	0.016*	114	108.3
Residence	0.997	0.995-0.999	-0.0022	0.001	0.028*	113	109.0
Behaviours outdoors	0.74	0.55-0.97	-0.30	0.144	0.037*	114	110
Relative Humidity (lagged 7 days)	1.18	1.0-1.26	0.112	0.056	0.046*	114	110.4
Relative Humidity (lagged 5 days)	1.11	1.05-1.18	0.10	0.029	$4.5x10^{-4}$***	114	110.5

5.3.3. Multivariable analysis of risk factors of infection

Suspected cases: GLM model results for CHIKV outbreak IgM & PCR:

Multivariable analysis of recent chikungunya infection in suspected cases revealed that mosquito bites inside the house, and behaviours indoors such as infrequent use of AC and leaving doors open had a significant positive effect in the probability of infection (Table 5.8). The model suggested that being bitten by mosquitoes inside the house posed a substantial risk, although the small number of observations (n = 30) and high variance caused extraordinarily large confidence intervals with little practical application. Validation of the model by bootstrapping indicated an optimistic index of 0.038 in Somer's D_{xy} index which resulted in a predictive probability of AUC = 0.918 and a necessary correction of the slope of 0.7135 (Table 5.9).

Table 5.8. Summary of CHIKV infection in suspected cases. No. observations: 30; No. of cases: 18; AIC: 22.5; Null deviance: 40.4; Residual deviance: 16.5 on 27 d.f.

	OR	Lower 0.95	Upper 0.95	Coef	S.E.	Pr(>|z|)
Intercept	$8.48x10^{-3}$	0.00	0.26	-4.77	2.56	0.0630.
Bitten in the house - Often	$4.52x10^{3}$	21.22	$4.2x10^{8}$	8.42	4.08	0.0391 *
Behaviour indoors: rare use of AC, doors open for > 30 minutes	15.45	2.46	613.4	2.74	1.34	0.0416 *

Table 5.9. Model validation: CHIKV infection in suspected cases (Bootstraping =200).

	Index orig	training	test	optimism	Index corrected	N
Dxy	0.8750	0.8936	0.8556	0.0380	0.8370	187
R2	0.7416	0.7803	0.7112	0.0691	0.6725	187
Intercept	0.0000	0.0000	0.0890	-0.0890	0.0890	187
Slope	1.0000	1.0000	0.7135	0.2865	0.7135	187

Sentinel models: GLM model results for CHIKV and ZIKV outbreak:

Similar multivariable analysis of recent infection in suspected cases by chikungunya and Zika suggested that the country of origin had the greatest effect in the probability of arbovirus infection (Table 5.10 and Figure 5.7). The suspected cases born in SKN had substantial higher risk than the suspected cases born in other countries (OR = 115.7, 95% C.I.: 9.64-4227.8, $p < 0.005$). Additionally, climatic variables were

analysed with a lag of 7 days prior to the sample collection which coincides roughly with the estimated duration of the incubation period of the virus in humans (Rudolph *et al*., 2014) including an additional time for the sample to be collected, showed higher daily temperature on the previous 25 days of sample collection (OR = 5.33, 95% C.I.: 1.70-27.7, p < 0.05), which stimulates mosquito development and reduces the extrinsic incubation period at the time when the mosquito becomes infective, and frequent mosquito bites resulted in higher risk of infection with an arbovirus (OR = 1.99, 95% C.I.: 1.22-3.64, p < 0.05). In contrast, heavy rainfall in the two days prior to the week of the sample being collected, (OR = 0.80; 95% C.I.: 0.65-0.91; p < 0.01), and high winds 10 days earlier than the sample taken had a negative effect (OR = 0.76; 95% C.I.: 0.60-0.92; p < 0.05), which decreased the number of mosquitoes when they were infective. An outlier identified (suspected case no. 1048) was removed from the model and improved the goodness of fit by reducing the residual deviance and AIC, although normality of the residuals and the predictive performance of the model were modest (Table 5.11).

*Table 5.10. Summary of model outcome of suspected cases for CHIKV and ZIKV. AIC = 82.0. Null deviance: 113.6 on 82 d.f. Residual deviance: 70.1 on 77 d.f. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1*

	OR	Lower 0.95	Upper 0.95	Coef	S.E.	Wald z	Pr(>|Z|)
Intercept	0.00	0.000	0.000	-45.79	19.87	-2.30	0.0212 *
Birth in SKN	115.7	9.64	4227.8	4.751	1.54	3.09	0.0020**
Temperature 18 day (lagged 7 days)	5.33	1.70	27.69	1.674	0.70	2.38	0.0174 *
Mosquito bites in the house	1.99	1.22	3.64	0.693	0.27	2.53	0.0113 *
Cumulative rainfall 2 days (lagged 7 days)	0.80	0.65	0.91	-0.227	0.081	-2.78	0.0055**
Wind speed (lagged 10 days)	0.76	0.60	0.92	-0.274	0.11	-2.51	0.0120 *

Table 5.11. Model validation: CHIKV and ZIKV infection in suspected cases (Bootstraping =200).

	Index orig	training	test	optimism	Index corrected	N
Dxy	0.7530	0.7874	0.7193	0.0681	0.6848	200
R2	0.5475	0.6000	0.5105	0.0894	0.4580	200
Intercept	0.0000	0.0000	-0.0584	0.0584	-0.0584	200
Slope	1.0000	1.0000	0.7509	0.2491	0.7509	200

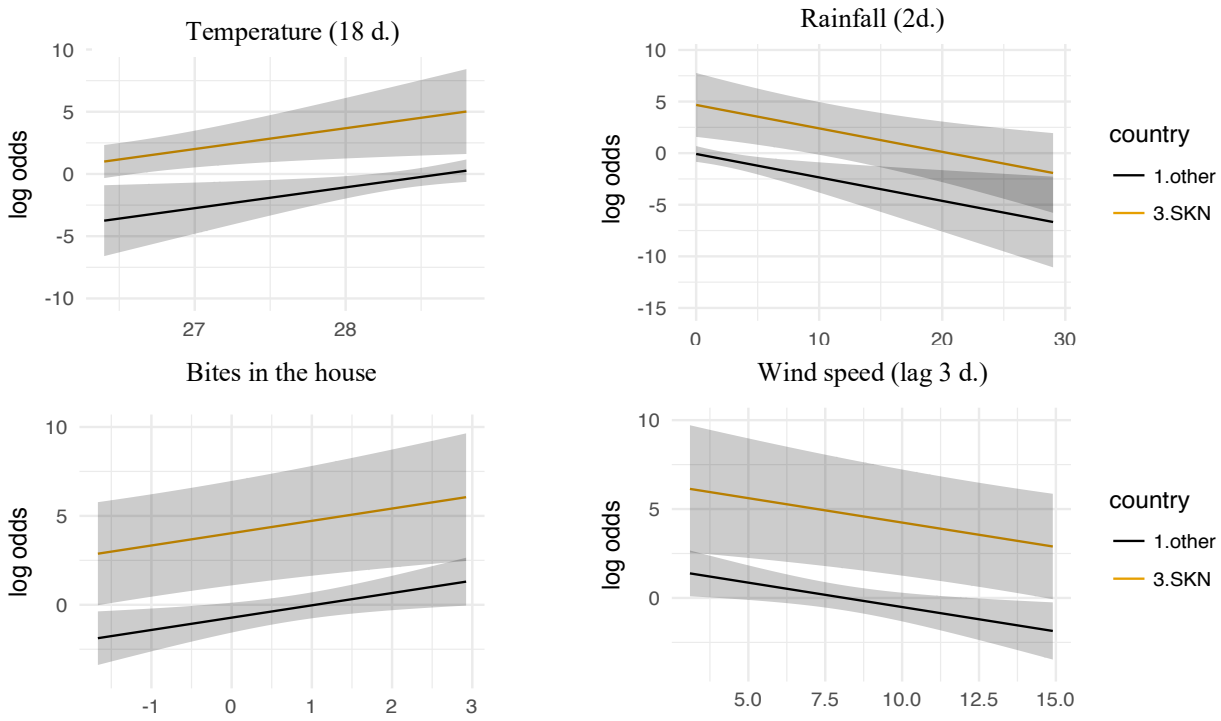


Figure 5.7. Effect plots: Multivariate analysis for CHIKV and ZIKV infections shown by country of origin with 95% C.I.(shaded areas). (Plots adjusted to: temp. (18 d.) = 28.5; Bites in the house = 0.701; Wind speed = 9; rain2 = 0.7)

Sentinel models: GEE model results for CHIKV IgG ELISA:

The analysis of sequential samples collected from the asymptomatic sentinels revealed that the two positive samples to chikungunya observed at the last sampling call in September 2016 occurred in sentinels that had missed several previous samplings, therefore, only the observations up to the sampling call in May 2016 were introduced in the model to reduce biased inferences. The sentinels' model for anti-CHIKV IgG antibodies supported previous findings in that climatic factors had a greater relevance in the level of antibodies than human or mosquito factors. Rainfall showed the greatest and most significant positive effect in the antibodies log-index levels (β = 41.4; 95% CI: 12.8-70.0; $p < 0.005$), as summarised in Table 5.12, and temperature increased the antibody titres until a peak was reached at optimal temperatures between 27ºC-28ºC (Figure 5.8). Mosquito bites in the house and mosquito abundance showed a small positive effect on the mean amount of antibodies measured by the log-ELISA index although only the later was significant (β =0.012; 95% CI: 0.0007-0.023; $p < 0.05$), which is consistent with mosquito ecology, and the length of residence had a negative effect in the mean antibody levels (β = -5.5x10^{-4}; 95% CI: -0.0001- -0.00006; $p < 0.05$) as most of the sentinels

were infected at the beginning of the study and the antibody titres declined over time with estimated mean IgG levels 20.1% lower after every year of residence. These model outcomes were consistent with infections occurring actively during the early epidemic phase and suggested that the virus became less active or disappeared over time. The estimated correlation parameter was 0.91 indicating that consecutive samples from the same sentinel are strongly correlated.

*Table 5.12. Summary of GEE model of sentinels CHIKV IgG results. Estimated Scale Parameters = 0.137, Std. err = 0.019. Correlation structure: AR1; Estimated Correlation Parameters: α = 0.91, Std. err = 0.035; Number of clusters = 181; Maximum cluster size = 5. QIC = -1009. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1*

Coefficients:	Estimate	Lower 0.95	Upper 0.95	Std.err	Pr(>|W|)
(Intercept)	-752.0	$-1.5x10^{-3}$	50.3	409.0	0.0662.
Daily mean temperature	52.0	-4.37	108	28.8	0.0706.
Rain (mm)	41.4	12.8	70.0	14.6	0.0045**
Bite in the house (Often)	0.059	$-9.8x10^{-3}$	0.128	0.035	0.0930.
Mosquito FADI	0.012	$9.7x10^{-4}$	0.023	$5.4x10^{-3}$	0.0326*
Length residence	$-5.5x10^{-04}$	$-1.03x10^{-3}$	$-6.4x10^{-5}$	$2.46x10^{-04}$	0.0264*
Daily mean temperature	-0.898	-1.89	0.09	0.504	0.0750.
Rainfall:Mean Temp.	-1.510	-2.55	-0.461	0.533	0.0047**

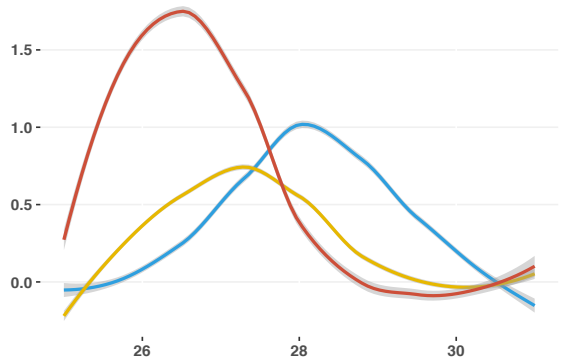


Figure 5.8. Left: Predicted mean ELISA index in the sentinels (y-axis) dependent on temperature in °C (x-axis) and rainfall (blue line = 1mm; yellow line = 2mm; red line = 3mm).

Sentinel models: GEE model results for CHIKV IgM ELISA:

The analysis of anti-CHIKV IgM agreed with previous models in the association with temperature and rainfall ($p < 0.001$) and the only risk factors associated to the sentinels were the level of affluence residence and occupancy ($p < 0.001$) and the higher risk of infection by frequenting bars and restaurants ($p < 0.05$) as summarised

in Table 5.13. Although the model predicted output follows closely the observed data (Figure 5.9), these results, however, need to be taken with caution as the residuals for this model showed weak normality and clustering of residuals against fitted values. Also, only the measurements collected until January 2016 were considered in the model as the analysis of samplings after that date produced spurious results which were attributed to a change in assay batches that increased artificially the index outputs.

*Table 5.13. Summary of model CHIKV IgM (sentinels). QIC = -727. Estimated Scale Parameters = 0.166, Std. err = 0.0354. Correlation structure: AR1; Estimated Correlation Parameters: α = 0.817, Std. err = 0.0354; Number of clusters = 179; Maximum cluster size = 4. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1*

	Estimate	Std.err	Wald	Pr(>|W|)
(Intercept)	87.814	7.3085	144.37	$< 2x10^{-16}$ ***
Daily mean temperature	15.477	1.2438	154.83	$< 2x10^{-16}$ ***
Daily maximum temperature	-16.961	1.3670	153.95	$< 2x10^{-16}$ ***
Rain (mm)	-1.0172	0.0805	159.51	$< 2x10^{-16}$ ***
Socio-economic, no. rooms, no. residents	-0.0508	0.0144	12.39	0.00043 ***
Visits to beach, bar or restaurants	0.0286	0.0125	5.19	0.02268 *

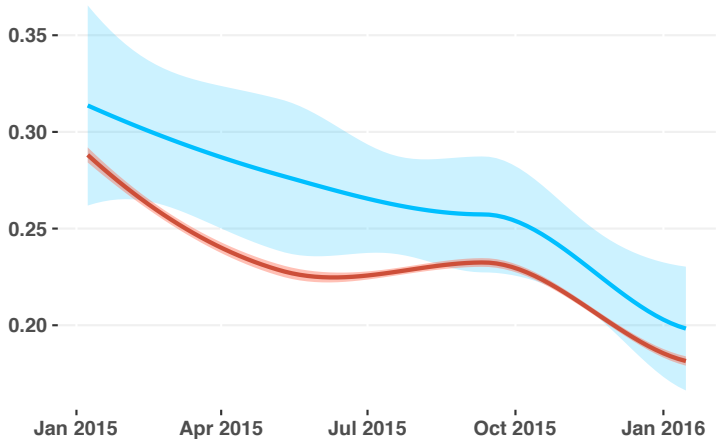


Figure 5.9. Predicted (blue) and observed (red) mean anti-CHIKV ELISA IgM index values with 95% C.I. (shaded areas).

Similarly, the analysis of DENV IgM and IgG ELISA index produced very weak models. The only significant factor associated with the sentinels was the negative effect of length of stay ($p < 0.001$) and climatic data. However, the residuals showed strong clustering and very weak normality therefore, inference from the model were discarded.

5.3.4. Time to event analysis in the target population

CHIKV:

The low number of seroconversions observed in the sentinel population (n = 9) and the low number of observations collected from suspected samples during the chikungunya outbreak (n = 30) made any inference from separate models for asymptomatic and suspected cases challenging, therefore, time to event of chikungunya infection was analysed for the overall population of sentinels and suspected cases. Since CHIKV was circulating several months before the arrival of the first cohort and therefore all the sentinels and suspected cases were exposed to infection, the initial time (t_0) was established as the beginning of exposure for each individual and events were defined as an anti-CHIKV IgM or IgG ELISA index greater than 0.8 or a RT-PCR Ct value < 40, which resulted in 46 events within 650 observations from 181 individuals. Initial models showed violation of the proportionality hazard assumptions which was addressed by stratification by socio-economic status and by using an extended Cox model adding an interaction with seasonality to accommodate the time varying covariates (Therneau and Grambsch, 2000). Influential observations (sentinels 1001 and 1048) were eliminated and produced a better fitted model with a slightly higher C concordance index (0.783) and R^2 statistic (0.088 over a max possible of 0.438) but also variations in the coefficients were observed suggesting that the estimates may not be very robust.

The model with the lowest AIC indicated that the factors with the greatest effect on the risk of infection were temperature and seasonality (Table 5.14). Averaged maximum temperature was highly significant and decreased considerably the risk of infection (HR = $2.5x10^{-3}$, 95% C.I.: $2.2x10^{-4}$-0.028, p < 0.001) alongside excessive rainfall (HR = 0.39, 95% C.I.: 0.26-0.60, p < 0.001). In contrast, seasonality associated with the rainy season increased the risk of infection (HR = 33.17, 95% C.I.: 4.61-238.9, p < 0.001) followed by the interaction of rainfall and seasonality (HR = 1.90, 95% C.I.: 1.34-2.69, p < 0.001), mosquito bites indoors (HR = 1.37, 95% C.I.: 1.11-1.69, p < 0.001), and mosquito abundance (HR = 1.20, 95% C.I.: 1.08-1.33, p < 0.001). Survival curves comparing the probability of survival at the lowest, mean and highest observed values are detailed in Figure 5.10 and the effect plots are shown in Figure 5.11.

Table 5.14. Summary of Cox PH model outcome for CHIKV transmission. N = 648, number of events = 46. Concordance statistic = 0.783 (SE = 0.072). R^2 = 0.088 (max possible = 0.438); LRT = 59.55 on 6 d.f. p < 0.001; Wald test = 63.4 (p < 0.001)

	HR	lower 0.95	upper 0.95	coef.	robust se	z	Pr(>|z|)
Seasonality	33.17	4.61	238.9	3.501	1.0073	3.47	0.0005***
Rainfall:seasonality	1.90	1.34	2.69	0.642	0.1778	3.61	0.0003***
Mosquito bites in house	1.37	1.11	1.69	0.316	0.1064	2.97	0.003 **
FADI	1.20	1.08	1.33	0.180	0.0526	3.42	0.0006 ***
Rainfall	0.39	0.26	0.60	-0.935	0.2142	-4.36	$1.3x10^{-5}$ ***
Max. Temp.	$2.5x10^{-3}$	$2.2x10^{-4}$	0.028	-5.975	1.2298	-4.86	$1.2x10^{-6}$ ***

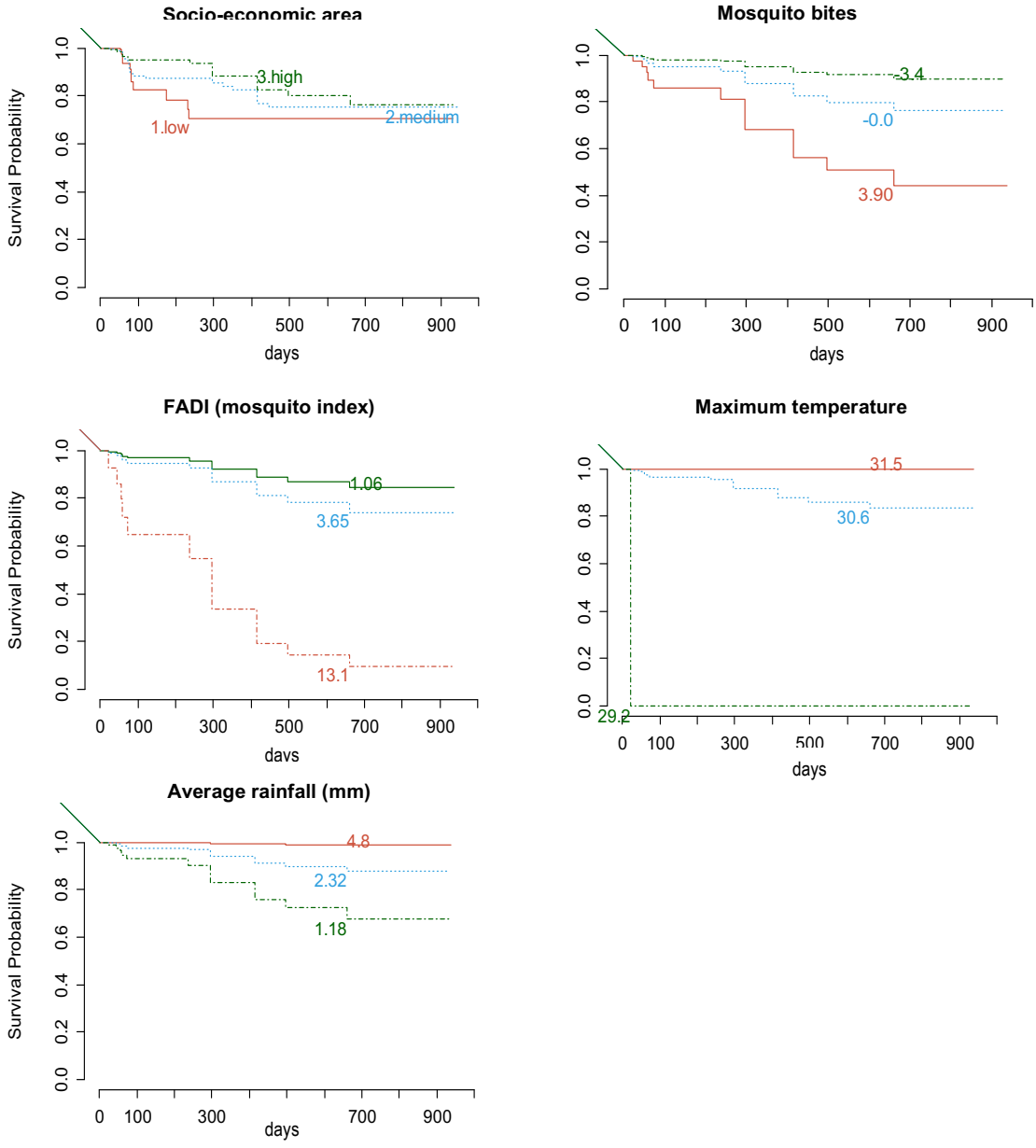


Figure 5.10. Comparison of Survival Probability curves in days for maximum (red), mean (blue) and minimum variable values (green).

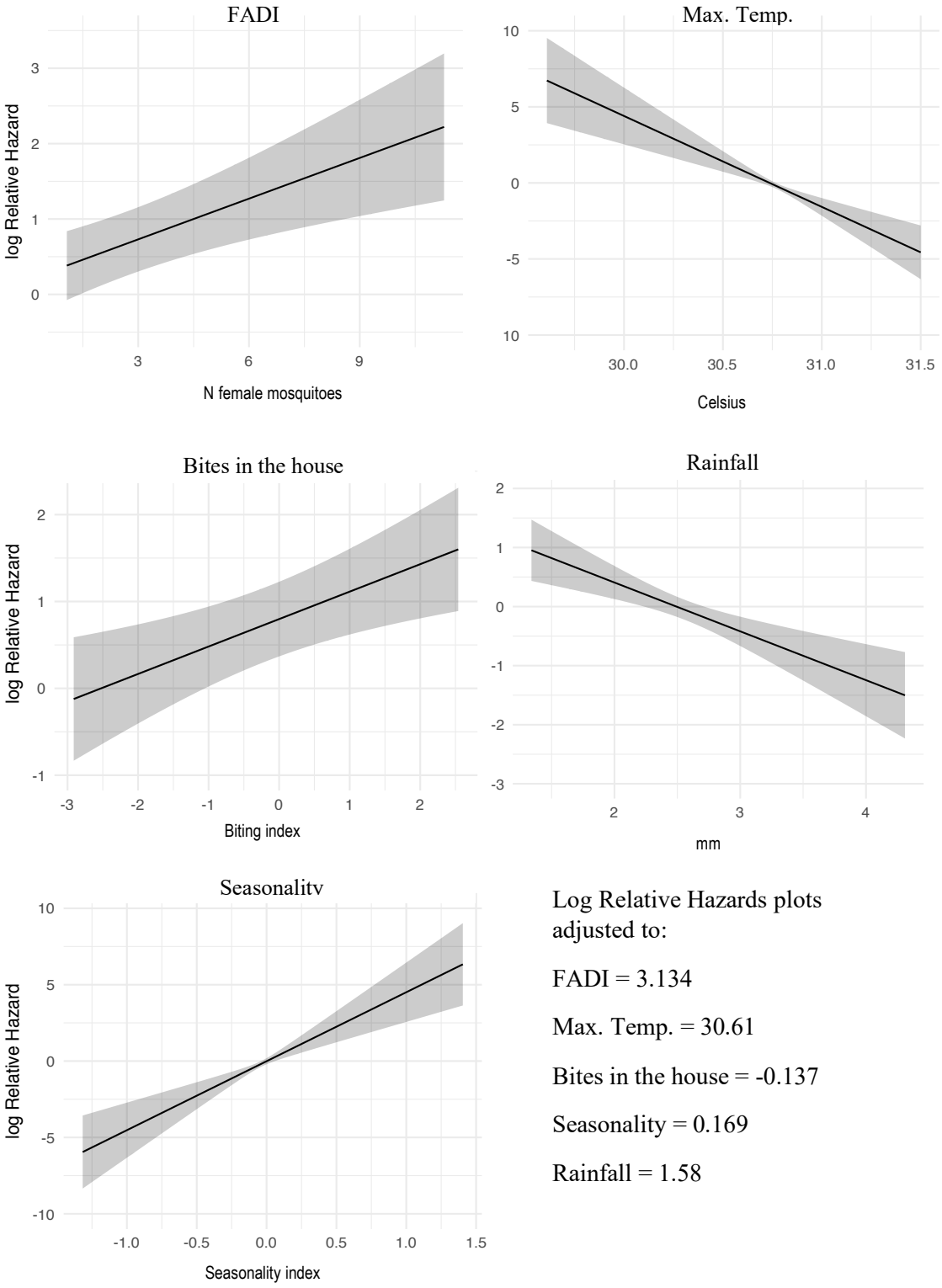


Figure 5.11. Log Relative hazard for variables for CHIKV with 95% C.I. (shaded areas). Index threshold for disease positivity = 0.8

The estimated C-statistics from the data indicated a good predictive probability of concordance between predicted and observed responses equivalent to an AUC = 0.783 (and close to the threshold of 0.8 of a strong model) and the LR = 59.5 on 6 df ($p < 0.001$) suggested a rough optimistic estimate of 0.101. Despite the slightly high

number of 6 degrees of freedom for 46 events (Babyak, 2004), internal validation of the model for slope shrinkage by bootstrapping with 200 re-samples to penalize for possible overfitting (Harrell, Lee and Mark, 1996) indicated a minor slope shrinkage of 0.9069 which is closely similar to the initial estimation and an optimistic R^2 index of 0.0383 (Table 5.15). The Cox-Snell residuals suggested a satisfactory goodness of fit.

Table 5.15. Validation model CHIKV 0.8 (Bootstrapping = 200)

	Index orig.	training	test	optimism	Index corrected	n
Dxy	0.0000	0.0026	0.0029	-0.0003	0.0003	200
R2	0.2006	0.2229	0.1847	0.0383	0.1623	200
Slope	1.0000	1.0000	0.9069	0.0931	0.9069	200

A similar model defining an event by anti-CHIKV IgM or IgG ELISA index greater than the stringent value of 1.1 or a Ct value on the RT-PCR < 40, resulted in 31 events and produced analogous coefficients, however, the degrees of freedom (6) were considered excessively high given the number of events (Harrell, Lee and Mark, 1996) and the model was discarded.

The power of the models to detect statistically significant differences in the hazard ratio of household environments and human behaviours, was rather low and below 0.70 in both univariate and full models (Table 5.16) suggesting that the probability of a Type II error was a concern and would require a substantially bigger sample size to detect difference in the hazard ratios of environmental and behavioural parameters in the study population that previously estimated.

Table 5.16. Power analysis Cox model CHIKV 0.8. HR observed, power in univariate model, power in full model, sample size required to detect observed HR, variance. No. observations: 650; no. individuals = 181. The contribution of variables to each PC are detailed in Appendix 30.

Covariates	HR	Power univar.	Power full model	σ^2	Sample size (n)
Max. Temp.	10^{-3}	1.0	1.0	0.487	29
Seasonality	33.12	1.0	1.0	1.041	39
Rainfall (mm)	0.39	0.88	0.85	0.987	561
FADI	1.2	0.34	0.32	6.501	2,322
PC1_bitten	1.38	0.28	0.26	1.62	2,948
PC1_envir2	0.71	0.25	0.24	1.279	3,312

(continued)

Covariates	HR	Power univar.	Power full model	σ^2	Sample size (n)
PC1_envir1	1.23	0.15	0.14	1.685	6,581
PC2_behav_pr	0.79	0.12	0.11	0.985	$9.38x10^3$
PC2_behav_out	0.84	0.08	0.08	1.02	$1.67x10^4$
PC1_behav_pr	0.88	0.07	0.07	1.336	$2.19x10^4$
PC1_behav_out	0.89	0.07	0.07	1.416	$2.40x10^4$
PC2_envir1	1.07	0.05	0.04	1.236	$8.08x10^4$
PC2_behav_in	0.96	0.03	0.03	1.053	$2.90x10^5$
PC1_behav_in	0.97	0.04	0.03	1.98	$2.77x10^5$
PC2_envir2	0.96	0.03	0.03	1.044	$2.93x10^5$
PC2_bitten	0.99	0.03	0.03	1.274	$3.84x10^6$

ZIKV

Whereas the first case of ZIKV was discovered in May 2016, very few observations occurred in the following weeks and the majority of the cases were collected after September 2016 which may explain the few events in the earlier weeks and a substantial drop in the survival probability after 120 days (Figure 5.12). A model for the samples collected from suspected cases during the Zika outbreak from 1st May 2016 (t_0) to December 2016 received from RUSVM health services was fitted as a Cox PH defining an event when evidence of viral genome was found by RT-PCR (Ct < 45 and 75.5°C < T_m <76°C) and assuming that the observations were right censored with events happening at the time of sampling, and censoring was non-informative and independent resulted in 17 events from 46 samples. The most parsimonious model with minimal risk of overfitting indicated that maximum temperatures had the greatest negative effect in viral infection (HR = 0.01, 95% C.I.: 0.0-0.39, p < 0.05) followed by socio-economic status had the greatest negative effect (HR = 0.26, 95% C.I.: 0.08-0.81, p < 0.05) with higher status being 26% less likely to be infected with ZIKV that lower status (Table 5.17).

Table 5.17. Summary of Cox PH model outcome for ZIKV. N = 46, number of events = 17. Concordance statistic = 0.792 (SE = 0.086). R^2 = 0.222 (max possible = 0.863); LRT = 11.54 on 2 d.f. p < 0.005; Wald test = 9.87 (p < 0.01); Score (logrank) test = 19.93 on 2 df, (p < 0.001).

	HR	Lower 0.95	Upper 0.95	coef	se(coef)	z	Pr(>|z|)
Max. Temp.	0.012	$3.5x10^{-4}$	0.389	-4.44	1.785	-2.489	0.0128 *
Socio-econ. (high)	0.262	0.084	0.811	-1.34	0.577	-2.323	0.0202 *

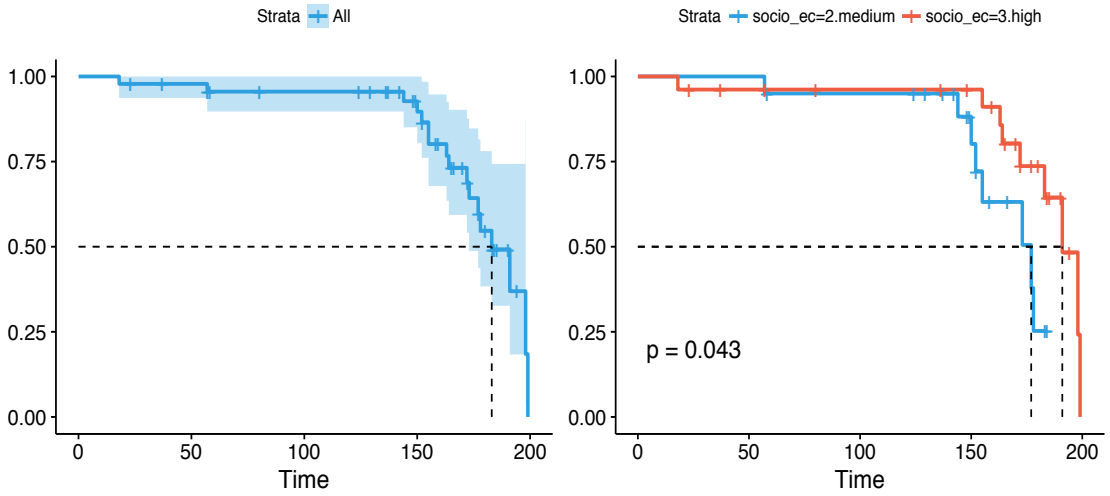


Figure 5.12. Baseline survival curve of ZIKV null model (left). Survival curve of ZIKV by socio-economic status (right). Time in days since estimated first infection

Schoenfeld residuals tests and examination of the residuals graph suggested that PH assumptions were met (global test p = 0.98). It was noted though that statistical testing suggested that the covariate temperature could be significant, but visual examination of residuals plot rejected this scenario and attributed the significance to the low number of samples and the long gaps between the earlier samples and later and the model was assumed to meet the proportional hazard assumption. One influential observation was found (suspected case no. 1065) and removed from the model, which changed substantially the coefficient for the maximum temperature (Table 5.18), suggesting that the estimation of this coefficient might not be very robust. The martingale residual plots showed linearity of the continuous coefficients overall and Cox-Snell residuals indicated an acceptable goodness of fit (Figure 5.13).

Table 5.18. Summary of Cox PH model outcome for ZIKV after removing influential observation. N = 45, number of events = 16. Concordance statistic = 0.777 (SE = 0.089). R2 = 0.227 (max possible = 0.845); LRT = 14.59 on 2 d.f. p < 0.001; Wald test = 7.99 (p < 0.05);

	HR	Lower 0.95	Upper 0.95	coef	se(coef)	z	Pr(>|z|)
Max. Temp.	$4.19x10^{-07}$	$4.7\ x10^{-13}$	0.373	-0.15	6.990	-2.101	0.03565*
Socio-econ. (high)	0.169	0.045	0.638	-1.78	0.677	-2.625	0.00867**

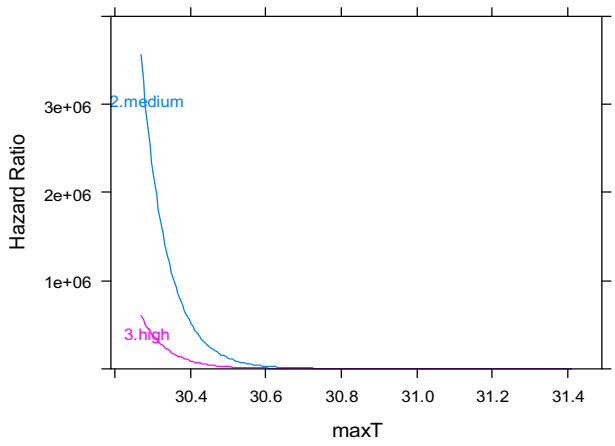


Figure 5.13. Hazard ratio for ZIKV model covariates: Red line: HR for high socio-economic status, Blue line: HR for medium socio-economic status

The C-statistics estimated from the data suggested a good predictive probability of concordance between predicted and observed responses equivalent to an AUC = 0.777 (close to the threshold of 0.8 of a strong model) and the LR = 14.6 on 2 df ($p < 0.001$) indicating a rough optimistic estimate of 0.137. Validation of the model Somer's D_{xy} for rank correlation between predicted log hazard and observed survival time, and for slope shrinkage by bootstrapping with 200 re-samples to penalize for possible overfitting (Harrell, Lee and Mark, 1996) showed a corrected C-statistics of 0.758, an optimistic index of 0.037 in the predictive ability of the model and a slope shrinkage of 0.866 which is closely similar to the initial estimation of 0.134 optimism (Table 5.19). Similar to the chikungunya scenario, the power of the model to detect significant effect of human related covariates was very low exposing the shortfalls from the number of samples collected (Table 5.20).

Table 5.19. Model validation ZIKV (Bootstraping =200)

	Index data	training	test	optimism	Index corrected	n
D_{xy}	0.5544	0.5841	0.5467	0.0374	0.5170	200
R2	0.3278	0.3563	0.3014	0.0549	0.2729	200
Slope	1.0000	1.0000	0.8659	0.1341	0.8659	200

Table 5.20. Power analysis. ZIKV Cox PH model. HR observed, power in univariate model, power in full model, sample size required to detect observed HR, variance. No. individuals = 46. The contribution of variables to each PC are detailed in Appendix 30.

Covariates	HR	Power	σ^2	Sample size (n)
Rainfall (mm)	0.13	1.00	0.554	10
Max. Temp.	0.019	1.00	0.113	13
Seasonality	0.273	0.94	0.445	30
Socio-economic	0.346	0.59	0.251	76
PC2_behav_in	0.688	0.37	1.118	139
PC1_envir2	0.819	0.28	2.823	190
PC1_behav_pr	0.834	0.22	2.518	258
PC1_envir1	1.179	0.21	2.973	266
PC1_behav_out	0.868	0.15	2.397	443
PC2_envir2	1.145	0.10	1.463	857
PC2_envir1	0.859	0.09	1.076	866
PC1_bitten	0.907	0.08	1.766	$1.27x10^3$
PC2_behav_out	0.931	0.05	1.40	$2.94x10^3$
PC1_behav_in	1.031	0.04	1.959	$1.14x10^4$
PC2_behav_pr	0.97	0.03	1.084	$2.20x10^4$
PC2_bitten	1.011	0.03	1.788	$9.86x10^4$

Finally, the outcome coefficients of the different models described previously in this chapter are summarised in Table 5.21.

Table 5.21. Summary of models outcomes. (1) CHIKV only; (2): CHIKV & ZIKV; (M) anti-CHIKV IgM; (G) anti-CHIKV IgG; NA: not analysed.

	Univariate (suspected cases)		**Binomial GLM (suspected cases)**		**GEE (sentinels)**		**Cox PH (all)**	
	Coef.	**p**	**Coef.**	**p**	**Coef.**	**p**	**Coef.**	**p**
Climatic:								
Temperature	-	-	$2.27^{(2)}$	<0.01	$15.5^{(M)}$	<0.001		
Max. Temp.	$0.53^{(1)}$	0.06	-	-	$-16.9^{(M)}$	<0.001	-5.97	<0.001
Rainfall < 7 d.	$-0.17^{(2)}$	<0.05	$-0.21^{(2)}$	<0.005	NA	NA	-	-
Rainfall 7-30 d.	$-3.58^{(1)}$	<0.01	-	-	NA	NA	-	-
Rainfall > 30 d.	-	-	-	-	$21.0^{(G)}$	<0.005	-0.94	<0.001
Rain + temp	-	-	-	-	-2.78e-2	<0.005	0.64	<0.001
Seasonality	-	-	-	-	-	-	3.50	<0.001
Relative Humidity	$0.11^{(2)}$	<0.05	-	-	-	-	-	-
Wind speed	$-0.19^{(2)}$	<0.05	$-0.27^{(2)}$	<0.01	-	-	-	-

(continued)

	Univariate (suspected cases)		Binomial GLM (suspected cases)		GEE (sentinels)		Cox PH (all)	
	Coef.	**p**	**Coef.**	**p**	**Coef.**	**p**	**Coef.**	**p**
Mosquito:								
FADI	0.22[(1)] 0.17[(2)]	<0.05 <0.05	-	-	1.15e-2	<0.05	- -	- -
Individual:								
Gender	-	-	-	-	-	-	-	-
Age	-	-	-	-	-	-	-	-
Length residence	-2e-3[(2)]	<0.05	-	-	-5.5e-4	<0.05	-	-
Origin (SKN)	2.35[(1)] 1.43[(2)]	<0.01 <0.001	5.45[(2)]	<0.005	-	-	-	-
Environment I: Household								
No. rooms			-	-	-0.051[(M)]	<0.001	-	-
No. rooms AC	0.64[(1)]	<0.05	-	-			-	-
No. occupants			-	-			-	-
Socio ec. (high)	-1.79[(1)]	0.08	-	-			-1.77	<0.01
Screens	-	-	-	-	-	-	-	-
Tap water	-	-	-	-	-	-	-	-
Materials	-	-	-	-	-	-	-	-
Environment II: larval habitats								
Pools	-	-	-	-	-	-	-	-
Trash area	-	-	-	-	-	-	-	-
Plant pots	-	-	-	-	-	-	-	-
Trash collection	-	-	-	-	-	-	-	-
Storage water	-	-	-	-	-	-	-	-
Behaviours indoors								
Time porch	-	-	-	-	-	-	-	-
Door open Rare use AC	0.63[(1)]	<0.05	2.74[(1)]	<0.05	-	-	-	-
Behaviours outdoors								
Freq. bar / beach	-0.30[(2)]	<0.05	-	-	0.028	<0.05	-	-
Hiking	-	-	-	-	-	-	-	-
Church	-	-	-	-	-	-	-	-
Behaviours protection								
Spray	-	-	-	-	-	-	-	-
Coils	-	-	-	-	-	-	-	-
Repellent	-	-	-	-	-	-	-	-

(continued)

	Univariate (suspected cases)		Binomial GLM (suspected cases)		GEE (sentinels)		Cox PH (all)	
	Coef.	**p**	**Coef.**	**p**	**Coef.**	**p**	**Coef.**	**p**
Mosquito bites								
Out-house	-	-	-	-	-	-	0.32	<0.005
In-house (often)	2.56[(1)]	<0.01	8.42[(1)]	<0.05	5.89e-2[(G)]	0.09		
at bar	0.33[(2)]	<0.05	2.46	<0.01	-	-	-	-
at beach					-	-	-	-
at hiking	-	-	-	-	-	-	-	-
at shop	-	-	-	-	-	-	-	-
at church	-	-	-	-	9.38e-2[(G)]	0.09	-	-

5.4. Discussion

The findings have illustrated the difficulty of determining the factors associated with arboviral infection, the diagnosis of subclinical Flavivirus, and the heterogeneity of arboviral transmission in the Caribbean. The conditions on St. Kitts are favourable for the transmission of dengue in that there is abundant mosquito vector (Belkin and Heinemann, 1976; Smith *et al.,* 2011; Mohammed *et al.,* 2015), a constant influx of susceptible population (Focks and Chadee, 1997) and sufficient contact between humans and mosquitoes (Wearing, Robert and Christofferson, 2016). Also, there is evidence of past dengue infections locally as all the samples (100%) from suspected cases born in St. Kitts showed high titres of anti-DENV IgG antibodies similar to earlier reports from the island (Mohammed *et al.,* 2012; Wood *et al.,* 2014), and other regional reports that have indicated 100% prevalence in Jamaica (Brown *et al*., 2009), 98% in Dominican Republic (Yamashiro *et al.*, 2004), 90.7% in Martinique and 96.2% in Guadeloupe (L'Azou *et al.*, 2015), 94.4% in Trinidad (Campbell *et al*., 2007), and 83.3% prevalence in St. Eustatius (Leslie *et al*., 2014). However, the Indirect IgG Panbio ELISA failed to identify seroconversions, defined as a four-fold rise in IgG antibodies between two sequential samples (Gubler, 1998; Endy *et al*., 2011), on the sentinels while residing on St. Kitts. The six sentinels with elevated anti-IgG titres were detected on their first sampling and came from areas where WNV and SLEV are prevalent (Mackenzie, Gubler and Petersen, 2004), had been vaccinated against YFV or were detected at the same time that ZIKV was circulating on St. Kitts, suggesting that infection occurred before coming to the island and that the cause of infection was inconclusive. In contrast, the in-house protocol developed by Miagostovich *et al.* (1999) showed a substantially higher number of positive samples amongst sentinels. Moreover, a small number of sentinels (1.8%) exhibited evidence of the short-term anti-DENV IgM at the time when WNV was discovered in

St. Kitts equine population (Bolfa *et al.*, 2017). These discrepancies may be explained by differences in performance between assays and that ELISA is often rather unspecific (Sang, Cuzzubbo and Devine, 1998; Vaughn *et al.*, 1999; Vazquez *et al.*, 2007; Hunsperger, 2012). In addition to the well-known cross-reactivity between flavivirus (Halstead, 1997, 2004; Gubler, 1998; Kuno, 2003), the predictive values of laboratory tests are also affected by the prevalence of disease in a population (Dohoo, Martin and Stryhn, 2012) and without the possibility of performing discriminatory PRNT tests (Gubler, 1998; Kuno, 2003), the prevalence of dengue in a population can be overestimated (Radke *et al.*, 2012). Nonetheless, simulation techniques indicated that the observed number of cases was significantly lower ($p < 0.05$) than the number of cases that were expected assuming active circulation of the virus (Mohammed *et al.*, 2012) and supporting proof of acute dengue infections by highly specific molecular methods remained elusive as no DENV genome was detected in any of the samples examined, suggesting that endemic circulation of DENV during the study period was low key or non-existent.

Further evidence of acute arboviral transmission in St. Kitts was found for CHIKV and ZIKV. A total of 33 samples presented signs of chikungunya infection, 20 of these corresponded to suspected cases detected between September 2014 and January 2015 resulting in an annual estimated incidence of 49.0%, nearly twice as high as the incidence of dengue reported in immunologically naïve children in Maracay, Venezuela (Comach *et al.*, 2009) and in Puerto Rico (Dayan *et al.*, 2015) and substantially higher than incidence rates of 6.3-7.3% reported in Thailand (Endy, Yoon and Mammen, 2010), and 5.05 per 1,000 population reported during the dengue outbreak in 2002 in Trinidad (Campbell *et al.*, 2007). The majority of the positive suspected cases (65%) never or rarely used the AC, 70% were bitten by mosquitoes often and 70.0% were born in SKN. Nine sentinels were detected during the routine sampling with evidence of chikungunya infection before May 2015 and at the last sampling call in September 2016. The estimated posterior median prevalence amongst sentinels was 12.8% (95% PI: 8.2-18.4%), similar to reports of 13.1% in Nicaragua (Kuan *et al.,* 2016), although lower than ~25% reported from Puerto Rico (Simmons *et al.*, 2016) and substantially lower than 69.6% prevalence detected in the suspected cases born in St. Kitts (95% C.I.: 47.1-86.8%), which was similar to earlier surveys indicating 69% prevalence in autochthonous population in Comoros (Sergon *et al.*, 2007) or 76% in the French Polynesia (Aubry *et al.*, 2017). The ratio of symptomatic to inapparent chikungunya was difficult to establish as

some of the sentinels reported unspecific signs within the past sampling period but linking symptoms with actual infections was unobtainable in most cases. Some sentinels were infected after only 12 days of arrival to the island and two other sentinels showed high levels of the long-term IgG antibody in September 2016 after a long monitoring gap. The time of infection for the latter cases is uncertain but due to the lack of chikungunya cases reported by the health authorities after January 2015 (R. Rosales 2016, personal communication), the high titres observed in the late samplings could be caused by an earlier infection undetected due to low titres, unspecific binding caused by a different antigen (Prat *et al*., 2014) such as in one sentinel who was vaccinated against YFV before a positive sampling or by the hypothetical presence of Mayaro virus (MAYV), a related Alphavirus which is known to have circulated in neighbouring Haiti (Lednicky *et al.*, 2016). Likewise, ZIKV genome was detected in 18 samples between May and December 2016, resulting in an estimated annual incidence of 36.6% and an apparent prevalence of 39.1%, lower than 49% previously reported in the French Polynesia (Aubry *et al.,* 2017) and 73% estimated in Yap, Micronesia (Duffy *et al*., 2009). Although the high ratio of asymptomatic infections reported in the literature (Kucharski *et al.*, 2016), suggests that the actual number of infections may be underestimated. Nonetheless, new cases of chikungunya and of Zika have remained undetected after the onset of the dry season after each outbreak (World Health Organization, 2017f)

Analysis of the data demonstrated distinct seasonality of infections in St. Kitts associated with rainfall and temperature were the latter was stronger, in addition to human socio-economic factors, mosquito density and mosquito biting indoors, in agreement with previous reports (Kuno, 1995; Vorndam and Kuno, 1997; Reiter *et al.*, 2003; Amarakoon *et al.*, 2008; David, Lourenço-de-Oliveira and de Freitas, 2009; Teurlai *et al.*, 2015). The effect of temperature and rainfall observed was consistent across several univariate and multivariable models and has also been widely described in the literature (Gubler *et al.*, 2001; Patz and Reisen, 2001; Reiter, 2001; Wu *et al.*, 2007; Wilder-Smith and Gubler, 2008; Hii *et al.*, 2009; Earnest, Tan and Wilder-Smith, 2012). Previous models of combined climatic variables and socio-economic factors built using dengue data from Mexico also concluded that temperature and humidity had the greatest effect in dengue incidence over the rest of the variables (Bouzid *et al.*, 2014), although other authors have reported annual cumulative precipitation as the greatest influence of ZIKV transmission (Messina *et al.*, 2016). Such a clear seasonal pattern of dengue fever transmission coinciding

with the rainy season has also been observed in Trinidad (Chadee, Williams and Kitron, 2005; Chadee *et al.*, 2007) and in Barbados (Kumar, Gittens-St Hilaire and Nielsen, 2013), although other authors have reported a small effect of seasonality (Lana *et al*., 2014), a lack of correlation between rainfall and severe dengue incidence (Chadee *et al.*, 2007) or, conversely, a peak of infections during the dry season which was linked to additional water storage (Porter *et al.*, 2005). In contrast, heavy rain and high wind around the estimated time of infection were associated with lower risk of infection in the suspected cases due to the challenging conditions for the infective mosquito vector as demonstrated in the literature (Rudolph *et al,* 2014). The dual effect of rainfall observed in the study, first by reducing transmission between 2-4 weeks, then increasing it around five weeks later is consistent with previous reports (Hii *et al.*, 2009; Riou, Poletto and Boëlle, 2017). Furthermore, time to event models in the sentinels indicated that temperatures between 26^{o}C and 29^{o}C were associated with higher risk of arboviral infections, in agreement with some studies (Carrington *et al.*, 2013; K. M. Campbell *et al.*, 2015; Mordecai *et al.*, 2017), but contrary to others that described a minimal effect in virus transmission of temperature variations between 22^{o}C and 29^{o}C (Riou, Poletto and Boëlle, 2017) or that temperature and relative humidity had a negative effect in dengue risk (Wu *et al.*, 2007). Temperatures above 31^{o}C had a strong negative effect in the transmission of arboviruses in St. Kitts which was consistent with the optimal temperature for mosquito survival and shorter duration of the gonotrophic cycle and extrinsic incubation period reported elsewhere (Fouque *et al.*, 2006; Carrington *et al.*, 2013; Johansson *et al.*, 2014; Goindin *et al.*, 2015). The collection of most sentinel samples occurred during the peak of the ENSO phenomenon in 2015 which has been indicated to have a negative effect on the number of dengue cases reported (Zambrano *et al.*, 2012) whereas, others linked higher sea temperatures with a greater dengue incidence 18 weeks later (Brunkard, Cifuentes and Rothenberg, 2008).

The human factor most consistently associated with arbovirus infection was birth in St. Kitts. Although rather than ethnical factors (Sierra, Kourí and Guzmán, 2007) the difference in risk observed was most likely due to the correlation discussed in section 2.3.5 between Kittitians and socio-economic risk factors as greater house occupants, leaving doors open, rare use of AC, and residence in deprived areas that have been associated with dengue infections in the wider literature (Gubler, 1998a; Reiter *et al.*, 2003; Cummings *et al.*, 2009; Brady *et al.*, 2014). Earlier studies have also indicated

higher rates of dengue prevalence associated with birth or long-term residence in a Caribbean island as opposed to birth in continental Europe or US and short-term residence in a Caribbean island (Mohammed *et al.*, 2012; Leslie *et al.*, 2014; L'Azou *et al.*, 2015) and with behavioural traits linked to risk denial in the form of familiarity with the disease in endemic areas such as the French Antilles (Mieulet and Claeys, 2014), lack of education (Siqueira-Junior *et al.*, 2008; Salyer *et al.*, 2014) or different health care seeking behaviour (Schiøler and Macpherson, 2009). In contrast, other authors have reported that adult age was the only significant factor for dengue prevalence and indicated little difference between genders and residential areas (Yamashiro *et al*., 2004). Length of residence has also been indicated as a risk factors in US travellers born or lived in endemic areas, although relationship with the number of years lived was only found for individuals born in endemic countries with the number of cases increasing every 5 years of residence (Sanchez-Vegas *et al.*, 2013).

Environmental variables observed in the study population showed inconsistent association with arbovirus transmission in St. Kitts. Whereas high numbers of occupants in suspected cases households was associated with an increased risk of chikungunya infection, in agreement with previous dengue studies (Gubler, 1997, 1998a; Cummings *et al.*, 2009; Kuno, 2009), the data suggested that high numbers of occupants in sentinels households was associated with lower risk of chikungunya infection. This contradictory effect may be explained as infections occurred at the beginning of the study and sentinels tended to move into smaller accommodations in affluent areas with longer time of residences as discussed in section 2.3.3. Other environmental factors such as housing materials or screened windows and doors indicated no significant associations with arboviral infection as in earlier reports (Gubler, 1997; Bloch *et al.*, 2016). Additionally, high female mosquito density was associated with increased risk of infection during outbreaks, as seen by (Padmanabha *et al.*, 2012; Ratanawong *et al.*, 2016), although other reports found inconclusive association of dengue infections with mosquito abundance (Focks, 2003; Wu *et al.*, 2007; Bowman, Runge-Ranzinger and McCall, 2014). Surprisingly, the presence of larval habitats such as debris and containers around the house, pots, or storage of water showed non-significant effect in the risk of infection to arboviruses, contrary to most reports (Ashford *et al.*, 2003; Porter *et al.*, 2005; Vanwambeke *et al.*, 2006; Åström *et al.*, 2012; Brady *et al.*, 2014).

Likewise, behaviours of the study population had little impact overall on the risk of infection. Although some authors have indicated that human activity, rather than weather, is the principal determinant of dengue prevalence and it is inappropriate to use climate based models only for viral predictions (Reiter, 2001), the models developed in this study showed inconsistent or non-significant association between human behaviours and arbovirus infections in St. Kitts. For instance, leaving doors or windows open regularly and scarce use of AC showed an increased risk of infection in suspected cases only, in agreement with previous findings (Reiter *et al.*, 2003; Radke *et al.*, 2012), and protective measures against mosquitoes such as use of coils and repellents showed no effect in the study population, in contrast with other reports (Ramos *et al*., 2008; Bloch *et al*., 2016;). Also, several of the models in the study indicated conflicting results regarding the risk of outdoor activities. Whereas sentinel models showed a small association between arboviral infection and outdoor activities, as observed by Vanwambeke *et al*. (2006), univariate models in suspected cases showed a lower risk of arboviral transmission for which the reasons are unclear.

Some challenges were encountered in the data analysis. The power of the time to event models was modest (Hoenig and Heisey, 2001), with very large standard errors rendering substantial confidence intervals which can be attributed to the small number of samples collected from suspected cases, the low number of events observed and the small hazard differences observed amongst sentinels' environments and behaviours as discussed in section 2.4. Also, some of the differences observed in transmission risk between viruses, suspected cases and sentinels can also be attributed to differences in the collection of samples from suspected cases which was heavily influenced by the health officer on duty, a concern previously highlighted in the region related to working ethic and weak motivation of staff that influences disease surveillance, under-reporting and disease control (Chadee, 2004, 2013; Suaya *et al.*, 2010).

Contrary to previous indications (Mohammed *et al.*, 2012), the analysis of the decreasing level of antibodies in the sentinel population and epidemiological data suggested that after short-lived and season-dependent epidemics, arboviruses may have failed to establish an endemic cycle in St. Kitts most likely due to the effect of herd immunity (Anderson and May, 1986; Kuno, 1995; Chadee, Doon and Severson, 2007) as humans are the most significant vertebrate hosts in urban settings and life-

long immunity follows infection (Petersen and Powers, 2016). The level of herd immunity required to stop outbreaks is unknown and varies with local transmission dynamics, such as human population size and mosquito abundance, but past outbreaks ceased or were greatly reduced after one transmission activity in small, isolated populations such as some Pacific and Caribbean Islands or other isolated areas with limited human populations of less than 200,000 (Schiøler and Macpherson, 2009; Weaver and Forrester, 2015; Petersen and Powers, 2016). Some authors have claimed a minimum level of immunized population of 250 to 500 thousand people to stop the epidemics of measles in populated islands (Anderson and May, 1986) or a proportion upwards of 50%-70% after which transmission ceased (Kuno, 1995; Sergon *et al*., 2007). Examples include Zika outbreaks in French Polynesia, where 94% population was infected although only 11.5% infections were reported, and the outbreak ended when the number of susceptible humans decreased (Kucharski *et al*., 2016). Furthermore, while there is an influx of naïve susceptible foreign residents arriving to St. Kitts consisting of around 6% of the island population, this incoming population is self-replacing, remains constant and there is little contact with high risk communities (Stoddard *et al*., 2013). The periodicity of dengue outbreaks in the region (San Martín *et al*., 2010) may be explained better by birth rate and the subsequent increase in the susceptible population from immunologically naïve children, and that re-emergence on St. Kitts is not an isolated event but is highly dependent on the introduction of genetically different virus from other areas (Chadee *et al.*, 2007; Cao-Lormeau *et al.*, 2014; Cauchemez *et al.*, 2014; Khan *et al.*, 2014) which appears to be predictable based on flight information, distance between countries, and climatic suitability (Tatem *et al.*, 2012).

5.5. Concluding remarks

Although there has been proof of epidemic arbovirus transmission on St. Kitts, there is little evidence to support the hypothesis of an endemic circulation cycle. Evidence suggest that viruses cause rapid outbreaks and die out later when climatic conditions become unfavourable for mosquito transmission or herd immunity reaches a critical threshold.

Chapter 6. Final discussion.

6.1. Introduction: summary of arbovirus transmission on St. Kitts

The emergence of mosquito-borne virus in a naïve population is a complex process that requires the presence of a suitable mosquito vector, the introduction of the virus and conditions favourable to the transmission of the virus (Wearing, Robert and Christofferson, 2016). However, persistence of a virus in the long term rests greatly on the efficiency of transmission which depends on the size of the population and the rate of replenishment of susceptible individuals (Anderson and May, 1986). The smaller islands in the Caribbean, such as St. Kitts with around 50 thousand inhabitants, offer less opportunities for an endemic cycle to be stablished, whereas the bigger islands with several hundred thousand inhabitants offer better opportunities for arboviruses to persist. The investigation of the hypothesis formulated in chapter 1 rested on the premise that dengue was endemic on St. Kitts which was based on numerous regional reports (Kumar, Gittens-St Hilaire and Nielsen, 2003; Chadee, 2007; World Health Organization, 2016; Elbrady *et al.,* 2017, PAHO / WHO, 2018b) and in earlier studies shortly after the last dengue outbreak in St. Kitts (Mohammed *et al.*, 2012). However, the evidence collected in this study demonstrates that, similar to the situation in other small islands in the Caribbean and the Pacific that have experienced recent explosive outbreaks of dengue, chikungunya or Zika (Weaver and Forrester, 2015; Leslie *et al*., 2014; Cao-Lormeau *et al*., 2014, 2016; Kucharski *el al*., 2016), these diseases have failed to establish an endemic cycle in St. Kitts.

Arbovirus transmission on St. Kitts was also associated with climatic variables connected to seasonality, mainly rainfall and temperature where the effect of the latter was stronger, as reported in other studies (Amarakoon *et al.*, 2008; Kumar, Gittens-St Hilaire and Nielsen, 2013), but also linked to socio-economic factors and different communities with distinct economic and cultural backgrounds experienced different levels of risk. Also, dengue transmission in a population is shaped by social connections and close interaction within infected individuals' households play a role in the spread of this vector-borne pathogen at fine spatial scales and are associated with increased odds of infection (Stoddard *et al*., 2013, Bloch *et al*., 2016). RUSVM, a for-profit institution with base in the US, is the largest university present in St. Kitts and is well known to its residents for being one of the major contributors to the local economy, however, social interactions between these communities are infrequent.

The areas of residence for university students and faculty are limited to certain neighbourhoods where RUSVM security staff patrol regularly due to safety concerns. Some of these areas closer to the university campus or in Basseterre town are also populated by local residents and the level of interaction is greater than in other wealthier neighbourhoods where the majority of residents are foreign nationals or tourists with little or no contact with the local population. The recreational areas (bars, restaurants, and beaches) frequented by university students and faculty are mostly in the south west of the island and along the peninsula where few locals visit. The other places where there is some interaction between communities are the grocery stores and church. Furthermore, the level of affluence of the students as a whole are better than the local population which may affect their living conditions and behaviours, such as the use of air-conditioning, and impact the risk of transmission of arbovirus, especially since electricity in St. Kitts is costly. Thus, a lack of social interactions between these communities and the difference in affluence may have predisposed them to different levels of prevalence and risks of transmission. Varying arboviral prevalence in foreign communities residing in endemic countries has previously been observed in studies investigating communities with varying levels of affluence such as in border towns between Mexico and the US (Reiter *et al*., 2003), foreign residents in endemic areas (Sanchez-Vegas *et al*., 2013), US military personnel (Sharp *et al.,* 1995; Hesse *et al*., 2017), and NGO workers (O'Leary *et al.*, 2002; Salyer *et al.*, 2014), which resulted generally in lower levels of infection in the visiting population than those reported in the local population (Balmaseda *et al.*, 2006, 2010; Dayan *et al.*, 2015; L'Azou *et al.*, 2015). Some reasons previously formulated to explain the lower risk of infections by visitors include higher awareness of arboviral diseases (Salyer *et al.*, 2014; L'Azou *et al.*, 2015) and of mechanism of protection against mosquitoes (O'Leary *et al.*, 2002),

Also, mosquito density and mosquito biting increased the risk of arbovirus transmission in the study population in St. Kitts. The abundance of the mosquito Aedes in urban areas of St. Kitts has been demonstrated to be associated with premises with debris and containers that can act as breeding sites, in accordance with numerous reports (Tun-Lin *et al*., 1996; Chadee, 2004; Gubler, 2011; Basker and Ezhil, 2012). The tidiness of the yard and ancillary areas was also correlated with the level of affluence and highly maintained gardens and yards were typically associated with lower household density, high degree of mosquito screen maintenance in up-market neighbourhoods whereas poorly maintained yards with

debris accumulating, including abandoned cars and other objects, was observed to occur more regularly in neighbourhoods reported to have high poverty levels (Pan American Health Organization, 2012). Thus, whereas individual mosquito biting is mostly influenced by personal characteristics (Bosch, Geier and Boeckh, 2000), deficient economic conditions increase the chances of contact with infective vectors.

In summary, socio-economic factors have a substantial effect in arboviroses (Gubler, 1998a; Reiter *et al.*, 2003; Cummings *et al.*, 2009; Brady *et al.*, 2014), and transmission, spread and persistence of arboviral infections in a population are determined by the interplay of multiple factors including presence and virulence characteristics of the circulating viral strain of virus, proportion of susceptible human population and climatic variables, and vector survival, abundance, behaviour and proportion of infected mosquitoes (Wearing, Robert and Christofferson, 2016).

6.2. Final conclusions

This study has aimed to answer the three formulated research hypothesis outlined in in the first chapter, namely:

I. The incidence and prevalence of arbovirus in a naïve population varies with (a) duration of residence; (b) seasonality; (c) individual behaviour; (d) exposure to the Aedes vector; (e) presence of Aedes vector;
II. The dynamics of different serotypes of DENV virus on St. Kitts is a key factor for determining outbreaks of symptomatic disease.
III. There is geospatial variation in the pattern of DENV serotype and CHIKV prevalence in the naïve population.

The samples and epidemiological data gathered from the sentinels and the suspected cases described in chapter 2 and the projection of vector abundance and exposure described in chapter 4 that were analysed by the laboratory assays detailed in chapter 3, assisted in the investigation of the inferences related to prevalence of arboviruses in humans and mosquitoes and their associations with the risk factors described in chapter 5 has led to the following conclusions:

Final conclusions: Hypothesis I

I. (a) The hypothesis that incidence and prevalence of arbovirus varies with the duration of residence cannot be rejected. Although the incidence of arboviruses on St Kitts was independent to the length of residence, the duration of residence had a dual effect on prevalence. Whereas the risk of chikungunya infection was higher at

the beginning of the study than at later stages, the risk or arboviral infection was only substantial during the duration of an outbreak and remained low or non-existent during inter-epidemic periods. However, long-term residents and individuals born in St. Kitts showed high levels of anti-dengue antibodies indicating an increased risk of residence during viral re-emergence and subsequent outbreaks, therefore, the prevalence of arboviruses varies with the duration of residence.

I. (b) The hypothesis that incidence and prevalence of arbovirus varies with seasonality cannot be rejected. Whereas the introduction of a mosquito-borne virus is strongly associated with events in neighbouring islands and can occur at any point in time, the evidence demonstrated that the peak of the outbreaks occurred during the rainy seasons and the risk of arbovirus infection was significantly lower after the onset of the dry season in which unfavourable conditions for the mosquito vector developed and the number of cases in humans disappeared.

I. (c) The hypothesis that incidence and prevalence of arbovirus varies with individual behaviour cannot be rejected. Although the risk of infection was significantly higher for behaviours associated with low socio-economic backgrounds (i.e. infrequent use of AC, occupancy), and a contradicting effect of outdoor activities was observed, the evidence collected were insufficient to demonstrate significant associations between arbovirus infection and human behaviours.

I. (d) The hypothesis that incidence and prevalence of arbovirus varies with exposure to Aedes vector cannot be rejected. Evidence collected indicated a significantly higher risk of infection in individuals that were prone to mosquito bites than in individuals that suffered no bites.

I. (e) The hypothesis that incidence and prevalence of arbovirus varies with presence of Aedes vector cannot be rejected. Although some models failed to find significant differences, other models indicated a significantly higher risk of arboviral infection in residential areas with high density of female *Ae. aegypti* than in areas with low mosquito density and greater female Aedes density was associated with greater risk of arbovirus infection in the sentinels.

Final conclusions: Hypothesis II

II. The hypothesis that the dynamics of different serotypes of DENV virus on St. Kitts is a key factor for determining outbreaks of symptomatic disease cannot be rejected. Evidence collected failed to identify any serotypes of dengue and therefore a

conclusion cannot be reached. Moreover, evidence collected from other arboviruses circulating failed to identify significant differences in the dynamics of CHIKV and ZIKV.

Final conclusions: Hypothesis III

III. The hypothesis that there is geospatial variation in the pattern of DENV serotype and CHIKV prevalence in the naïve population cannot be rejected. Evidence collected from mosquitoes and humans failed to identify significant differences in the prevalence of arboviruses based on geographical variation and arbovirus transmission were found in all the areas surveyed in the island.

6.3. Final remarks

Socio-economic factors appear frequently in the wider literature associated with the epidemiology of arboviruses, either directly related to an increased exposure to the infective mosquito vector but also indirectly by the decay in public health infrastructures due to lack of resources and critical shortages of trained specialists who understand and can develop effective prevention and control programs for vector-borne diseases (Gubler, 1998b; Horstick, Tozan and Wilder-Smith, 2015). Dengue is also regarded as a neglected tropical disease (NTD), a group of 20 diseases characterised by high morbidity and low mortality connected with poverty, inequality, lack of adequate health care, sanitation, housing, education (Bangert *et al.*, 2017) and close contact with vectors and livestock that affect more than one billion people worldwide and cost developing economies billions of dollars every year (Hotez, 2013; World Health Organization, 2015, 2017e). Although neglected tropical diseases are increasing their presence in countries within the G20 group, Sub-Saharan Africa remains a key region affected and the global burden of the major NTDs were roughly equivalent to the disability-adjusted life-years (DALYs) lost from any of the ‘‘big three’’ conditions, i.e., HIV/ AIDS, tuberculosis, and malaria (Hotez, 2013). The multifaceted causes are being acknowledged and addressed through the Sustainable Development Goals (SDGs) framework by reducing impact on poverty and hunger, improving education, work and economic growth and thereby reducing inequalities (World Health Organization, 2015; Bangert *et al.*, 2017). However, NTD’s receive minimal attention overall with the exception of dengue which, in contrast with the other NTD’s, affects both rich and poor populations, infections are short-lived and do not lead to disability and social stigma, and dengue is high on the public agenda (Horstick, Tozan and Wilder-Smith, 2015). Some authors have argued

that the increase in dengue research funding has been linked to the perceived threat of virus invasion in Western countries (Horstick, Tozan and Wilder-Smith, 2015), which is feared to happen through international sports competitions (Massad *et al.*, 2014; Wilder-Smith and Macary, 2014) or tourism (Wilder-Smith and Macary, 2014; World Health Organization, 2017b).

Current research funding, which more than three-quarters of global public-sector health investments come from the Governments of the United States and United Kingdom, has been considered inadequate (Hotez, 2013) and further examples of unequal concerns for diseases could be observed in early 2016 when Zika was declared a Public Health Emergency of International Concern after reports of new-borns affected in Western countries (World Health Organization, 2016) and later, by July 2016, when nearly US$700 million were diverted by the US Congress from the Ebola fund and other public agencies research portfolios for the development of a Zika vaccine even to the underfunding of other lethal diseases (*Nature*, 2016). Moreover, USAID, one of the world's leading international development agencies, allocated in 2016 a budget for Zika of up to US$155 million, whereas the budget for all NTD diseases combined was US$75 million and focused on scaling up preventive drug treatments for seven NTDs (Appendix 38) (U.S. Department of State, 2018a). To put things in perspective, in 2015, there were 2.35 million cases of dengue reported in the Americas alone, of which 10,200 cases were diagnosed as severe dengue causing 1,181 deaths (World Health Organization, 2017b). In contrast, Chagas disease, also included in the NTD list, affects approximately 6 to 8 million people, causes an annual incidence of 28,000 cases, and about 12,000 deaths per year in the Americas (PAHO / WHO, 2018a).

Thus, the different concern in NTD's versus other diseases appears to be based on priorities other than the severity or burden of the disease on the global population but on the segment of the population affected which may be attributed to a 'differential valuation of life in which some lives are deemed more important than others' (Kelly, 2015). This concept has been linked to bio-political imperialism in which control is exerted by using scientific knowledge to care for and enhance the lives of certain populations in order to enhance the power of some states and the wealthy which in turn derives from the long-recognised form of international dependence known as neo-colonialism (Kelly, 2015).

The term neo-colonialism was coined in the 1960's to describe initially the economic and cultural relationship of European countries with their former colonies although neo-colonial dynamics have evolved as new geopolitical powers emerged (Nkrumah, 1965; Quist-Adade, 2017). The essence of neo-colonialism is that, whereas the formal presence of a metropolis and colonial administration are absent nowadays and the subject state is nominally independent and has all the symbols of sovereignty, in reality their economic system and political policy are directed by external force through economic means (Nkrumah, 1965; Kelly, 2015; Quist-Adade, 2017). Neo-colonial control is exercised by a consortium of financial interests, which may not necessarily be identifiable with any particular state (Nkrumah, 1965). Moreover, the rulers of neo-colonial states derive their authority to govern from the support they obtain from their neo-colonialist masters, and have little interest in developing education and the needs of the population are often ignored, leaving issues such as healthcare, education, development, and poverty unresolved (Nkrumah, 1965; Quist-Adade, 2017, citing Leong, 2015) which causes difficulties to implement SDG's and the perpetuation of NTD's in those territories.

Many of these traits hindering economic development and healthcare can be observed in the Caribbean, a region with high variance between territories, especially in poverty and economic rates but with common colonial experiences and vulnerabilities, dependent economies and prone to natural disasters (Downes and Downes, 2003; Downes, 2010; Caribbean Development Bank, 2016). For instance, the fallout of 2008-2009 financial crisis severely affected the situation of the population in St. Kitts and agreements with the International Monetary Fund (IMF) after a debt burden in 2012 of EC $2.7 billion[1] left the island authorities 'very little space for measures to address the harsh conditions facing the majority of the population' (Vassell, 2014). In addition, the single crop economy based on sugar cane was abandoned in 2005 and replaced by tourism and off-shore activities, namely citizenship by investment and international finance services (Government of St. Kitts and Nevis, 2006; Vassell, 2014) of which the last two have raised international concerns (Government of St. Kitts and Nevis, 2016; Council of the European Union, 2018a, 2018b; U.S. Department of State, 2018b). The number of tourists arriving to St. Kitts and Nevis annually reaches 23-fold the islands population, mainly as day cruisers from North America and Europe (St. Kitts Statistics Office,

[1] US $1= EC $2.7

2015) and recent developments in the island infrastructure are subordinated to meet the demands of tourism, including the construction of high-end marinas, additional cruise ships docking piers and improved roads to luxury resorts. Such expansion will increase the number of visitors further and the risk of introducing new and emergent diseases, deplete local resources and have little benefit on the inhabitants whereas inequality, healthcare and the perpetuation of crime and violence are of great concern in St. Kitts and Nevis (Vassell, 2014). An indirect consequence of such inequality is that St. Kitts and Nevis is excluded from development programmes such as access to research grants and other government aid that promotes the economic development and welfare of developing countries health, sanitation, education as part of the Official Development Assistance (ODA) from the Organisation for Economic Co-operation and Development (OECD), as the federation is considered a high income country based on gross national income (GNI) per capita as published by the World Bank (OECD, 2018; The World Bank, 2018).

In summary, neglected tropical diseases are a group of diseases mainly due to poverty and actions aimed to achieving the Sustainable Development Goals (SDGs) and improving health infrastructure by reducing poverty and inequality are necessary. However, financial interests related to neo-colonial dynamics enhance such inequalities and dependence on external forces and economies in detriment of affected states' national interest which perpetuates the negative impact on the health of their inhabitants.

6.4. Further research

This study has highlighted several gaps in knowledge and additional information is required. The level of herd immunity threshold in small island countries to which infection stops is mainly unknown. A better understanding of population immunity in isolated territories would assist in a more efficient allocation of resources during outbreaks and surveillance in inter-epidemic periods.

Survey of other arboviruses in St. Kitts. A number of arboviruses, such as Mayaro virus, have been recorded in other islands of the Caribbean and it would be reasonable to fear that those have also spread to other territories.

Also, the contribution of tourism, in particular cruise ships, to the spread of infectious disease and vectors among islands and territories remains mainly unknown and is needed for predicting dynamics of future epidemics.

Appendix 1 ***Historical arboviral outbreaks (Vasilakis and Weaver, 2008)***

Year	Location	Probable virus
1635	Martinique, Guadeloupe	DENV?
1699	Panama	DENV?
1780	Philadelphia	DENV
1818	Peru	DENV
1827-1828	Caribbean. Gulf of Mexico. South US	CHIKV?
1845-1849	New Orleans, Cuba, Brazil	DENV?
1850	US (New Orleans, Mobile, Charleston, Augusta, and Savannah). Havana, Cuba	DENV
1851	Lima, Peru	DENV
1873	New Orleans	DENV
1879-1880	Southern US port cities	DENV
1880	Brazil	DENV?
1889	Chile	DENV?
1885-1886 & 1897	Texas	DENV?
1898-1899	Florida	DENV?
1897	Havana, Cuba	DENV?
1882	Bahamas, Bermuda	DENV?
1904, 1912	Panama	DENV?
1915	Virgin Islands. Puerto Rico	DENV?
1916	Rio Grande do Sol, Brazil. Corrientes and Entre Rios, Argentina	DENV?
1918-1922	Galveston, Texas & Louisiana	DENV?
1934	Miami, Florida. Georgia	DENV?
1941	Texas	DENV?
1941-1942	Panama Canal	DENV2
1944	Havana, Cuba	DENV?
1945	Puerto Rico	DENV?
1945-46	Caracas, Venezuela. Bermuda. Bahamas	DENV?

Appendix 2 *Aedes Flight Range*

Location	Released	Overall Recaptured	Days after release	Distance from release (of recaptured)		Reference:
				Minimum	Maximum	
Laboratory flight mill			12-day old		11.3 km per day 17.5km per day	(Rowley and Graham, 1968)
Kenya	Unfed mosquitoes	11.4% 1.7% 0.2%	>1 day		200m 400m 800m	(McDonald, 1977)
Mombassa, Kenya	Captured mosquitoes	55%		1 house	5 houses from released	(Trpis and Hausermann, 1986)
Puerto Rico	3-day old 13-day old	35% 16%	1-10 days	Same house: 76% 79%	79m	(Harrington *et al.*, 2001)
Puerto Rico	Adults	34% 23%	3 days	Same house: 80-87%	>43m: 8-14%	(Harrington *et al.*, 2005)
Thailand	Adults (several releases)	4-9%	3 days	Same house: 66%	> 3 houses: 11%	
Puerto Rico	Rb-marked gravid females	Eggs	1-4 days		840m	(Reiter *et al.*, 1995)
Rio de Janeiro, Brazil	Rb-marked gravid females	Eggs	Day 6		1,600m	(Honório *et al.*, 2003)
China	Adults	1.3-2.8%	6 days	<15m: 81% females	>75m: 0-3% females	(Tsuda *et al.*, 2001)
Australia	Adults	13% 3.6%	7 days	< 50m for 75%	160m	(Muir and Kay, 1998)
Brazil	Adults	5-12.2%	1-10 days	Mean distance = 57-122m	263m	David, (David, Lourenço-de-Oliveira and de Freitas, 2009)

Appendix 3 *Informed Consent form for Student Sentinels in the Epidemiology of Dengue/ Chikungunya on St Kitts*

Informed Consent form for Student Sentinels in the Epidemiology of Dengue and Chikungunya on St Kitts

Purpose of the study:
Drs Pat Kelly and Michel Vandenplas from Ross University School of Veterinary Medicine (RUSVM) together with researchers at Newcastle University, England (Drs Inaki Deza-Cruz, Steve Rushton and Aileen Mill), and James Cook University, Australia (Dr Graham Burgess), are carrying out a research project on factors affecting how people on St Kitts become infected with the Dengue and Chikungunya viruses.

Who can be in the study?
Only the students of RUSVM can participate in this study.

What will happen if you decide to participate in this study?
You will be required to answer an online questionnaire to give us background information on the risk factors you might have which will make you more or less likely to get Dengue and/or Chikungunya. You will also need to donate 5 ml of blood which we will test for antibodies against Dengue and Chikungunya virus indicating you have been exposed in the past. We will Email you the results of this test when they are completed, usually within a month. You will be able to discuss the results of the test with one of the project investigators or your health care provider.

Are there benefits for participating in this study?
By participating you will contribute to our study which will provide information that can help us get a better understanding of Dengue and Chikungunya on St. Kitts, and even around the world. This might enable us to develop better ways of preventing and controlling Dengue and Chikungunya.

The blood test will tell us if you have had Dengue which is important information you need to give to your healthcare worker. The problem is that you can have Dengue a number of times and when you get it for a second or third time you are more likely to develop a severe form of the disease. If your healthcare worker knows you have had Dengue before he/she will be aware of the possibility of the severe form developing if you get Dengue again and this will enable her/him to take early appropriate action. Unfortunately there is no specific treatment for Dengue but the severe form can be made less dangerous with early supportive treatments such as intravenous drips.

1

Finally, you will be entered into a raffle for 6 cinema tickets which will be drawn after the blood collections are performed. Winners will be notified by Email.

Are there risks for participating in this study?
Although there is risk with the donation of a blood sample, this will be done by highly experienced people who take blood samples for a commercial diagnostic laboratory in Basseterre. Usually the only discomfort is some mild pain when the needle is inserted into your arm. Occasionally people giving blood samples may feel lightheaded and can feint. There might also be some bruising at the site where the needle enters the skin but this quickly goes away over a few days. If you believe you have suffered an injury related to the study you should make an appointment with the doctor you regularly see.

What about confidentiality?
Your information will be coded and stored in a password protected file on a secure computer. Only Dr Kelly will know the code and the password. Your test results and questionnaire responses will be kept strictly confidential and will not be shared with anyone except you.

Voluntary participation:
It is your choice to be in this study. If you decide to be in this study, you may change your mind at any time and leave the study. Should you leave the study, we will destroy all your samples and any information relating to your participation. If you decide not to be in this study you will not be discriminated against in any way.

What will it cost?
The cost of the sample collection and Dengue and Chikungunya tests are covered by the research project. The costs of medical care for illnesses that occur during the study period will not be paid for by this study.

Who is responsible for this study?
This study is paid for by an Intramural Research Grant from Ross University School of Veterinary Medicine in collaboration with the University of Newcastle.

If you experience any physical or mental injury as a direct result of your participation in this study, medical treatment will be provided at a local clinic or hospital. There will be no monetary compensation available if any injury were to occur as a result of participating in this study. You are not waiving any legal rights by signing this consent form.

Any questions or concerns that you may have about this study should be directed to the principal investigator on St Kitts, Dr. Patrick Kelly. His contact information is below.

Who can I call if I have problems or question about the study?
If you have questions about the study or believe that you were harmed by being in the study, you can call Dr. Patrick Kelly (the Principal Investigator) at (869) 465-4161 extension 1108.

Authorization
I have read and understand this consent form, and I volunteer to participate in this study. I understand that I will receive a copy of this form. I voluntarily choose to participate, but I understand that my consent does not take away any legal rights in the case of negligence or other legal fault of anyone who is involved in this study. I further understand that nothing in this consent form is intended to replace any applicable Federal, state, or local laws.

Participant Name (Printed): .....

Participant Signature: ..... Date:.....

Anonymized Sample Donation
I further consent to my blood sample being anonymized and used in future research projects. I realize that my blood sample will be de-identified and there will be no connection between it and myself. It will therefore not be possible for me to be informed of the research that is performed on the sample in the future or the results of these studies.

Participant Name (Printed): .....

Participant Signature: ..... Date:.....

Appendix 4 ***Initial Questionnaire for Student Sentinels - Epidemiology of Dengue / Chikungunya on St Kitts***

Initial Questionnaire for Student Sentinels – Epidemiology of Dengue / Chikungunya

1. Are you willing to participate as a sentinel in this study?
   - Yes
   - No

2. What is your name?
   - Surname
   - First name

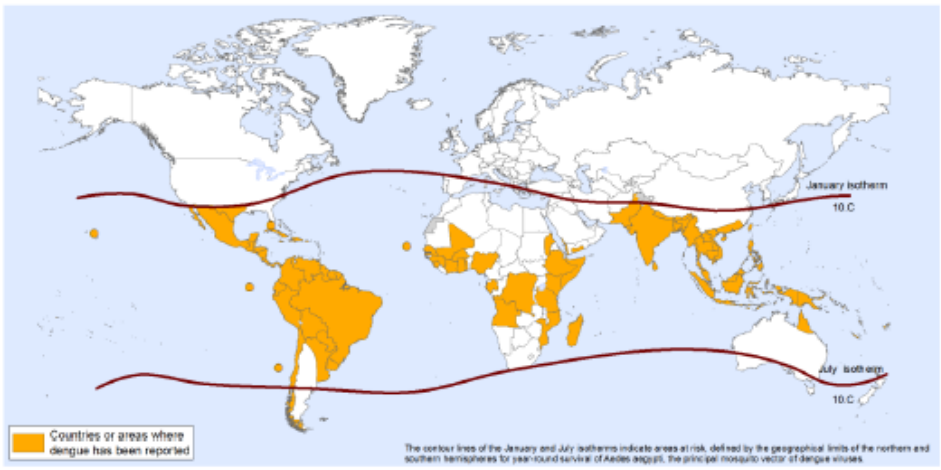
   Contact details:
   Phone no.:
   email:

3. What is your gender?
   - Female
   - Male

4. What is your date of birth
   (MM/DD/YYYY)

5. If born outside St Kitts where were you born?
   - Town/City
   - State (if applicable)
   - Country

Dengue, countries or areas at risk, 2013



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: World Health Organization
Map Production: Health Statistics and Information Systems (HSI)
World Health Organization

World Health Organization

6. Have you ever lived in or visited any of the countries within the red bands above (excluding the southern United States and Western Europe)?
   - Yes
   - No

7. When and for how long?

8. Where will you be living in St Kitts for your first semester of study?
   RUSVM Dormitory ..... Douglas
   Columbus
   Woods
   Cleghorn
   Tegroman.
   Parish/ Suburb
   Street

9. Did you live somewhere else on St Kitts before moving to the above address?
   Parish/ Suburb
   Street
   How long

10. Before coming to St Kitts had you ever been diagnosed with dengue fever?
   No
   Yes

11. In what year were you diagnosed with dengue fever?
   Year

12. Was a blood test done to confirm the diagnosis?
   No
   Yes
   Don't know

13. Have you ever been diagnosed with a West Nile virus (WNV) infection?
   No
   Yes

14. Have you ever been vaccinated for Yellow Fever?
   No
   Yes
   Don't know

15. If YES, in what year?
   Year

16. Have you ever been vaccinated for Japanese encephalitis virus?
   No
   Yes
   Don't know

17. If YES, in what year?

   Year

18. Have you ever been diagnosed with a Chikungunya virus infection?
   Yes
   No

19. Have you been diagnosed with a medical condition?

No

Yes, (please, specify)

20. Are you taking any treatment for the above condition?

END OF QUESTIONNAIRE

Appendix 5 ***Questionnaire for Student Sentinels - Epidemiology of Dengue / Chikungunya on St Kitts***

Questionnaire for Student Sentinels - Epidemiology of Dengue

Section 1. Personal information

What is your First Name? *	
What is your Last Name? *	
What semester are you commencing?	

If this is your FIRST sample, please, answer the questions in 'grey', otherwise, skip to the next 'clear' question

What is your Phone number?	
What is your email?	
What is your gender?	
What is your Date of Birth? (MM/DD/YYYY)	
Town/City	
State (if applicable)	
Country	
If born outside of St. Kitts, when did you first arrived on St. Kitts (MM/DD/YYYY)	
Before coming to St. Kitts for the first time, have you ever lived in or visited any of the countries within the red bands above (excluding the southern United States and Western Europe)?	Yes / No
Which country?	
How long (weeks)	
How long (months)	
Previous country - 1	
How long (weeks)	
How long (months)	
Previous country - 1	
How long (weeks)	
How long (months)	
Previous country - 1	
How long (weeks)	
How long (months)	
dengue fever	Yes / No
in what year?	
Was a blood test done to confirm the diagnosis?	Yes / No
Have you ever been diagnosed with a West Nile virus (WNV) infection?	Yes / No
Have you ever been diagnosed with Yellow Fever?	Yes / No
Have you ever been vaccinated for Yellow Fever?	Yes / No
If answered, 'yes', in what year were you vaccinated?	
Have you ever been diagnosed with Japanese encephalitis?	Yes / No
Have you ever been vaccinated for Japanese encephalitis?	Yes / No
If answered, 'yes', in what year were you vaccinated?	
Have you ever been diagnosed with a Chikungunya virus infection?	Yes / No
Do you smoke?	No / Occasionally / Daily / Several times a day

Page 1 of 6

Have you been diagnosed with a medical condition?	Yes / No
If you have been diagnosed with a medical condition, please, specify	
If you have been diagnosed with a medical condition, please, specify	
During the last 4 months (or since last giving a blood sample for the project) have you traveled outside of St. Kitts?	Yes / No
Which country?	
How long (weeks)	
How long (months)	
Previous country - 1	
How long (weeks)	
How long (months)	
Previous country - 2	
How long (weeks)	
How long (months)	
Previous country - 3	
How long (weeks)	
How long (months)	
Have you had any visitors staying with you over the past 4 months?	
Where had they been in the two weeks before coming to St Kitts?	
How long (weeks) did your visitors stay?	
Were they ill at the time or shortly afterwards?	

Where do you now live on St. Kitts and how long have you lived there since you were last tested for dengue / chikungunya on the project?

Dorm (if applicable)	
Area: (Frigate Bay, West Farm, etc)	
Street Name or Apartment Complex name (e.g. Golfview, Silver Reef, Horizons, Manor by the Sea (etc.):	
Duration of stay (in months)	
Did you live somewhere else on St Kitts before moving to the above address and since you were last tested for dengue/ chikungunya on the project?	
Dorm (if applicable)	
Area:	
Street Name or Apartment Complex name (e.g. Golfview, Silver Reef, Horizons, Manor by the Sea (etc.):	
Duration of stay (in years)	
Duration of stay (in months)	
In your current residence, how many front or back doors had screens in good condition (with no holes or tears)?	All / Some / None / Don't know
Do you ever leave the front or back doors that had no or defective screens open for 30 minutes or more?	Often / Sometimes / No
Do you have air-conditioning in your home?	
Number of rooms with air-conditioning	
Number of rooms in your home	

On average, how often do you use the air-conditioning?	Less than once per month / Monthly / Every couple of weeks / Once a week / More than once a week
Other than air-conditioning, do you have any other type of air-cooling system?	Yes, fans / Yes, other / No
If you selected 'Other' for having any other type of air-cooling system, please, specify:	
Which of the following materials is your house made from?	Concrete / Wood / Both concrete and wood / Don't know
On average, do you have pipe-borne water (tap water) available at your home 24 hours a day?	No / Yes / Don't know
Do you store water for household use (other than what you purchased in a bottle) in an open container?	Always / Sometimes / No
Is the water you store from the tap, purchased, or was it from rainwater? Click all that apply.	From the tap / Purchased / Rainwater / Don't know
Which containers do you use to store water? Click all that apply.	55 gallon drum / Rooftop cistern / Cistern /Tank / 5 gallon containers / Don't know
How often is trash collected from your neighborhood?	Less than once per month / Monthly / Every couple of weeks / Once a week / More than once a week
If you selected 'Other' for how often trash is collected from your neighborhood, please specify:	
Is there trash (open cans, tires, etc) in the immediate area surrounding your home?	No / Yes, a little / Yes, a lot / Don't know
Including yourself, how many people live in your house or apartment?	
How much time did you spend in your porch/yard/ garden during the week or the weekend?	None / Less than one hour per day / More than one hour per day
What parts of the day would you spend in the porch/yard? Click all that apply.	Morning / Afternoon / Evening
Do you have potted plants?	Yes / No
If yes, about how many plants?	
and, are they inside or outside or both?	Inside / Outside / Both inside and outside
Do you have pools of water that last a few days in your yard?	Yes / No
If you do have pools of water that last a few days in your yard, please state where they occur.	
Approximately, what distance (in feet) was there between your house and the closest neighboring house?	
Do mosquitoes bite you INSIDE your house?	Often / Sometimes / No
When do the mosquitoes bite you inside your house?. Click all that apply	Day / Evening / Night
Do mosquitoes bite you when sitting OUTSIDE your house?	Often / Sometimes / No
When do the mosquitoes bite you outisde your house?. Click all that apply	Day / Evening / Night
Do you use mosquito coils?	Yes / No
If you answered 'yes', how frequently did you use the mosquito coils?	Less than once per month / Monthly / Every couple of weeks / Once a week / More than once a week
If you selected 'Other' for how frequently you used the mosquito coils, please, specify:	
Do you use an insecticide spray (like Baygon, Bop, etc) in or around the house?	Yes / No

How often did you use the insecticide spray?	Less than once per month / Monthly / Every couple of weeks / Once a week / More than once a week
Do you have professional pest exterminators spraying your house?	Every…(enter below) moths / Sometimes / Rarely / Never / Don't know
Every ….. months	
Do you wear mosquito repellent (Off, Cutter, etc) when you spend time outdoors?	Always / Often / Sometimes / Rarely / Never / Don't know
Did you visit a beach during the day or evening?	Yes / No
If you answered 'yes', which one?, if not, leave blank.	
If you click 'other', please, specify:	
How often did you visit that beach	Less than once per month / Monthly / Every couple of weeks / Once a week / More than once a week
Do you usually get bitten by mosquitoes there?	Often / Sometimes / No
Other beach - 2	
If you click 'other', please, specify:	
How often did you visit that beach	Less than once per month / Monthly / Every couple of weeks / Once a week / More than once a week
Do you usually get bitten by mosquitoes there?	Often / Sometimes / No
Other beach - 3	
If you click 'other', please, specify:	
How often did you visit that beach	Less than once per month / Monthly / Every couple of weeks / Once a week / More than once a week
Do you usually get bitten by mosquitoes there?	Often / Sometimes / No
Did you go to a bar or restaurant during the day or evening?	Yes / No
If so, which one?	
If you click 'other', please, specify:	
How often did you go to that bar / restaurant?	Less than once per month / Monthly / Every couple of weeks / Once a week / More than once a week
Do you usually get bitten by mosquitoes there?	Often / Sometimes / No
Bar / restaurant - 2	
If you click 'other', please, specify:	
How often did you go to that bar / restaurant?	Less than once per month / Monthly / Every couple of weeks / Once a week / More than once a week
Do you usually get bitten by mosquitoes there?	Often / Sometimes / No
Bar / restaurant - 3	
If you click 'other', please, specify:	
How often did you go to that bar / restaurant?	Less than once per month / Monthly / Every couple of weeks / Once a week / More than once a week
Do you usually get bitten by mosquitoes there?	Often / Sometimes / No
Did you attend a church during the day or evening?	Yes / No
Name of Church	

How often did you visit that church	Less than once per month / Monthly / Every couple of weeks / Once a week / More than once a week
Do you usually get bitten by mosquitoes there?	Often / Sometimes / No
Where do you do your grocery shopping and how often do you go there?	Yes / No
If you click 'other', please, specify:	
How often did you go to that supermarket / shop?	Less than once per month / Monthly / Every couple of weeks / Once a week / More than once a week
Do you usually get bitten by mosquitoes there?	Often / Sometimes / No
Name of supermarket/shop - 2	
If you click 'other', please, specify:	
How often did you go to that supermarket / shop?	Less than once per month / Monthly / Every couple of weeks / Once a week / More than once a week
Do you usually get bitten by mosquitoes there?	Often / Sometimes / No
Name of supermarket/shop - 3	
If you click 'other', please, specify:	
How often did you go to that supermarket / shop?	Less than once per month / Monthly / Every couple of weeks / Once a week / More than once a week
Do you usually get bitten by mosquitoes there?	Often / Sometimes / No
Where do you spend most of your time at Ross?	
On average, how many hours per day?	
When? Click all that apply	Day / Evening / Night
Which other rooms (classrooms, laboratories, library areas, etc) do you spend most of your time in at Ross?	
Location - 1	
On average, how many hours per day?	
When? Click all that apply	Day / Evening / Night
Location - 2	
On average, how many hours per day?	
When? Click all that apply	Day / Evening / Night
Location - 3	
On average, how many hours per day?	
When? Click all that apply	Day / Evening / Night
Location - 4	
On average, how many hours per day?	
When? Click all that apply	Day / Evening / Night
Did you do hiking during the day or evening?	Yes / No
If you selected 'Yes' for doing hiking, please list the areas:	
Do you usually get bitten by mosquitoes there?	Often / Sometimes / No
Are there any other places you frequent where you are bitten by mosquitoes?	
place1	
place2	
place3	
dengue fever	Yes / No

in what year?	
Was a blood test done to confirm the diagnosis?	Yes / No
Have you ever been diagnosed with a West Nile virus (WNV) infection?	Yes / No
Have you ever been diagnosed with Yellow Fever?	Yes / No
Have you ever been vaccinated for Yellow Fever?	Yes / No
If answered, 'yes', in what year were you vaccinated?	
Have you ever been diagnosed with Japanese encephalitis?	Yes / No
Have you ever been vaccinated for Japanese encephalitis?	Yes / No
Have you been diagnosed with a Chikungunya virus infection?	Yes / No
Have you been diagnosed with a medical condition?	Yes / No
If you have been diagnosed with a medical condition, please, specify	
If you have been diagnosed with a medical condition, please, specify	
Are you willing to participate as a sentinel in this study?	Yes, I have read and understood the 'Consent Form' and I am willing to participate as a sentinel in this study / I further consent to my blood sample being anonymized and used in future research projects. / No
Date of completion (MM/DD/YYYY)	

Appendix 6 ***Questionnaire for Acute Cases - Epidemiology of Dengue/ Chikungunya on St Kitts.***

Questionnaire for Patients with Clinical Dengue/ Chikungunya / Zika

Before you begin

We realize you are not feeling well but we do need to get some information while it is fresh in your mind. Please complete at least the first 10 Questions – "Immediate information we would like to have". If you feel you cannot complete more then we can arrange for you to complete the "Additional Information" section once you have recovered.

Immediate information we would like to have

1. Are you willing to participate in this study?			
	Yes		
	No		
3. What is your gender?		**4. What is your date of birth (MM/DD/YYYY)**	
Female		**Male**	
5. What is your current address:			

6. Which places did you visit in the 3 – 7 days before you became ill? Please tick those that you remember and give names if possible.		**7. Did you notice mosquitoes or were bitten by them? Tick which sites and put an exclamation mark if there were really a lot of mosquitoes or you were badly bitten at the site.**	**8. Are there usually monkeys around the sites you visited? Tick which sites and put an exclamation mark if there are a lot of monkeys there.**
Workplace			
Home			
Friends and relatives			
Restaurant			
Bar			
Beach			
Sports event			
Nature walk			
Church			
Other			

Informed Consent Form for Acute Clinical Dengue/Chikungunya/Zika Patients

Purpose of the study:
Drs Pat Kelly and Michel Vandenplas from Ross University School of Veterinary Medicine (RUSVM) together with researchers at Newcastle University, England (Drs Inaki Deza-Cruz, Steve Rushton and Aileen Mill), and James Cook University, Australia (Dr Graham Burgess), are carrying out a research project on factors affecting how people on St Kitts become infected with the Dengue or Chikungunya virus.

Dr Pat Kelly from Ross University School of Veterinary Medicine (RUSVM) together with Dt Ted Ross of the Vaccine and Gene Therapy Institute of Florida (VGTIF), USA, are carrying out a research project on the immune responses people make against the Chikungunya virus.

Who can be in the study?
Students of RUSVM can participate in this study through their local doctors or the Student Health Services. Members of the public can participate in the study if referred by their local doctor.

What will happen if you decide to participate in this study?
You will be required to provide a blood sample (40ml) which we will test for the presence of antibodies to Dengue or Chikungunya using a standard commercial kit. Your healthcare worker will collect the blood sample or you will be referred to a laboratory where professional technicians will draw the sample. The test is normally performed on the same day the sample is received into the research Laboratory of the RUSVM and the results communicated to your healthcare worker who will then contact you to discuss the results of the test and possible treatments/ further diagnostics.

Are there benefits for participating in this study?
The blood test will tell us if you have Dengue or Chikungunya now and this will allow your healthcare worker to give you the most appropriate treatment. If the test indicates you do not have Dengue or Chikungunya your healthcare worker can consider the other possibilities for your illness and the best ways to diagnose them.

Dengue and Chikungunya are transmitted by mosquitoes so if you are found to be infected you will be able to seek advice about the best ways of killing mosquitoes in your home and thus help prevent further infections in your family, neighbors and visitors.

Finally, by participating you will be contributing to our study which will provide information that can help us get a better understanding of Dengue and Chikungunya on St. Kitts, and even around the world. This might enable us to develop better ways of preventing and controlling Dengue and Chikungunya.

Are there risks for participating in this study?
Although there is risk with the donation of a blood sample, this will be done by highly experienced phlebotomists from a commercial diagnostic laboratory in Basseterre who take blood samples on a regular basis each working day. Usually the only discomfort is some mild pain when the needle is inserted. Occasionally there might be lightheadedness and feinting. There might also be some bruising but this resolves quickly over a few days. If participants in the study believe they have suffered an injury related to the study they are encouraged to contact their regular doctor.

What about confidentiality?
The project will ensure your anonymity and only your healthcare worker and Dr Kelly will know the results of your test. This will be achieved by Dr Kelly giving your sample a number when it arrives in the RUSVM laboratory. This number is the only identifier that laboratory staff working on your sample will see. They will not see or know your name. Dr Kelly will enter your name and the corresponding sample number in a password protected computer file on a secure computer in his office.

Only Dr Kelly will know the password to the file and only Dr Kelly will be able to link your name to the sample number. All information collected for the project will be stored under the sample number and once we have information from your healthcare worker on how you recovered from the Chikungunya (uneventfully over a couple of days/ weeks or whether there were ongoing problems, such as joint pain, for months) Dr Kelly will delete the computer file linking your name to the sample number. This will mean your sample has been anonymized – that is, it will be impossible for anyone to link your name to your sample and vice versa. The only information that will be available for your sample will be the patient information we collect (your age group – youth, adult (20-45y), middle-aged (45-60y), senior (>60y), sex, and the parish where you reside as well as the outcome of your infection (recovered uneventfully or developed chronic signs). The only place your name will be linked to your Chikungunya test result will be in your healthcare worker's files.

If you wish your blood sample to be used in future research on other diseases that might be investigated at RUSVM or the VGTIF please sign the Anonymized Sample Donation option at the end of the document. Remember that although this will help future researchers gain knowledge about diseases and lessen the suffering they cause, there will be no link between you and your anonymized sample so you cannot be informed of future tests on your sample or the results. If you do not sign this part of the form you samples will be destroyed upon completion of the project or after 5 years, whichever occurs first.

Voluntary participation:
It is your choice to be in this study. If you decide to be in this study, you may change your mind at any time and leave the study. Should you leave the study, we will destroy all your samples and any information relating to your participation. If you decide not to be in this study you will not be discriminated against in any way.

What will it cost?
The cost of the sample collection and Dengue / Chikungunya tests are covered by the research project. The costs of medical care for illnesses that occur during the study period will not be paid for by this study.

If you experience any physical or mental injury as a direct result of your participation in this study, medical treatment will be provided at a local clinic or hospital. There will be no monetary compensation available if any injury were to occur as a result of participating in this study. You are not waiving any legal rights by signing this consent form.

Any questions or concerns that you may have about this study should be directed to the principal investigator on St Kitts, Dr. Patrick Kelly. His contact information is below.

Who is responsible for this study?
This study is paid for by the research link between RUSVM and Newcastle University.

If you experience any physical or mental injury as a direct result of your participation in this study, medical treatment will be provided at a local clinic or hospital. There will be no monetary compensation available if any injury were to occur as a result of participating in this study. You are not waiving any legal rights by signing this consent form.

Any questions or concerns that you may have about this study should be directed to the principal investigator on St Kitts, Dr. Patrick Kelly. His contact information is below.

Who can I call if I have problems or question about the study?
If you have questions about the study or believe that you were harmed by being in the study, you can call Dr. Patrick Kelly (the Principal Investigator) at (869) 465-4161 extension 1108.

Authorization
I have read and understand this consent form, and I volunteer to participate in this study. I understand that I will receive a copy of this form. I voluntarily choose to participate, but I understand that my consent does not take away any legal rights in the case of negligence or other legal fault of anyone who is involved in this study. I further understand that nothing in this consent form is intended to replace any applicable Federal, state, or local laws.

Participant Name (Printed or Typed): .....

Participant Signature: ..... Date: .....

Anonymized Sample Donation

I further consent to my blood sample being anonymized and used in future research projects. I realize that my blood sample will be de-identified and there will be no connection between it and myself. It will therefore not be possible for me to be informed of the research that is performed on the sample in the future or the results of these studies.

Participant Name (Printed): .....

Participant Signature: ..... Date:.....

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Additional information

Ref. Case No:	

2. Were you born in St. Kitts & Nevis?			
Yes		**No**	

4. During the last 3 weeks have you travelled outside of St Kitts?	
Yes	**No**

1. Are you willing to participate in this study?	
Yes	
No	

3. If born outside St Kitts where were you born?	
Town/City	
State (if applicable)	
Country	

If you have travelled outside of St. Kitts list countries and duration of your stay in each:

Country and city/ town		Duration	
Country and city/ town		Duration	
Country and city/ town		Duration	
Country and city/ town		Duration	

5. Have you had any visitors staying with you over the past 4 months?	**Yes**		**No**

6. If you had visitors where had they been in two the weeks before coming to St Kitts?

Country and city/ town		Duration	
Country and city/ town		Duration	
Country and city/ town		Duration	

Were they ill at the time or shortly afterwards?	**Yes**		**No**

8. How long have you lived at your current address in St. Kitts?			
Years		**Months**	

9. Did you live somewhere else on St Kitts before moving to the above address?					
Parish/ Suburb					
Street					
How long	**Years**		**Months**		

10. In your current residence, how many front or back doors had screens in good condition (with no holes or tears)?	
All	
Some	
None	
Don't know	
12. Do you have air-conditioning in your home?	

11. Do you ever leave the front or back doors that had no or defective screens open for 30 minutes or more?	
Never	
Sometimes	
Most of the time	
13. How many rooms in your home have air-conditioning units?	

No	
Yes (Central air-conditioning)	
Yes (Air-conditioner units)	

Number of rooms with air conditioning	
Number of rooms in your home	

14. On average, how often do you use the air-conditioning?	
Daily	
Once a week	
A few times a week	
A few times a month	
A few times a year	
Never	

15. Other than air-conditioning, do you have any other type of air-cooling system?	
No	
Yes, fans	
Other (please specify):	

16. Which of the following materials is your house made from?	
Concrete	
Wood	
Both concrete and wood	
Don't know	

17. On average, do you have pipe-borne water (tap water) available at your home 24 hours a day?	
No	
Yes	
Don't know	

18. Do you store water for household use (other than what you purchased in a bottle)?	
Yes, always	
Yes, sometimes	
No	

19. Is the water you store from the tap, purchased, or was it from rainwater? Click all that apply.	
Purchased	
From the tap	
Don't know	
Rainwater	

20. Which containers do you use to store water? Click all that apply.	
55 gallon drum	
Rooftop container	
Cistern	
Tank	
5 gallon containers	
Don't know	

21. How often is trash collected from your neighborhood?	
Never	
A few times per week	
Once per week	
Once per month	
Other (please specify)	

22. Is there trash (open cans, tires, etc) in the immediate area surrounding your home?	
No	
Yes, a little	
Yes, a lot	
Don't know	

23. Including yourself, how many people live in your house or apartment?	
Number of people	

24. How much time did you spend in your porch/yard/ garden during the week or the weekend?	
None	
Less than one hour per day	
More than one hour per day	

25. What parts of the day would you spend in the porch/yard? Click all that apply	
Morning	
Afternoon	
Evening	

26. Do you have pot plants? If yes, about how many and are they inside or outside or both?				
		Yes		No
How many				

Inside			**27. Do you have pools of water that last at least a few days in your yard? If yes please state where they occur.**				
outside							
both			Yes		No		
28 Approximately what distance is there between your house and the closest neighboring house?							
Distance in feet							
29. Do mosquitoes bite you in your house?			When do the mosquitoes bite you?				
No			Day				
Yes, sometimes			Evening				
Yes, often			Night				
30. Do mosquitoes bite you when sitting <u>outside</u> at your house?			When do the mosquitoes bite you?				
No			Day				
Yes, sometimes			Evening				
Yes, often			Night				
31. Do you use mosquito coils?	Yes		No				
32. How frequently did you use the mosquito coils?			**33. Do you use an insecticide spray (like Baygon, Bop, etc) in or around the house?**				
Daily			Yes		No		
Weekly			**34. How often did you use the insecticide spray?**				
Monthly			Daily				
Other (please specify):			A few times a week				
			A few times a month				
			Don't know				
35. Do you have professional pest exterminators spray your house?			**36. Do you wear mosquito repellent (Off, Cutter, etc) when you spend time outdoors?**				
Every …… months			Always				
Sometimes			Often				
Rarely			Sometimes				
Never			Rarely				
Don't know			Never				
			Don't know				
Which kind of mosquito repellent do you use?							

37. Which beaches do you frequent during the day and how often?					**Do you get bitten by mosquitoes?**		
Name of Beach	Daily	Couple of times a week	Once a week	Every couple of weeks	No	Sometimes	Often
1.							
2.							
3.							
4.							
5.							

38. Which bars or restaurants do you frequent during the day or the evening and how often?					Do you get bitten by mosquitoes there?		
Name of bar / restaurant	Daily	Couple of times a week	Once a week	Every couple of weeks	No	Sometimes	Often
1.							
2.							
3.							
4.							
5.							

39. Do you attend a church?		Yes		No	
Name of Church					
How often do you attend do you get bitten by mosquitoes there?					

40. If you are a Ross student, where do you study at Ross and at which times?					
Location	1.	2.	3.	4.	5.
hours per day/night					

If you are a student of a member of staff, which rooms (classrooms, laboratories, library areas, etc) do you spend most of your time in at Ross?					
Location:	1.	2.	3.	4.	5.
hours per day/night					

42. Do you do a lot of hiking? If yes, please list the areas.			
Yes		No	
Areas	1.	2.	
3.		4.	

43. Are there any other places you frequent where you are bitten by mosquitoes:	
1.	4.
2.	5.
3.	6.

44. Have ever been diagnosed with any of the diseases below?	
Dengue fever	
Chikungunya	
West Nile virus	
Yellow fever	
Japanese encephalitis virus	
If yes, indicate which year	

45. Have ever been vaccinated against any of the diseases below?	
Dengue fever	
Chikungunya	
West Nile virus	
Yellow fever	
Japanese encephalitis virus	
If yes, indicate which year	

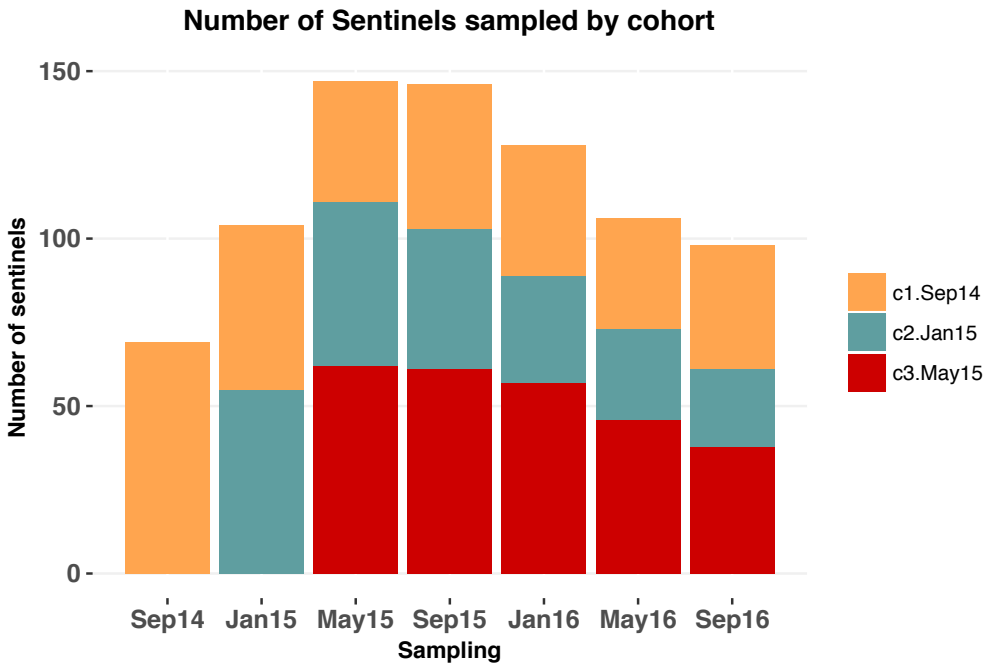
END OF QUESTIONNAIRE

Appendix 7 *Proportion of the most commonly unanswered questions*

Cohort	Cohort test No.	House materials	People live in house	Distance to neighbour	Use of spray	Freq. pest control	Visit church	Bitten at church	Hiking	Bitten at shop	At RUSVM during day	At RUSVM during evening	At RUSVM during night	Hours at RUSVM
Sep.14	2	14.80%	2.50%	12.30%	17.30%	82.70%	23.50%	23.50%	4.90%	6.20%	8.60%	8.60%	8.60%	48.10%
Sep.14	3	18.60%	7.00%	20.90%	20.90%	46.50%	11.60%	11.60%	2.30%	9.30%	2.30%	2.30%	2.30%	90.70%
Sep.14	4	13.20%	4.40%	0.00%	5.90%	38.20%	29.40%	29.40%	0.00%	1.50%	2.90%	2.90%	2.90%	58.80%
Sep.14	5	9.10%	15.90%	13.60%	11.40%	40.90%	13.60%	13.60%	11.40%	18.20%	9.10%	9.10%	9.10%	25.00%
Sep.14	6	10.80%	5.40%	18.90%	5.40%	32.40%	21.60%	21.60%	2.70%	5.40%	0.00%	0.00%	0.00%	10.80%
Sep.14	7	28.60%	26.20%	21.40%	14.30%	54.80%	23.80%	23.80%	23.80%	23.80%	23.80%	23.80%	23.80%	26.20%
Jan.15	2	22.60%	†17.00%	20.80%	20.80%	69.80%	24.50%	24.50%	17.00%	17.00%	18.90%	18.90%	18.90%	†92.50%
Jan.15	3	4.30%	20.30%	† 1.40%	7.20%	43.50%	†36.20%	†36.20%	1.40%	7.20%	5.80%	5.80%	5.80%	†60.90%
Jan.15	4	0.00%	28.60%	31.40%	5.70%	40.00%	20.00%	20.00%	20.00%	14.30%	22.90%	22.90%	22.90%	†25.70%
Jan.15	5	3.40%	27.60%	13.80%	0.00%	48.30%	17.20%	17.20%	13.80%	17.20%	10.30%	10.30%	10.30%	13.80%
Jan.15	6	0.00%	8.70%	0.00%	8.70%	26.10%	26.10%	26.10%	†26.10%	21.70%	26.10%	26.10%	26.10%	26.10%
May.15	2	30.90%	†21.60%	4.10%	18.60%	†56.70%	33.00%	33.00%	4.10%	15.50%	13.40%	13.40%	13.40%	58.80%
May.15	3	12.30%	9.20%	16.90%	18.50%	53.80%	20.00%	20.00%	15.40%	18.50%	9.20%	9.20%	9.20%	† 18.50%
May.15	4	10.40%	18.70%	25.00%	12.50%	60.40%	20.80%	20.80%	20.80%	10.40%	10.40%	10.40%	10.40%	† 18.70%
May.15	5	23.70%	21.10%	13.20%	18.40%	63.20%	†26.30%	†26.30%	28.90%	15.80%	18.40%	18.40%	18.40%	21.10%
	Mean	**13.50%**	**15.60%**	**14.30%**	**12.40%**	**50.50%**	**23.20%**	**23.20%**	**12.80%**	**13.50%**	**12.10%**	**12.10%**	**12.10%**	**39.70%**

† Significant values ($p < 0.05$). Figures >25% of non-responses highlighted in red.

Appendix 8 *Number of sentinels sampled by cohort*



Appendix 9 *Number of samples collected from sentinels and Drop-out rate and lost to follow-up:*

Table 6.1. Number of samples collected from sentinels

No. samplings:	1	2	3	4	5	6	7	Total
Sep. 2014	69	-	-	-	-	-	-	**69**
Jan. 2015	56	48	-	-	-	-	-	**104**
May 2015	74	42	31	-	-	-	-	**147**
Sep. 2015	18	57	41	30	-	-	-	**146**
Jan. 2016	6	14	49	31	28	-	-	**128**
May. 2016	1	6	10	36	28	25	-	**106**
Sep. 2016	0	2	7	13	29	22	25	**98**
Total	**224**	**169**	**138**	**110**	**85**	**47**	**25**	**798**

Table 6.2. Drop'out rate and lost to follow-up:

cohort	Sep.14				Jan.15				May.15				Overall		
sample date	in	out	Pop.	test	in	out	Pop.	test	in	out	Pop.	test	in	out	Pop.
Sep.14	69	-	69	69	-	-	-	-	-	-	-	-	69	0	69
Jan.15	1	0	70	49	55	-	55	55	-	-	-	-	56	0	125
May.15	2	8	64	36	10	1	64	49	62	-	62	62	74	9	190
Sep.15	1	4	61	43	1	1	64	42	16	0	78	61	18	5	203
Jan.16	0	2	59	39	1	4	61	32	5	1	82	57	6	7	202
May.16	0	2	57	33	1	3	59	27	0	4	78	46	1	9	194
Sep.16	0			37	0			23	0			38			
Overall	73	16			68	9			83	5			224	30	194

Appendix 10 ***InBios DENV DetectTM IgM Capture ELISA Protocol***

1. Dilution of samples and controls to 1/100 using the provided DENV Sample Dilution Buffer.
2. Apply 50 µl per well of 1/100 diluted test sera, Dengue IgM Negative Control, and Dengue IgM Positive Control to the plate
3. Cover the plate with parafilm on the well opening surface.
4. Incubate the plate at 37ºC for 1 hour
5. After the incubation, wash the plate 6 times with an automatic plate washer using 1x wash buffer. Use 300 µl per well in each wash cycle.
6. Add 50µl per well of DENRA into rows A-D and 50µl per well of NCA into rows E-H by multi-channel pipette (as in plate layout).
7. Cover the plate with parafilm just on the well opening surface.
8. Incubate the plate at 37ºC for 1 hour.
9. Wash the plate 6 times with an automatic plate washer using 1x wash buffer. Use 300 µl per well in each wash cycle.
10. Add 50 µl per well of ready to use Enzyme-HRP conjugate into all wells by multi-channel pipette.
11. Cover the plate with parafilm just on the well opening surface.
12. Incubate the plate at 37ºC for 1 hour.
13. Wash the plate 6 times with an automatic plate washer using 1x wash buffer. Use 300 µl per well in each wash cycle.
14. Add 150µl per well of EnWash into all wells by multi- channel pipette.
15. Incubate the uncovered plate at room temperature for 5 minutes.
16. Wash the plate 6 times with an automatic plate washer using 1x wash buffer. Use 300 µl per well in each wash cycle
17. Add 75 µl/well of Liquid TMB substrate into all wells using a multi-channel pipette.
18. Incubate the plate at room temperature (20-25^{o}C) in a dark place (or container) for 10 minutes without any cover on the plate.
19. Add 50µl/well of Stop solution into all wells by multi-channel pipette and incubate at room temperature for 1 minute without cover on the plate.
20. Read the RAW OD 450 nm (optical density at 450 nm) value with a Microplate reader. Do not subtract or normalize for any blank values or wells. This may result in low NCA values and incorrect ISR values.

Plate layout:

	1	2	3	4	5	6	7	8	9	10	11	12	
A	Neg												DENRA
B	Neg												
C	Pos												
D	Pos												
E	Neg												NCA
F	Neg												
G	Pos												
H	Pos												

Appendix 11 *In-house Miagostovich ELISA Protocol*

Anti-DENV IgG ELISA

Test:_____ Date: _____

	1	2	3	4	5	6	7	8	9	10	11	12	
A	S8	S7	S6	S5	S4	S3	S2	S1	HP	LP	N2	N1	1:40
B													1:160
C													1:640
D													1:2,560
E													1:10,240
F													1:40,960
G													1:163,840
H													1:655,360

Serum Dilutions ↓

___ 1. Cover plate with 100µl/well of **4G2 Monoclonal Ab** (Lot # 0303046) diluted at 1:30,000 ; incubate O/N at 4°C.
Plates sensitized on:_____

___ 2. Wash plate 3X with PBS

___ 3. Fill wells to the top with **standard diluent**: PBS + 0.05% Tween 20 + 3% NGS. Incubate **uncovered 1 hour**/ 37°C.

___ 4. Tap plates to remove blocking buffer (do not wash).

___ 5. Prepare D1-4 antigen mix in **standard diluent** (need 8ml/ plate). Add 75µL/well; incubate **covered 1 hour**/ 37°C.

Antigen Mix

Serotype	Lot Number	Dilution	ml
D1	FCTC 00080-06-1	1:640	
D2	FCTC 00091-07-1	1:640	
D3	FCTC 00101-08	1:320	
D4	FCTC 00087-07-1	1:640	

___ 6. Wash 3X with PBS

___ 7. Dilute test and control serum 1:40 in **PBS + 0.05% Tween + 3% NFDM;** then mix in vortex.
N1_____ Low Positve _____
N2 _____ High Positive _____

___ 8. Dispense 100 µL/well of each diluted sample in row A according to template. Add 75µl/well of serum diluent to the rest of the plate and serially dilute 1:4 by transferring 25µl down the plate (see template) .

___ 9. Incubate covered for **1 hour** at 37°C.

___ 10. Wash 3X with PBS

___ 11, Dilute HRP-conjugated anti-human IgG 1:10,000 in PBS 0.05% tween/ 3% NFDM. Use 4 mL for 1 plate.
Add 40 µl/well ; incubate **1 hour** at room temperature. **Place solutions A and B at room temperature.**
Conjugate Lot # : 103344
Dilution: 1: 10,000

___ 12. Wash 6X with PBS

___ 13. Combine solutions A and B 1:1 once they are at room temperature. Add 100 µL per well. Incubate in dark at room temperature for **30-60 min**. Read at 405-410 nm. OD values ≥ 0.150 are considered positive.
The endpoint will be the highest dilution in which OD value is positive.

Appendix 12 ***Panbio Dengue IgG indirect ELISA Protocol***

1. Remove the required number of microwells from the foil sachet and insert into strip holder.
2. Using suitable test tubes dilute the Positive Control (P), Negative Control (N), Calibrator (CAL) and patient samples. To 10µl of samples add 1,000µl of Sample Diluent and mix well.
3. Pipette 100 µl of diluted patient sample, controls and calibrator into their respective microwells (as in plate layout)
4. Cover plate and incubate for 30 minutes at 37°C
5. Wash six times with diluted Wash Buffer
6. Pipete 100 µl HRP Conjugated Anti-human IgG to each well
7. Cover plate and incubate for 30 minutes at 37°C
8. Wash six times with diluted Wash Buffer
9. Pipette 100µl of TMB into each well
10. Incubate 10 minutes at room temperature (20-25oC)
11. Pipette 100µl of Stop Solution into all wells in the same sequence
12. Within 30 minutes read the absorbance of each well at a wavelength of 450nm with a reference filter of 600-650nm.

Plate layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cal											
B	Cal											
C	Cal											
D	Pos											
E	Neg											
F												
G												
H												

Appendix 13 ***EUROIMMUN Anti-Chikungunya virus ELISA (IgM / IgG)***

Sample incubation: (1st step)

(i) Transfer 100 µl of the calibrator, positive and negative controls or diluted patient samples into the individual microplate wells (as plate layout).

(ii) Cover the finished test plate with the protective foil

(iii) Incubate for 60 minutes at 37°C

Washing:

(iv) Remove the protective foil, empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.

(v) Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells.

Conjugate incubation:

(vi) Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgM) into each of the microplate wells.

(vii) Incubate for 30 minutes at room temperature (+18°C to +25°C).

Washing: (as steps 3 and 4 above)

Substrate incubation:

(viii) Pipette 100 µl of chromogen/substrate solution into each of the microplate wells.

(ix) Incubate for 15 minutes at room temperature (+18°C to +25°C), protect from direct sunlight.

Stopping:

(x) Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced

Measurement:

(xi) Photometric measurement at a wavelength of 450 nm and a reference wavelength between 620-650 nm within 30 minutes of adding the stop

solution. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Plate layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cal.1											
B	Cal.2											
C	Cal.3											
D	Pos											
E	Neg											
F												
G												
H												

Appendix 14 ***RNA Extraction Protocol: QIAamp® Viral RNA Mini Kit (QIAGEN®).***

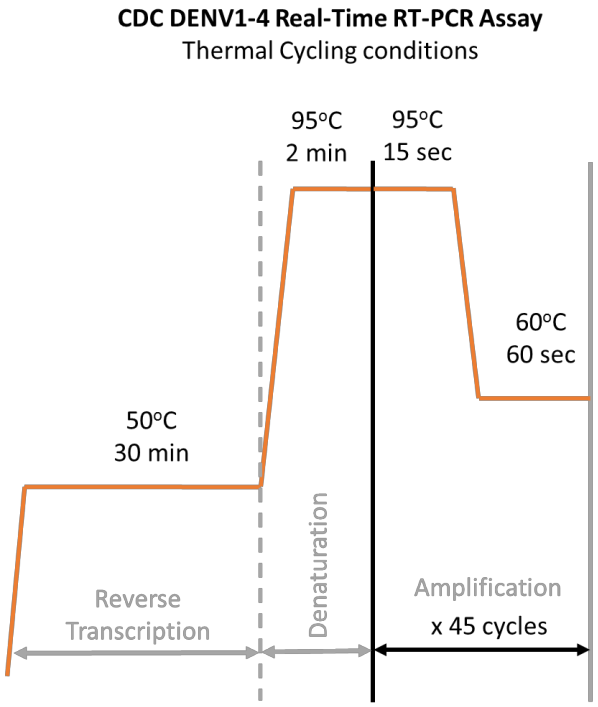
1. Pipet 560 µl of Buffer AVL containing carrier RNA into a 1.5 ml microcentrifuge tube in duplicate.
2. Add 140 µl plasma, serum or urine to the Buffer AVL-carrier RNA in the microcentrifuge tube and mix by pulse-vortexing for 15 s.
3. Incubate at room temperature for 10 minutes and briefly centrifuge the tube to remove drops from the inside of the lid.
4. Add 560 µl of ethanol (96-100%) to the sample and mix by pulse-vortexing for 15 s. Briefly centrifuge the tube to remove drops from inside the lid.
5. Carefully apply 630 µl of the solution from step 5 to the QIAamp Mini column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini column into a clean 2 ml collection tube, and discard the tube containing the filtrate.
6. Carefully open the QIAamp Mini column, and repeat step 6.
7. Carefully open the QIAamp Mini column, and add 500 µl of Buffer AW1. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini column in a clean 2 ml collection tube, and discard the tube containing the filtrate.
8. Carefully open the QIAamp Mini column, and add 500 µl of Buffer AW2. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.
9. Place the QIAamp Mini column in a new 2 ml collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.
10. Place the QIAamp Mini column in a clean 1.5 ml microcentrifuge tube. Discard the old collection tube containing the filtrate.
11. Carefully open the QIAamp Mini column and add 60 µl of Buffer AVE equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min. Centrifuge at 6000 x g (8000 rpm) for 1 min.
12. Store yielded RNA at -80^{o}C.

Appendix 15 *RT-PCR Protocol: DENV 1-4* (Johnson *et al.*, 2005)

DENV 1-4 Real-Time Assay

DENV MASTER MIX	
	ul
Water	2.2
2X Mix	12.5
Primer D1-F	0.5
Primer D1-R	0.5
Primer D2-F	0.25
Primer D2-R	0.25
Primer D3-F	0.5
Primer D3-R	0.5
Primer D4-F	0.25
Primer D4-R	0.25
Probes (1-4)	0.45
Probes (1-4)	0.45
Probes (1-4)	0.45
Probes (1-4)	0.45
TaqMan	0.5
Total =	20.00
Add 20ul in each well + 5 ul RNA	

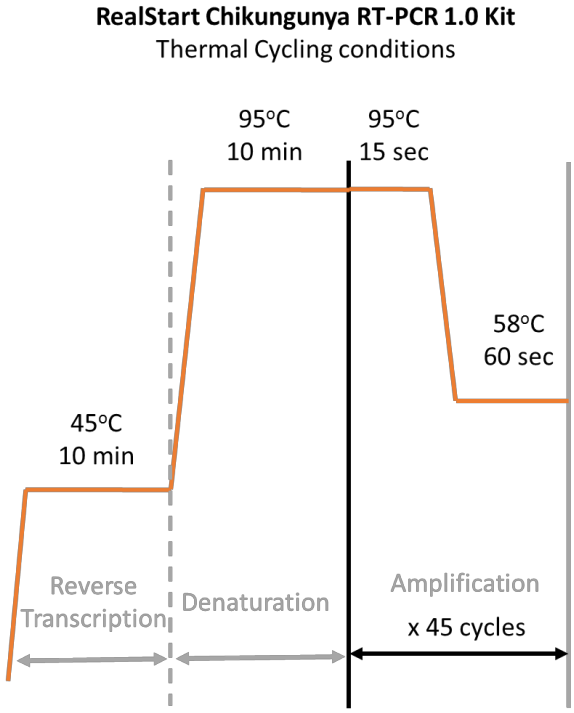
HSC MASTER MIX	
	ul
Water	5.5
2X Buffer Mix	12.5
Primer RP-F	0.5
Primer RP-R	0.5
Probe	0.5
TaqMan	0.5
Total =	20.00



Appendix 16 *RT-PCR Protocol: CHIKV RealStar Chikungunya 1.0 Assay (Altona Diagnostics, Germany)*

CHIKV MASTER MIX	
	ul
Mix Master A	2.5
Mix Master B	7.5
Internal Control	0.5
Total =	10.50

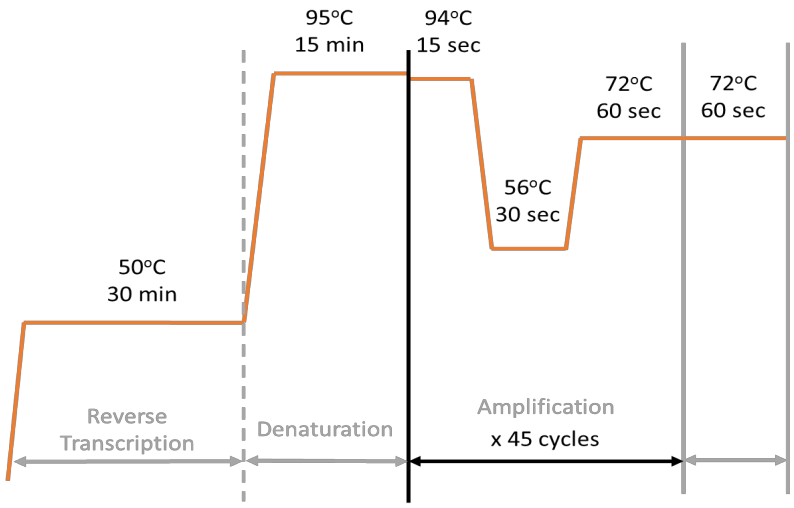
Add 10ul in each well + 5 ul RNA



Appendix 17 *PCR Protocol: CHIKV One-Step RT-PCR QIAGEN Assay*

ONE STEP RT-PCR QIAGEN	ul
Water	7.5
5x QIAGEN OneStep RT-PCR Buffer	5.0
dNTP Mix	1.0
Primer FG1 (100uM)	0.25
Primer FG2 (100uM)	0.25
OneStep RT-PCR Enzyme Mix	1.0
Total*=	15.00
Add 15ul of Master Mix and 10ul of RNA in each tube	

One-Step RT-PCR QIAGEN Assay
Thermal Cycling conditions



2% Agarose Gel	
TAE	100 ml
Agarose	2 g
EtBr	10 ul

	template	dye
Ladder	5.0	1.0
Sample	5.0	1.0
Load 5ul in each well		
Run:	70 Volt *(5 V/cm)*	40 minutes

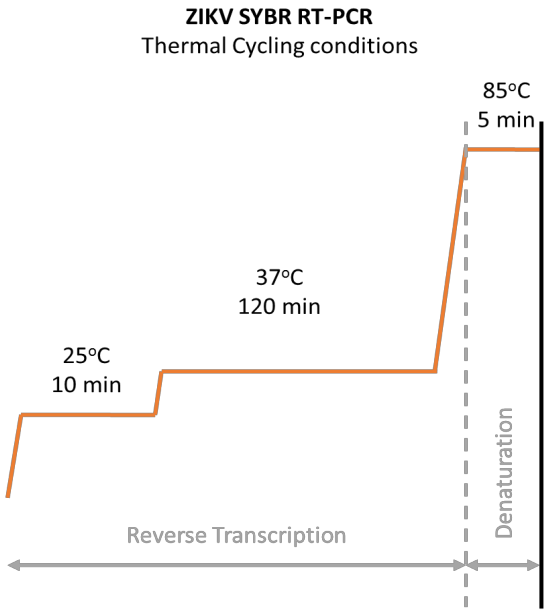
Gel Layout																
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Ladder																

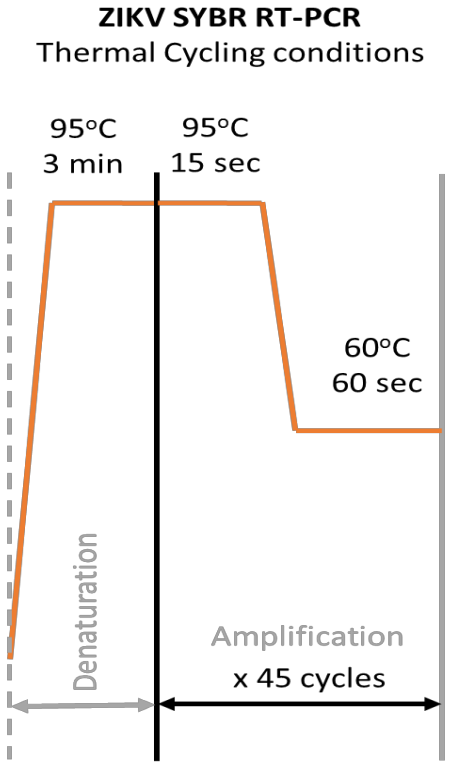
Appendix 18 *RT-PCR Protocol: ZIKV SYBR RT-PCR Assay (Lanciotti et al., 2008)*

REVERSE TRANSCRIPTION	
	ul
Water	4.2
10x RT Buffer	2.0
25X dNTP Mix	0.8
10X Random Primer	2.0
Multi ReverseTr	1.0
Total* =	10.00
Add 10ul of Master Mix and 10ul of RNA in each tube	

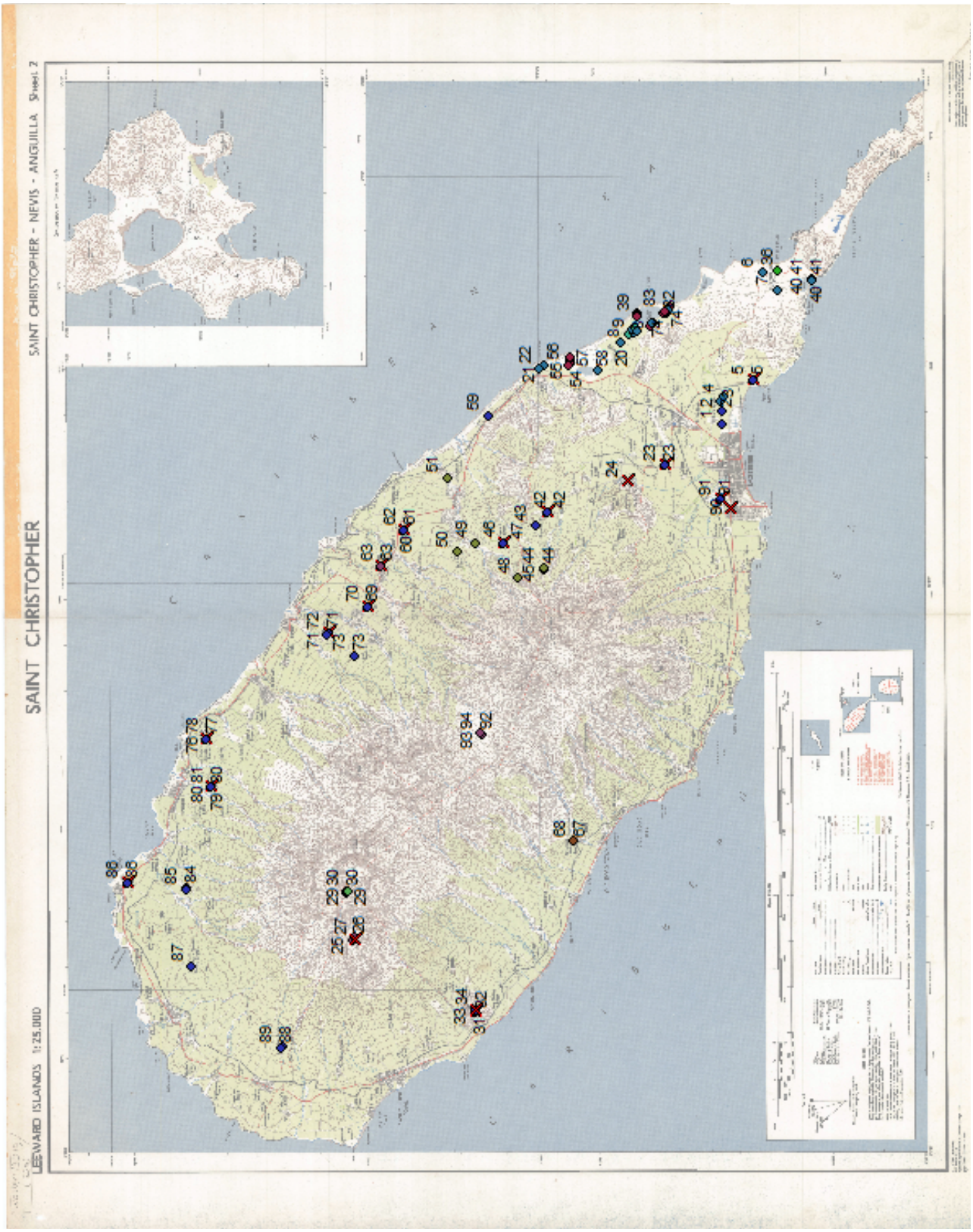
SYBR Green	ul
Water	4.8
Master Mix	10.0
Primer F Zika	0.1
Primer R Zika	0.1
Total =	15.00
Add 15 ul of Master Mix and 5ul cDNA	

HSC MASTER MIX	
	ul
Water	5.5
2X Buffer Mix	12.5
Primer RP-F	0.5
Primer RP-R	0.5
Probe	0.5
TaqMan	0.5
Total =	20.00

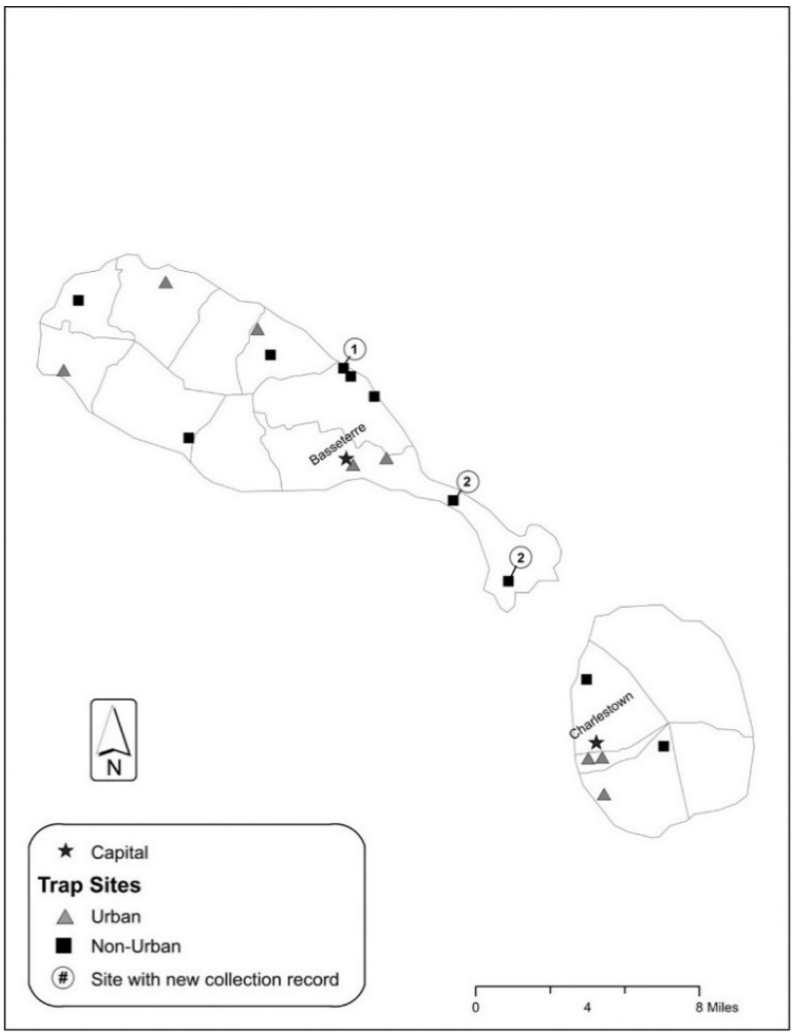




Appendix 19 ***Location of mosquito collection sites of survey performed by Belkin and Heinemann (1976). Illustration created with data from the authors.***



Appendix 20 ***Location of trap sites in the survey performed in 2010 by Mohammed et al., (2015). Reproduced with permission from the author.***



Appendix 21 ***Summary of mosquitoes captured***

2015	Species	Sex	Total captured	% per species	% total	Mosq. per trap	Maximum per trap	Minimum per trap
	Ae. aegypti	Female	488	50.4	22.7	4.31	23	0
		Male	474	49.0	22.0	4.79	44	0
	Aedes spp.	Male	3	0.3	0.1	3.00	3	3
	Ae. taeniorhynchus	Female	3	0.3	0.1	1.00	1	1
	Cx. quinquefasciatus	Female	478	40.4	22.2	2.73	21	1
		Male	614	51.9	28.6	8.77	72	1
	Culex spp.	Female	10	0.9	0.5	1.12	2	1
		Male	80	6.8	3.8	3.89	40	1
	Total		2,150				$\bar{x}$min = 25.75	$\bar{x}$min = 1
2016	Species	Sex	Total captured	% per species	% total	Mean per trap	Maximum per trap	Minimum per trap
	Ae. aegypti	Female	486	48.6	14.3	4.32	23	0
		Male	513	51.2	15.1	3.89	72	0
	Ae. taeniorhynchus	Female	2	0.2	0.1	1.00	1	1
	Cx. quinquefasciatus	Female	721	30.0	21.2	3.37	36	1
		Male	169	7.0	5.0	8.89	46	1
	Culex spp.	Female	69	2.9	2.0	1.77	7	1
		Male	1,442	60.5	42.6	20.03	185	1
	Total		3,402				$\bar{x}$max = 52.8	$\bar{x}$max = 0.74

Appendix 22 ***Distance between traps (meters).***

Area	Min.	Max.	Mean
Bird Rock	76	1,465	636.6
Bladens	54	54	-
Boyds'	78	357	170.6
Camps	74	679	322.9
Conaree	-	-	-
Frigate Bay	87	1234	530.7
Half Moon Bay	94	924	362.2
Mattingley	130	870	340.0
RUSVM Campus	9	458	212.6
West Farm	29	720	290.2
Reggae Beach	427	427	213.7
Shipwreck Beach	-	-	-
Monkey Hill	43	4484	2040.5
Shadwell	-	-	-
Peninsula	-	-	-
Sandy Point	271	271	135.6
Mean	85.8	746.4	350.3

Appendix 23 ***Percentage of trappings***

No.	Name of area	Land use	No. sites	No. trappings	% trappings
1	Airport	other	1	1	0.4
2	Basseterre Health Centre	other	1	1	0.4
3	Bird Rock	residential	6	19	8.2
4	Bladens	residential	2	6	2.6
5	Boyds	residential	18	26	11.3
6	Camps	residential	6	20	8.7
7	Conaree	residential	7	9	3.9
8	Frigate Bay	residential	9	24	10.4
9	Half Moon Bay	residential	7	19	8.2
10	Mattingley	residential	6	18	7.8
11	Monkey Hill	residential	3	16	6.9
12	Peninsula	residential	1	2	0.9
13	Reggae Beach	recreational	2	10	4.3
14	RUSVM Campus	study	8	22	9.5
15	Sandy Point	residential*	5	6	2.6
16	Shadwell	residential	1	1	0.4
17	Shipwreck Beach	recreational	1	4	1.7
18	West Farm	residential	11	27	11.7

Appendix 24 ***Entomological indices (Aedes aegypti both trapping seasons):***

2015 & 2016	Adults							Females						
address	ATI	Adults	A house 10	A house 30	A person 10	A person 30	ADI	ATI	Females	A house 10	A house 30	A person 10	A person 30	FADI
Airport	0.0	0	0.00	0.00	0.00	0.00	0.00	0.0	0	0.00	0.00	0.00	0.00	0.00
Basseterre Health Centre	0.0	0	0.00	0.00	0.00	0.00	0.00	0.0	0	0.00	0.00	0.00	0.00	0.00
Bird Rock	94.7	355	1.13	1.13	0.42	0.42	18.68	89.5	174	0.69	0.69	0.26	0.26	9.16
Bladens	100.0	56	0.93	0.93	0.35	0.35	9.33	83.3	24	0.50	0.50	0.19	0.19	4.00
Boyds	65.4	83	0.17	0.09	0.06	0.03	3.19	57.7	52	0.14	0.07	0.05	0.03	2.00
Camps	100.0	133	0.35	0.35	0.13	0.13	6.65	90.0	67	0.22	0.22	0.08	0.08	3.35
Conaree	77.8	36	0.34	0.20	0.13	0.07	4.00	66.7	21	0.25	0.15	0.09	0.05	2.33
Frigate Bay	79.2	130	0.19	0.09	0.07	0.03	5.42	66.7	71	0.13	0.06	0.05	0.02	2.96
Half Moon Bay	42.1	27	0.05	0.04	0.02	0.02	1.42	42.1	16	0.04	0.03	0.01	0.01	0.84
Mattingley	61.1	192	1.07	0.77	0.40	0.28	10.67	61.1	86	0.60	0.43	0.22	0.16	4.78
Monkey Hill	93.8	213	0.73	0.47	0.27	0.18	13.31	93.8	136	0.59	0.38	0.22	0.14	8.50
Peninsula	100.0	4	0.40	0.40	0.15	0.15	2.00	100.0	2	0.25	0.25	0.09	0.09	1.00
Reggae Beach	100.0	282	5.64	1.88	1.41	0.47	28.20	100.0	100	2.50	0.83	0.62	0.21	10.00
RUSVM Campus	77.3	151	0.40	0.22	0.05	0.01	6.86	68.2	72	0.24	0.13	0.03	0.01	3.27
Sandy Point	50.0	29	0.45	0.17	0.17	0.06	4.83	50.0	16	0.31	0.11	0.11	0.04	2.67
Shadwell	100.0	13	1.30	1.30	0.48	0.48	13.00	100.0	4	0.50	0.50	0.19	0.19	4.00
Shipwreck Beach	100.0	112	5.60	1.87	1.40	0.47	28.00	75.0	44	2.75	0.92	0.69	0.23	11.00
West Farm	81.5	145	0.35	0.21	0.13	0.08	5.37	74.1	89	0.27	0.16	0.10	0.06	3.30

Appendix 25 ***Appendix. Entomological indices (adult Aedes aegypti per trapping season):***

	2015							2016						
address	ATI	Adults	A house 10	A house 30	A person 10	A person 30	ADI	ATI	Adults	A house 10	A house 30	A person 10	A person 30	ADI
Airport	-	-	-	-	-	-	-	0.0	0	0.00	0.00	0.00	0.00	0.00
Basseterre Health Centre	-	-	-	-	-	-	-	0.0	0	0.00	0.00	0.00	0.00	0.00
Bird Rock	92.3	220	1.05	1.05	0.39	0.39	16.92	100.0	135	1.29	1.29	0.48	0.48	22.50
Bladens	100.0	56	0.93	0.93	0.35	0.35	9.33							
Boyds	83.3	28	0.35	0.35	0.13	0.13	4.67	60.0	55	0.14	0.06	0.05	0.02	2.75
Camps	100.0	84	0.40	0.40	0.15	0.15	7.64	100.0	49	0.29	0.29	0.11	0.11	5.44
Conaree	100.0	15	1.50	1.50	0.56	0.56	15.00	75.0	21	0.22	0.12	0.08	0.05	2.62
Frigate Bay	84.6	108	0.37	0.16	0.14	0.06	8.31	72.7	22	0.06	0.03	0.02	0.01	2.00
Half Moon Bay	20.0	11	0.04	0.04	0.02	0.01	1.10	66.7	16	0.05	0.04	0.02	0.02	1.78
Mattingley	54.5	166	1.51	1.38	0.56	0.51	15.09	71.4	26	0.37	0.20	0.14	0.07	3.71
Monkey Hill	87.5	76	0.58	0.36	0.22	0.13	9.50	100.0	137	0.86	0.57	0.32	0.21	17.12
Peninsula	100.0	4	0.40	0.40	0.15	0.15	2.00	-	-	-	-	-	-	-
Reggae Beach	100.0	53	5.30	1.77	1.32	0.44	26.50	100.0	229	5.72	1.91	1.43	0.48	28.62
RUSVM Campus	88.9	54	0.30	0.16	0.05	0.02	6.00	69.2	97	0.50	0.29	0.05	0.01	7.46
Sandy Point	-	-	-	-	-	-	-	50.0	29	0.45	0.17	0.17	0.06	4.83
Shadwell	100.0	13	1.30	1.30	0.48	0.48	13.00	-	-	-	-	-	-	-
Shipwreck Beach	-	-	-	-	-	-	-	100.0	112	5.60	1.87	1.40	0.47	28.00
West Farm	100.0	74	2.47	0.78	0.91	0.29	12.33	76.2	71	0.19	0.12	0.07	0.05	3.38

Appendix 26 ***Entomological indices (Female Aedes aegypti per trapping season):***

2015								2016						
address	**FATI**	**Females**	**A house 10**	**A house 30**	**A person 10**	**A person 30**	**FADI**	**FATI**	**Females**	**A house 10**	**A house 30**	**A person 10**	**A person 30**	**FADI**
Airport	-	-	-	-	-	-	-	0.0	0	0.00	0.00	0.00	0.00	0.00
Basseterre Health Centre	-	-	-	-	-	-	-	0.0	0	0.00	0.00	0.00	0.00	0.00
Bird Rock	84.6	105	0.62	0.62	0.23	0.23	8.08	100.0	69	0.82	0.82	0.30	0.30	11.50
Bladens	83.3	24	0.50	0.50	0.19	0.19	4.00	-	-	-	-	-	-	-
Boyds	83.3	21	0.33	0.33	0.12	0.12	3.50	50.0	31	0.10	0.05	0.04	0.02	1.55
Camps	90.9	46	0.27	0.27	0.10	0.10	4.18	88.9	21	0.15	0.15	0.06	0.06	2.33
Conaree	100.0	9	1.12	1.12	0.42	0.42	9.00	62.5	12	0.16	0.09	0.06	0.03	1.50
Frigate Bay	84.6	59	0.25	0.11	0.09	0.04	4.54	45.5	12	0.04	0.02	0.01	0.01	1.09
Half Moon Bay	20.0	7	0.03	0.03	0.01	0.01	0.70	66.7	9	0.04	0.03	0.01	0.01	1.00
Mattingley	54.5	69	0.78	0.72	0.29	0.27	6.27	71.4	17	0.30	0.16	0.11	0.06	2.43
Monkey Hill	87.5	46	0.44	0.27	0.16	0.10	5.75	100.0	90	0.70	0.47	0.26	0.17	11.25
Peninsula	100.0	2	0.25	0.25	0.09	0.09	1.00	-	-	-	-	-	-	-
Reggae Beach	100.0	24	3.00	1.00	0.75	0.25	12.00	100.0	76	2.38	0.79	0.59	0.20	9.50
RUSVM Campus	77.8	27	0.19	0.10	0.03	0.01	3.00	61.5	45	0.29	0.17	0.03	0.01	3.46
Sandy Point	-	-	-	-	-	-	-	50.0	16	0.31	0.11	0.11	0.04	2.67
Shadwell	100.0	4	0.50	0.50	0.19	0.19	4.00		-	-	-	-	-	-
Shipwreck Beach	-	-	-	-	-	-	-	75.0	44	2.75	0.92	0.69	0.23	11.00
West Farm	100.0	45	1.88	0.59	0.69	0.22	7.50	66.7	44	0.14	0.09	0.05	0.04	2.10

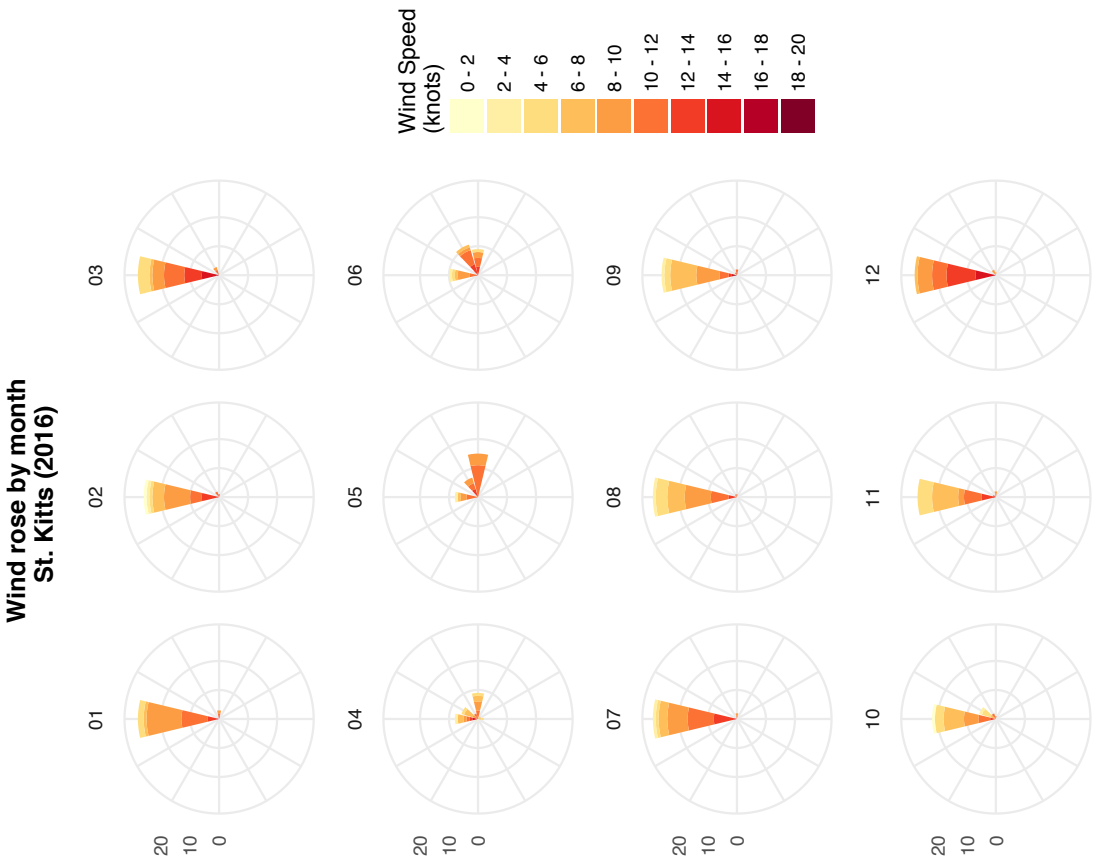
Appendix 27 ***Entomological indices (Outdoors only Female Aedes aegypti per trapping season):***

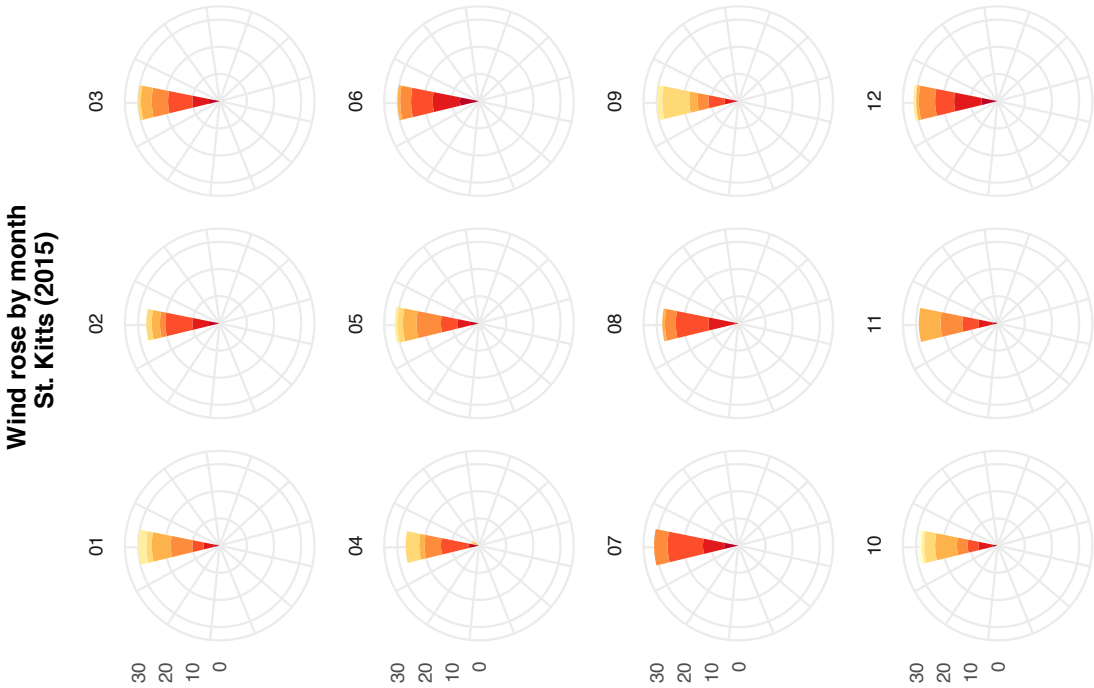
Female address	2015							2016						
	Positive traps	N traps	ATI	Total	Adults trap	Adults house	Adults person	Positive traps	N traps	ATI	Total	Adults trap	Adults house	Adults person
Bird Rock	11	13	84.6	105	8.1	0.2	0.1	6	6	100	69	11.5	0.2	0.1
Bladens	5	6	83.3	24	4	0.2	0.1	-	-	-	-	-	-	-
Boyds	5	6	83.3	21	3.5	0.1	0	3	5	60	7	1.4	0	0
Camps	10	11	90.9	46	4.2	0.3	0.1	8	9	88.9	21	2.3	0.1	0
Conaree	1	1	100	9	9	0	0	2	2	100	7	3.5	0	0
Frigate Bay	11	13	84.6	59	4.5	0.2	0.1	5	11	45.5	12	1.1	0	0
Half Moon Bay	2	10	20	7	0.7	0	0	6	9	66.7	9	1	0	0
Mattingley	6	11	54.5	69	6.3	0.8	0.3	4	6	66.7	16	2.7	0.2	0.1
Monkey Hill	7	8	87.5	46	5.8	0.1	0	8	8	100	90	11.2	0.1	0
Peninsula	2	2	100	2	1	0.1	0	-	-	-	-	-	-	-
Reggae Beach	2	2	100	24	12	8	0.1	6	6	100	48	8	16	0.2
RUSVM Campus	7	9	77.8	27	3	1.6	0	8	12	66.7	45	3.8	2.6	0
Sandy Point	-	-	-	-	-	-	-	2	2	100	15	7.5	NA	NA
Shadwell	1	1	100	4	4	0.8	0.3	-	-	-	-	-	-	-
Shipwreck Beach	-	-	-	-	-	-	-	3	3	100	44	14.7	14.7	0.2
West Farm	6	6	100	45	7.5	0.9	0.3	12	16	75	31	1.9	0.6	0.2
	76	99	83.3	34.9	5.3	1	0.1	73	95	76.8	31.8	5.4	2.9	0.1

Appendix 28 *Ae. aegypti captured by household PCI scores:*

PCI house	PCI yard	PCI shade	Sum of PCI scores	No. collections	Adults		Female	
					No. Mosquitoes	No. per collection	No. Mosquitoes	No. per collection
1	1	1	3	44	172	3.91	98	2.23
1	1	2	4	61	225	3.69	118	1.93
2	1	1	4	12	51	4.25	36	3.00
1	1	3	5	2	24	12.00	11	5.50
1	2	2	5	1	13	13.00	4	4.00
2	1	2	5	5	14	2.80	10	2.00
2	2	1	5	15	94	6.27	41	2.73
1	2	3	6	7	80	11.43	42	6.00
2	2	2	6	15	202	13.47	87	5.80
2	2	3	7	21	218	10.38	116	5.52
2	3	2	7	12	70	5.83	31	2.58
3	3	1	7	3	21	7.00	13	4.33
2	3	3	8	16	381	23.81	215	13.44
3	3	2	8	3	16	5.33	9	3.00
3	3	3	9	14	380	27.14	143	10.21
-	-	-	Total	231	1,961	11.9	974	5.64

Appendix 29 ***Wind rose representing the wind speed (in knots) and wind direction in 2015 and 2016 by month (Adapted from Clifton, 2013)***





Appendix 30 *Contribution of variables to PC's dimensions*

Environment I: Household	**Dim.1**	**Dim.2**	**Dim.3**	**Dim.4**
No. rooms in the house	42.3282292	4.86786232	0.00064384	6.1866178
Air-Conditioning	26.5406992	21.5749699	2.82478608	2.6711806
No. occupants in the house	21.2486749	29.5166842	0.07367732	0.6820782
Socio-economic area	6.0975763	36.8152023	2.50476009	7.0570058
Intact mosquito screens	2.2480100	1.42791200	23.0985212	33.933855
Water supply	1.1471780	0.03249957	47.1506340	1.1807557
Construction materials	0.3896324	5.76486964	24.3469773	48.288506
Environment II: larval habitats	**Dim.1**	**Dim.2**	**Dim.3**	**Dim.4**
Pools water	34.1113443	1.779994	11.1211742	3.640482
Debris around house	33.3376006	4.230024	0.3621111	30.451615
Plant pots	28.8433547	7.254406	0.1165734	56.050667
Trash collection	3.6000784	43.649354	39.1092572	3.632134
Storage water	0.1076221	43.086223	49.2908841	6.225102
Behaviours indoors	**Dim.1**	**Dim.2**	**Dim.3**	**Dim.4**
Length time in the porch	37.1355415	0.4183222	0.45835363	0.2367084
Part day in the porch: evening	21.7776104	3.9294285	27.40119181	0.3966712
Part day in the porch: mrng	19.8086023	0.4101717	1.92729928	11.0946269
Part day in the porch: aftrn	12.0821757	1.1155327	69.34852628	0.0100662
Door open for > 30 min.	8.8639214	25.1363478	0.78120140	61.5539264
Use of AC	0.3321488	68.9901972	0.08342759	26.7080010
Behaviours outdoors	**Dim.1**	**Dim.2**	**Dim.3**	**Dim.4**
Visit to bars / restaurants	41.748490	2.109160	12.602228514	43.540122
Visit to beach	41.316188	9.416458	0.008282589	49.259071
Hiking	15.46615	4 24.336883	58.386033022	1.810930
Visit to church	1.469168	64.137499	29.003455875	5.389878
Behaviours protection	**Dim.1**	**Dim.2**	**Dim.3**	**Dim.4**
Use of house spray	46.34377	1.937053	51.719181	-
Use of house coils	42.65448	10.839900	46.505621	-
Use of house repellent	11.00176	87.223046	1.775198	-
Mosquito bites	**Dim.1**	**Dim.2**	**Dim.3**	**Dim.4**
Bites outside / around the house	29.318531	0.69312896	18.2485751	0.01223108

Bites inside the house	25.024199	11.27623863	15.3780803	0.37528657
Bites at bar / restaurants	12.843735	26.48309070	6.3423692	9.94568816
Bites at beach	12.391001	32.50558879	0.3789936	0.35561179
Bites hiking	9.430406	0.04315494	12.7517381	74.11041496
Bites at grocery store	6.940748	17.37815989	10.1237212	0.37946289
Bites in church	4.051378	11.62063809	36.7765225	14.82130455

Appendix 31 *PCA Eigen values*

Environment I: Household	eigenvalue	Variance percent	Cumulative variance percent
Dim.1	1.6804736	24.006766	24.00677
Dim.2	1.2104094	17.291563	41.29833
Dim.3	1.1049278	15.784683	57.08301
Dim.4	0.9946125	14.208750	71.29176
Dim.5	0.9116988	13.024268	84.31603
Dim.6	0.7675713	10.965304	95.28133
Dim.7	0.3303066	4.718666	100.00000
Environment II: larval habitats			
Dim.1	1.3386301	26.77260	26.77260
Dim.2	1.0607597	21.21519	47.98780
Dim.3	0.9710537	19.42107	67.40887
Dim.4	0.8480979	16.96196	84.37083
Dim.5	0.7814585	15.62917	100.00000
Behaviours indoors			
Dim.1	1.9915471	33.19245	33.19245
Dim.2	1.0710560	17.85093	51.04339
Dim.3	0.9770489	16.28415	67.32753
Dim.4	0.8484882	14.14147	81.46900
Dim.5	0.7399924	12.33321	93.80221
Dim.6	0.3718674	6.19779	100.00000
Behaviours outdoors			
Dim.1	1.4332425	35.83106	35.83106
Dim.2	1.0844629	27.11157	62.94263
Dim.3	0.8618009	21.54502	84.48766
Dim.4	0.6204938	15.51234	100.00000
Behaviours protection			
Dim.1	1.3428492	44.76164	44.76164
Dim.2	0.9629795	32.09932	76.86096
Dim.3	0.6941713	23.13904	100.00000
Mosquito bites			
Dim.1	1.7435400	24.90771	24.90771
Dim.2	1.2758961	18.227087	43.13480
Dim.3	1.0585078	15.12154	58.25634
Dim.4	0.9042094	12.91727	71.17362
Dim.5	0.8648959	12.35565	83.52928
Dim.6	0.6286267	8.980382	92.50966
Dim.7	0.5243240	7.490343	100.00000

Appendix 32 *DENV sentinel positive cases.*

Table 6.3. No. of positive sentinel DENV cases according to the different cutoff indices. N: number of samples; n: number of positive cases; AP: Apparent Prevalence (%); new: number of new positive cases; I: incidence proportion (%). (Bottom) No. of positive DENV cases according to the different cutoff indices of potential seroconversions while on St. Kitts.

		InBios DENV DetectTM IgM Capture ELISA								Panbio Dengue IgG indirect ELISA								In-house (Miagostovich *et al*. protocol)							
		1.65				2.84				0.45				1.10				0.27				0.76			
Sampling	N	n	AP	new	I	n	AP	new	I	n	AP	new	I	n	AP	new	I	n	AP	new	I	n	AP	new	I
Sep. 2014	69	5	7.2	5	7.2	1	1.4	1	1.4	2	2.9	2	2.9	0	0.0	2	2.9	17	24.6	17	24.6	1	1.4	1	1.4
Jan. 2015	104	24	23.1	23	22.1	0	0.0	0	0.0	4	3.8	4	3.8	2	1.9	2	1.9	26	25.0	22	21.2	4	3.8	4	3.8
May 2015	147	44	29.9	34	23.1	3	2.0	2	1.4	5	3.4	3	2.0	3	2.0	1	0.7	62	42.2	42	28.6	17	11.6	13	8.8
Sep. 2015	146	31	21.2	20	13.7	2	1.4	2	1.4	4	2.7	1	0.7	3	2.1	0	0.0	52	35.6	10	6.8	5	3.4	0	0.0
Jan. 2016	128	5	3.9	1	0.8	0	0.0	0	0.0	3	2.3	1	0.8	2	1.6	0	0.0	19	14.8	0	0.0	5	3.9	0	0.0
May 2016	106	1	0.9	0	0.0	0	0.0	0	0.0	1	0.9	0	0.0	1	0.9	0	0.0	18	17.0	1	0.9	0	0.0	0	0.0
Sep. 2016	98	3	3.1	2	2.0	0	0.0	0	0.0	4	4.1	1	1.0	2	2.0	0	0.0	39	39.8	12	12.2	5	5.1	3	3.1
Suspected		1		1		0				0		1		1		1		-				-			
Total	224			86	38.4			5	2.2			13	5.8			6	2.7			104	46.4			21	9.4

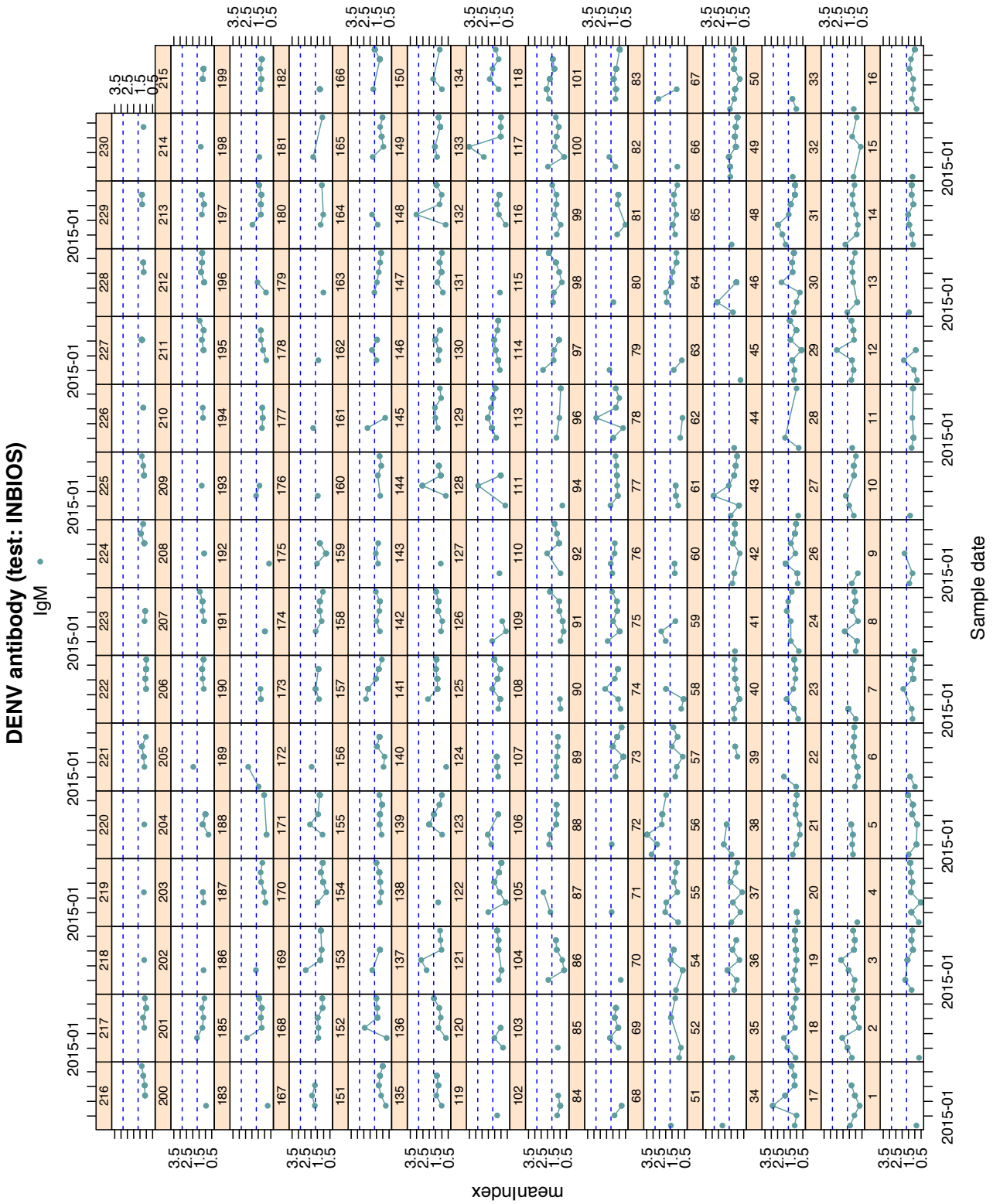
Appendix 33 *Number of positive samples for each virus*

virus		N positive	N sampled	%	95% C.I.
DENV	Sentinels	9	224	4.0%	1.9-7.5%
	Suspected	34	69	49.3%	37.0-61.6%
	Total	43	293	14.7%	10.8-19.3%
CHIKV	Sentinels	9	224	4.0%	1.9-7.5%
	Suspected	24	75	32.0%	21.7-43.8%
	Total	33	292	11.3%	7.9-15.5%
ZIKV	Sentinels	-	-	-	-
	Suspected	18	46	39.1%	25.1-54.6%
All arboviruses		73	303	24.1%	19.4-29.3%

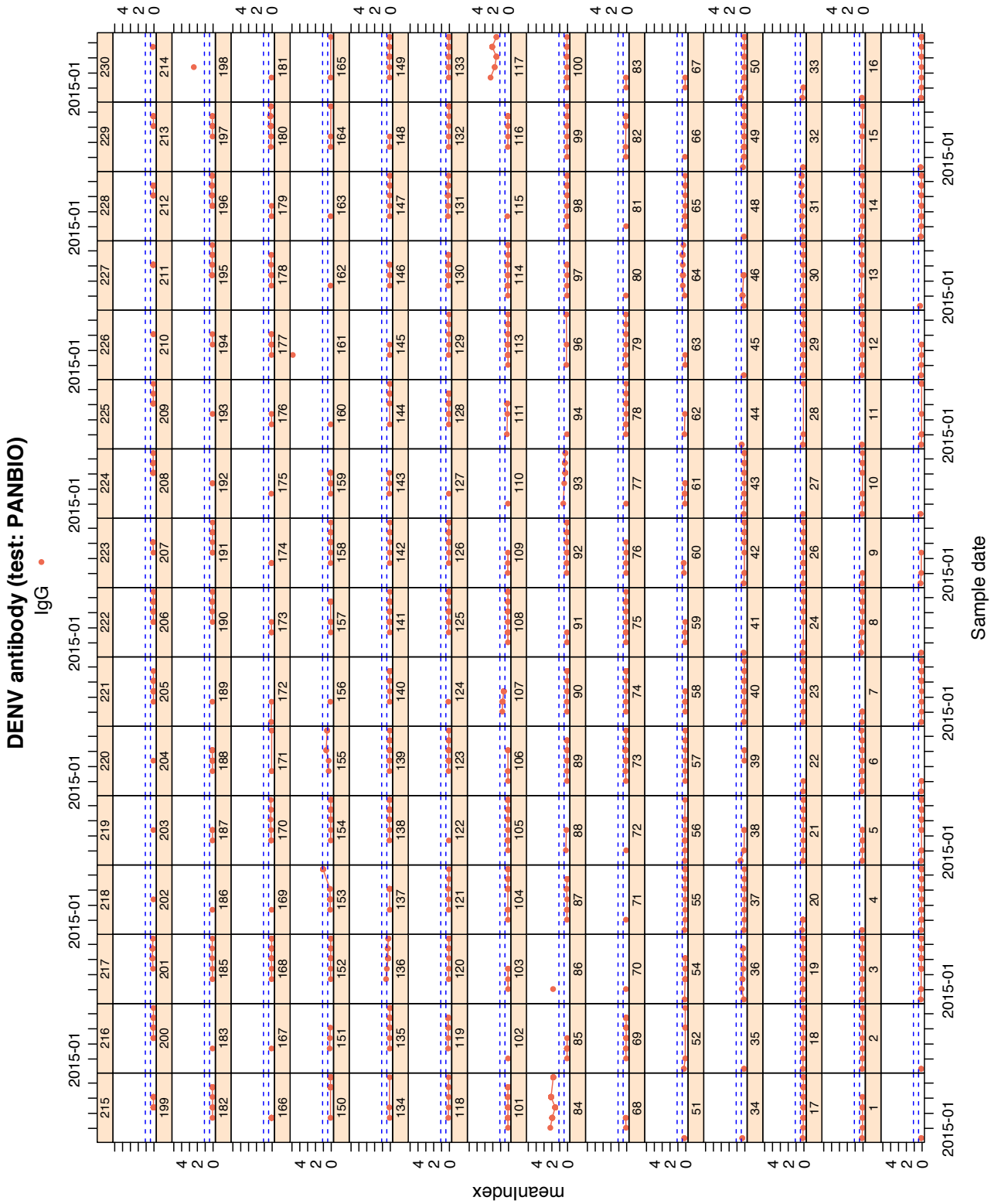
Table 6.4. Asymptomatic DENV ELISA positive cases. N: number of sentinels tested; AP: Apparent prevalence; I.P: Incidence Proportion

Sampling call	N	No. positive samples	AP	New positive samples	I.P.	Suspected seroconversions
Sep. 2014	69	1	1.4%	1	2.9%	0
Jan. 2015	104	2	1.4%	2	5.8%	0
May 2015	147	6	4.1%	4	10.2%	2
Sep. 2015	146	4	2.7%	2	1.4%	1
Jan. 2016	128	2	1.5%	0	0.0%	0
May 2016	106	1	0.9%	0	0.0%	0
Sep. 2016	98	2	2.0%	0	3.1%	1
Total no. of sentinels	224	18	8.0%	9	4.0%	4

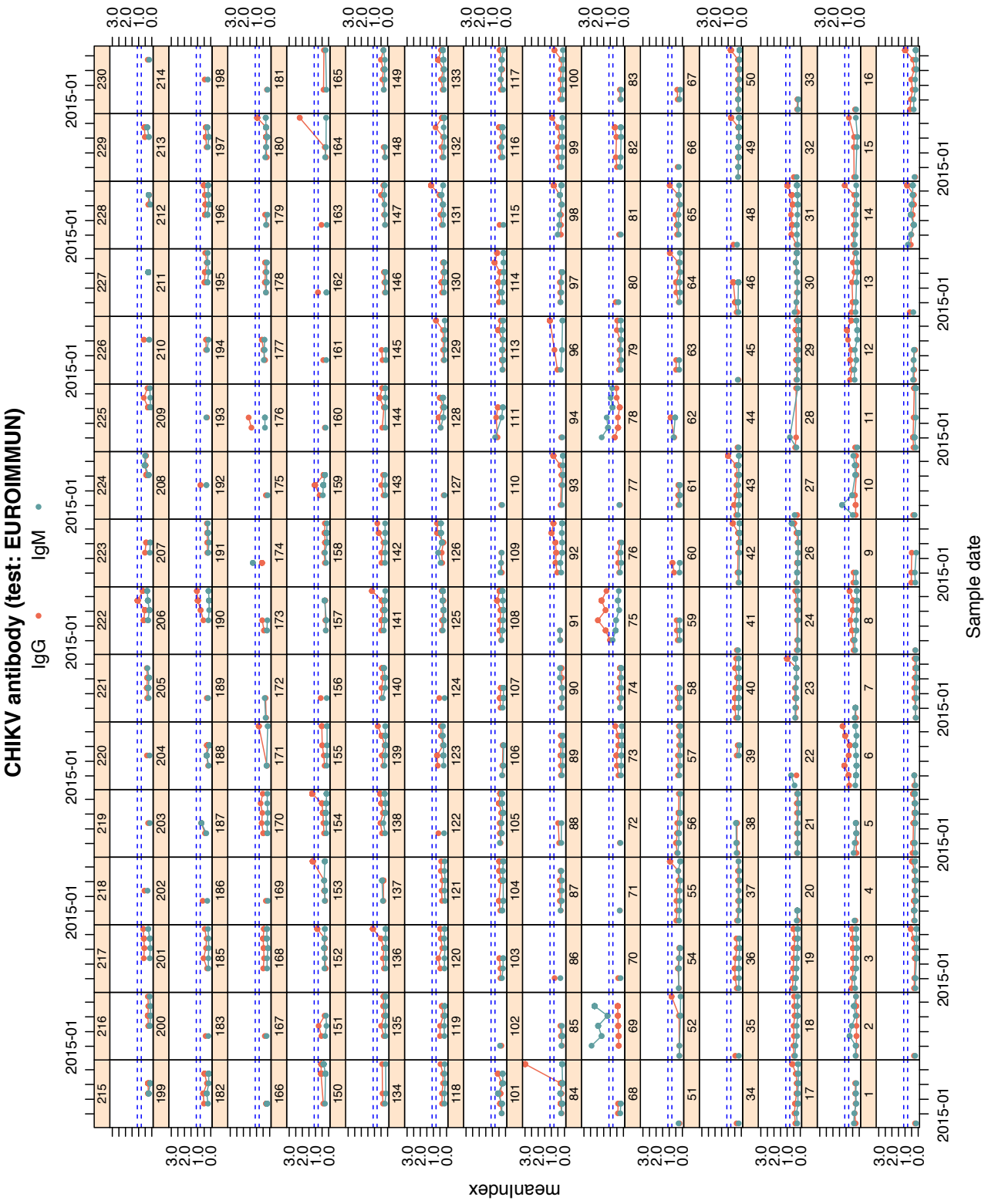
Appendix 34 *DENV index for sentinels (IgM – Inbios)*



Appendix 35 *DENV index for sentinels (IgG – Panbio)*



Appendix 36 *CHIKV index for sentinels*



Appendix 37 *List of R packages*

Name of package and Version	Reference
cccrm_1.2.1	(Carrasco and Martinez, 2015)
coda_0.19-1	(Plummer *et al.*, 2016)
devEMF_3.6	(Johnson, 2017)
dplyr_0.7.4	(Wickham *et al.*, 2017)
effects_4.0-0	(Fox *et al.*, 2017)
factoextra_1.0.5	(Kassambara and Mundt, 2017)
FactoMineR_1.39	(Husson *et al.*, 2017)
forecast_8.2	(Hyndman *et al.*, 2017)
Formula_1.2-2	(Zeileis and Croissant, 2017)
geepack_1.2-1	(Højsgaard, Halekoh and Yan, 2016)
ggcorrplot_0.1.1	(Kassambara, 2016)
ggformula_0.6.1	(Kaplan and Pruim, 2018)
ggmap_2.6.1	(Kahle and Wickham, 2016)
ggplot2_2.2.1	(Wickham and Chang, 2016)
ggpubr_0.1.6	(Kassambara, 2017a)
glmmADMB_0.8.3.3	(Skaug *et al.*, 2016)
Hmisc_4.1-1	(Harrell Jr, 2018)
knitr_1.18	(Xie, 2017)
lattice_0.20-35	(Sarkar, 2017)
lubridate_1.7.1	(Spinu, Grolemund and Wickham, 2017)
magrittr_1.5	(Bache and Wickham, 2014)
MASS_7.3-48	(Ripley, 2017)
mice_2.46.0	(van Buuren and Groothuis-Oudshoorn, 2017)
mosaic_1.1.1	(Pruim, Kaplan and Horton, 2017)
mosaicData_0.14.0	(Pruim, Kaplan and Horton, 2016)
MuMIn_1.40.0	(Barton, 2017)
nlme_3.1-131	(Pinheiro, Bates and R-core, 2017)
powerSurvEpi_0.0.9	(Qiu *et al.*, 2015)
R_3.4.1	(R Core Team, 2017)
RColorBrewer_1.1-2	(Neuwirth, 2014)

Name of package and Version	Reference
Reshape_0.8.7	(Wickham, 2017a)
reshape2_1.4.3	(Wickham, 2017b)
rjags_4-6	(Plummer, 2016)
rms_5.1-1	(Harrell Jr, 2017)
runjags_2.0.4-2	(Denwood, 2016)
shiny_1.0.5	(Chang *et al.*, 2015)
survival_2.41-3	(Therneau, 2017)
survminer_0.4.1	(Kassambara and Kosinski, 2017)
tidyr_0.7.2	(Wickham and Henry, 2017)

Appendix 38 *US Congressional Budget for Global Health Programs*

Table 6.5. US Congressional Budget for Global Health Programs by Strategic Framework ($ in thousands). (*) preventive drug treatments for seven of the most prevalent NTDs – schistosomiasis, onchocerciasis, lymphatic filariasis, trachoma, and three unspecificied soil-transmitted helminth. Adapted from: Department of State (2018).

		FY 2018 Total	HIV / AIDs	Tuberculosis	Malaria	NTD's	Nutrition/ Maternal and Child Health
Africa		4,220,525	3,450,000	63,750	364,000	-	342,775
East Asia and Pacific		131,500	64,500	31,850	12,000	-	23,150
Europe and Eurasia		33,880	30,000	3,880	-	-	-
Near East		3,500	-	-	-	-	3,500
South and Central Asia		112,785	17,500	26,660	-	-	68,625
Western Hemisphere		145,000	120,000	-	4,000	-	21,000
USAID Asia Regional		2,250	-	-	-	-	2,250
GH - Global Health		155,550	-	34,600	44,000	-	76,950
GH – International Partnerships	Vaccine Alliance	290,000	-	-	-	-	290,000
	NTD's(*)	75,000	-	-	-	75,000	-
	TB Drug Facility	13,500	-	13,500	-	-	-
	MDR Financing	4,160	-	4,160	-	-	-
S/GAC - Global AIDS Coordinator and Health Diplomacy	International Partnerships	1,125,000	1,125,000	-	-	-	-
	Oversight / Management	168,000	168,000	-	-	-	-
TOTAL		6,480,500	4,975,000	178,400	424,000	75,000	828,100

References:

van Aalst, M. *et al.* (2017) 'Long-term sequelae of chikungunya virus disease: A systematic review'. doi: 10.1016/j.tmaid.2017.01.004.

Ackroyd, S. and Hughes, J. A. (1981) *Data collection in context*. Longman (Aspects of modern sociology). Available at: https://books.google.com/books?id=HKTrAAAAMAAJ.

Akaike, H. (1973) 'Information theory and an extension of the maximum likelihood principle', in *2nd Inter. Symposium on Information Theory*. Budapest, pp. 267–281.

Althouse, B. M. *et al.* (2016) 'Potential for Zika virus to establish a sylvatic transmission cycle in the Americas', *PLoS Neglected Tropical Diseases*, 10(12), pp. 1–11. doi: 10.1101/047175.

Alto, B. W. and Juliano, S. A. (2001) 'Precipitation and Temperature Effects on Populations of Aedes albopictus (Diptera: Culicidae): Implications for Range Expansion', *J Med Entomol*, 38(5), pp. 646–656. doi: 10.1007/s11103-011-9767-z.Plastid.

Amarakoon, D. *et al.* (2008) 'Dengue epidemics in the Caribbean-temperature indices to gauge the potential for onset of dengue', *Mitigation and Adaptation Strategies for Global Change*, 13(4), pp. 341–357. doi: 10.1007/s11027-007-9114-5.

Anderson, R. M. and May, R. M. (1986) 'The invasion, persistence and spread of infectious diseases within animal and plant communities', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 314(1167), pp. 533–570. Available at: http://rstb.royalsocietypublishing.org/content/314/1167/533.short.

Ashford, D. A. *et al.* (2003) 'Outbreak of dengue fever in Palau, Western Pacific: Risk factors for infection', *American Journal of Tropical Medicine and Hygiene*, 69(2), pp. 135–140.

Åström, C. *et al.* (2012) 'Potential distribution of dengue fever under scenarios of climate change and economic development', *EcoHealth*, 9(4), pp. 448–454. doi: 10.1007/s10393-012-0808-0.

Atkinson, B. *et al.* (2016) 'Detection of zika virus in semen', *Emerging Infectious Diseases*, 22(5), p. 940. doi: 10.3201/eid2205.160107.

Aubry, M. *et al.* (2017) 'Zika virus seroprevalence, French Polynesia, 2014–2015', *Emerging Infectious Diseases*, 23(10), pp. 1751–1753. doi: 10.3201/eid2310.170583.

Aubry, M. *et al.* (2018) 'Seroprevalence of dengue and chikungunya virus antibodies, French Polynesia, 2014-2015', *Emerging Infectious Diseases*, 24(3), p. In press. doi: 10.3201/eid2403.171149.

de Ázara, T. M. F. *et al.* (2013) 'The impact of CO2 on collection of Aedes aegypti (Linnaeus) and Culex quinquefasciatus say by BG-sentinel traps in Manaus, Brazil', *Memorias do Instituto Oswaldo Cruz*, 108(2), pp. 229–232.

Babyak, M. A. (2004) 'What you see may not be what you get: A brief, nontechnical introduction to overfitting in regression-type models', *Psychosomatic Medicine*, 66(3), pp. 411–421. doi: 10.1097/01.psy.0000127692.23278.a9.

Bache, S. M. and Wickham, H. (2014) 'magrittr: A Forward-Pipe Operator for R'. Available at: https://cran.r-project.org/package=magrittr.

Ball, T. S. and Ritchie, S. R. (2010) 'Sampling biases of the BG-sentinel trap with respect to physiology, age, and body size of adult Aedes aegypti (Diptera: Culicidae).', *Journal of*

medical entomology. England, 47(4), pp. 649–56. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20695281.

Ballinger, G. A. (2004) 'Using Generalized Estimating Equations for Longitudinal Data Analysis', *Organizational Research Methods*, 7(2), pp. 127–150. doi: 10.1177/1094428104263672.

Balmaseda, A. *et al.* (2006) 'High seroprevalence of antibodies against dengue virus in a prospective study of schoolchildren in Managua, Nicaragua', *Tropical Medicine and International Health*, 11(6), pp. 935–942. doi: 10.1111/j.1365-3156.2006.01641.x.

Balmaseda, A. *et al.* (2010) 'Trends in patterns of dengue transmission over 4 years in a pediatric cohort study in Nicaragua.', *The Journal of infectious diseases*, 201, pp. 5–14. doi: 10.1086/648592.

Bangert, M. *et al.* (2017) 'The cross-cutting contribution of the end of neglected tropical diseases to the sustainable development goals', *Infectious Diseases of Poverty*. Infectious Diseases of Poverty, 6(1), pp. 1–20. doi: 10.1186/s40249-017-0288-0.

Banu, S. *et al.* (2015) 'Impacts of El Niño Southern Oscillation and Indian Ocean Dipole on dengue incidence in Bangladesh', *Scientific Reports*, 5. doi: 10.1038/srep16105.

Bargielowski, I. E., Lounibos, L. P. and Carrasquilla, M. C. (2013) 'Evolution of resistance to satyrization through reproductive character displacement in populations of invasive dengue vectors', *Proceedings of the National Academy of Sciences*, 110(8), pp. 2888–2892. doi: 10.1073/pnas.1219599110.

Barton, K. (2017) 'MuMIn: Multi-Model Inference'. Available at: https://cran.r-project.org/package=MuMIn.

Barzon, L. *et al.* (2016) 'Isolation of infectious zika virus from saliva and prolonged viral RNA shedding in a traveller returning from the dominican republic to Italy, January 2016', *Eurosurveillance*, 21(10), pp. 1–5. doi: 10.2807/1560-7917.

Basker, P. and Ezhil, R. (2012) 'Study on the Correlation of Premises Condition Index and the Presence of Larvae of Aedes Species Mosquitoes in Human Dwellings of the Cuddalore District of Tamil Nadu, India', *Osong Public Health and Research Perspectives*. Elsevier, 3(1), pp. 3–7. doi: 10.1016/j.phrp.2011.11.046.

Belkin, J. N. and Heinemann, S. J. (1976) 'Collection Records of the Project "Mosquitoes of Middle America". 4. Leeward Islands: Anguilla (ANG), Antigua (ANT), Barbuda (BAB), Monserrat (MNT), Nevis (NVS), St. Kitts (KIT)', *Mosquito Systematics*, 8(2), pp. 123–162.

Belkin, J. N., Heinemann, S. J. and Page, W. A. (1970) 'The Culicidae of Jamaica', *Contrib. Amer. Ent. Inst.*, 6(1), pp. 1–458. doi: 10.1007/s13398-014-0173-7.2.

Bhatt, S. *et al.* (2013) 'The global distribution and burden of dengue', *Nature*, 496(7446), pp. 504–507. doi: 10.1038/nature12060.

Biswas, H. H. *et al.* (2012) 'Early clinical features of dengue virus infection in Nicaraguan children: A longitudinal analysis', *PLoS Neglected Tropical Diseases*, 6(3). doi: 10.1371/journal.pntd.0001562.

Blacksell, S. D. *et al.* (2012) 'Short report: Comparison of performance of serum and plasma in panbio dengue and Japanese encephalitis virus enzyme-linked immunosorbent assays', *American Journal of Tropical Medicine and Hygiene*. United States, 87(3), pp. 573–575. doi: 10.4269/ajtmh.2012.12-0043.

Bland, J. M. and Altman, D. G. (1986) 'Statistical Methods for Assessing Agreement Between Two Methods of Clinical Measurement', *The Lancet*. Elsevier, 327(8476), pp. 307–310. doi: 10.1016/S0140-6736(86)90837-8.

Bloch, D. *et al.* (2016) 'Use of Household Cluster Investigations to Identify Factors Associated with Chikungunya Virus Infection and Frequency of Case Reporting in Puerto Rico', *PLoS Neglected Tropical Diseases*, 10(10). doi: 10.1371/journal.pntd.0005075.

Bolfa, P. *et al.* (2017) 'Detection of West Nile Virus and other common equine viruses in three locations from the Leeward Islands, West Indies', *Acta Tropica*. Elsevier, 174(April), pp. 24–28. doi: 10.1016/j.actatropica.2017.06.023.

Bolker, B. *et al.* (2012) 'Getting started with the glmmADMB package', *R package ver. 2.0–8*, p. 12.

Booth, G. D. (1994) *Identifying proxy sets in multiple linear regression : an aid to better coefficient interpretation*. 1st edn. Ogden, UT: U.S. Dept. of Agriculture. Available at: https://search.library.wisc.edu/catalog/999759331802121.

Bosch, O. J., Geier, M. and Boeckh, J. (2000) 'Contribution of fatty acids to olfactory host finding of female Aedes aegypti.', *Chemical senses*. England, 25(3), pp. 323–330.

Bouzid, M. *et al.* (2014) 'Climate change and the emergence of vector-borne diseases in Europe: case study of dengue fever', *BMC Public Health*, 14(1), p. 781. doi: 10.1186/1471-2458-14-781.

Bowman, L. R., Runge-Ranzinger, S. and McCall, P. J. (2014) 'Assessing the Relationship between Vector Indices and Dengue Transmission: A Systematic Review of the Evidence', *PLoS Neglected Tropical Diseases*, 8(5). doi: 10.1371/journal.pntd.0002848.

Brady, O. J. *et al.* (2012) 'Refining the Global Spatial Limits of Dengue Virus Transmission by Evidence-Based Consensus', *PLoS Neglected Tropical Diseases*, 6(8). doi: 10.1371/journal.pntd.0001760.

Brady, O. J. *et al.* (2013) 'Modelling adult Aedes aegypti and Aedes albopictus survival at different temperatures in laboratory and field settings.', *Parasites & vectors*, 6(1), p. 351. doi: 10.1186/1756-3305-6-351.

Brady, O. J. *et al.* (2014) 'Global temperature constraints on Aedes aegypti and Ae. albopictus persistence and competence for dengue virus transmission', *Parasites & Vectors*, 7(1), p. 338. doi: 10.1186/1756-3305-7-338.

Branscum, A. J., Gardner, I. A. and Johnson, W. O. (2005) 'Estimation of diagnostic-test sensitivity and specificity through Bayesian modeling', *Preventive Veterinary Medicine*, pp. 145–163. doi: 10.1016/j.prevetmed.2004.12.005.

Brouard, C. *et al.* (2008) 'Estimated risk of chikungunya viremic blood donation during an epidemic on reunion island in the indian ocean, 2005 to 2007', *Transfusion*, 48(7), pp. 1333–1341. doi: 10.1111/j.1537-2995.2008.01646.x.

Brown, J. E. *et al.* (2011) 'Worldwide patterns of genetic differentiation imply multiple "domestications" of Aedes aegypti, a major vector of human diseases', *Proceedings of the Royal Society B: Biological Sciences*. England, 278(1717), pp. 2446–2454. doi: 10.1098/rspb.2010.2469.

Brown, M. G. *et al.* (2009) 'Seroprevalence of dengue virus antibodies in healthy Jamaicans', *Human Antibodies*, 18(4), pp. 123–126. doi: 10.3233/HAB-2009-0207.

Browning, R. (1855) ‘Andrea del Sarto’, p. 1. Available at: https://www.poetryfoundation.org/poems/43745/andrea-del-sarto.

Brunkard, J. M. *et al.* (2007) ‘Dengue Fever Seroprevalence and Risk Factors , Texas – Mexico’, *Emerging Infectious Diseases*, 13(10), pp. 1477–1483. doi: 10.3201/eid1310.061586.

Brunkard, J. M., Cifuentes, E. and Rothenberg, S. J. (2008) ‘Assessing the roles of temperature, precipitation, and ENSO in dengue re-emergence on the Texas-Mexico border region’, *Salud Publica de Mexico*, 50(3), pp. 227–234. doi: 10.1590/S0036-36342008000300006.

Buonaccorsi, J. P., Harrington, L. C. and Edman, J. D. (2003) ‘Estimation and Comparison of Mosquito Survival Rates with Release-Recapture-Removal Data’, *Journal of Medical Entomology*, 40(1), pp. 6–17. doi: 10.1603/0022-2585-40.1.6.

Burke, D. S. *et al.* (1988) ‘A prospective study of dengue infections in Bangkok’, *American Journal of Tropical Medicine and Hygiene*. United States, 38(1), pp. 172–180. doi: 10.4269/ajtmh.1988.38.172.

Burnham, K. P. and Anderson, D. R. (2002) *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach (2nd ed)*, *Ecological Modelling*. doi: 10.1016/j.ecolmodel.2003.11.004.

van Buuren, S. and Groothuis-Oudshoorn, K. (2017) ‘mice: Multivariate Imputation by Chained Equations’. Available at: https://cran.r-project.org/package=mice.

Campbell, C. A. *et al.* (2007) ‘Seroprevalence of dengue in Trinidad using rapid test kits: A cord blood survey’, *Acta Tropica*, 101(2), pp. 153–158. doi: 10.1016/j.actatropica.2006.11.009.

Campbell, K. M. *et al.* (2015) ‘Weather regulates location, timing, and intensity of dengue virus transmission between humans and mosquitoes’, *PLoS Neglected Tropical Diseases*, 9(7), pp. 1–26. doi: 10.1371/journal.pntd.0003957.

Campbell, L. P. *et al.* (2015) ‘Climate change influences on global distributions of dengue and chikungunya virus vectors’, *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370(1665), pp. 20140135–20140135. doi: 10.1098/rstb.2014.0135.

Cao-Lormeau, V.-M. *et al.* (2014) ‘Zika virus, French Polynesia, South Pacific, 2013’, *Emerging infectious diseases*, 20(11), p. 1960. doi: 10.3201/eid2011.141380.

Caraguel, C. G. B. *et al.* (2011) ‘Selection of a Cutoff Value for Real-Time Polymerase Chain Reaction Results to Fit a Diagnostic Purpose: Analytical and Epidemiologic Approaches’, *Journal of Veterinary Diagnostic Investigation*, 23(1), pp. 2–15. doi: 10.1177/104063871102300102.

Carey, D. E. (1971) ‘Chikungunya and dengue: a case of mistaken identity?’, *Journal of the history of medicine and allied sciences*. England, 26(3), pp. 243–262.

Caribbean Development Bank (ed.) (2016) *The Changing Nature of Poverty and Inequality in the Caribbean: New issues, new solutions*. Wildey, St. Michael, Barbados: Caribbean Development Bank.

Carod-Artal, F. J. *et al.* (2013) ‘Neurological complications of dengue virus infection’, *The Lancet Neurology*, 12(9), pp. 906–919. doi: 10.1016/S1474-4422(13)70150-9.

Carpenter, S. and LaCasse, W. J. (1974) *Mosquitoes of North America*, *University of*

California Press. University of California Press.

CARPHA (2015) *Total Reported Cases of Chikungunya in Caribbean Countries or Territories and Neighbouring Mainland Countries (Reporting Dates: December, 2013 - May, 2015)*. Available at: http://carpha.org/What-We-Do/Public-Health-Activities/Chikungunya (Accessed: 14 April 2017).

Carrasco, J. L. and Martinez, J. P. (2015) 'cccrm: Concordance Correlation Coefficient for Repeated (and Non-Repeated) Measures'. Available at: http://cran.r-project.org/package=cccrm.

Carrington, L. B. *et al.* (2013) 'Effects of Fluctuating Daily Temperatures at Critical Thermal Extremes on Aedes aegypti Life-History Traits', *PLoS ONE*, 8(3). doi: 10.1371/journal.pone.0058824.

Cash, R. A. and Narasimhan, V. (2000) 'Impediments to global surveillance of infectious diseases: Consequences of open reporting in a global economy', *Bulletin of the World Health Organization*, 78(11), pp. 1358–1367. doi: 10.1057/palgrave.development.1110097.

Cassadou, S. *et al.* (2014) 'Emergence of chikungunya fever on the French side of Saint Martin island, October to December 2013', *Eurosurveillance*, 19(13), pp. 1–4. doi: 10.2807/1560-7917.ES2014.19.13.20752.

Cauchemez, S. *et al.* (2014) 'Local and regional spread of chikungunya fever in the Americas', *Eurosurveillance*, 19(28), p. 20854. doi: 10.2807/1560-7917.ES2014.19.28.20854.

Chadee, D. D. (2004) 'Key premises, a guide to Aedes aegypti (Diptera: Culicidae) surveillance and control', *Bulletin of Entomological Research*, 94(3), pp. 201–207. doi: 10.1079/BER2004297.

Chadee, D. D. *et al.* (2007) 'Climate, mosquito indices and the epidemiology of dengue fever in Trinidad (2002–2004)', *Annals of Tropical Medicine & Parasitology*, 101(1), pp. 69–77. doi: 10.1179/136485907X157059.

Chadee, D. D. *et al.* (2009) 'Aedes aegypti in Jamaica, West Indies: container productivity profiles to inform control strategies.', *Tropical medicine & international health : TM & IH*. England, 14(2), pp. 220–227. doi: 10.1111/j.1365-3156.2008.02216.x.

Chadee, D. D. (2009) 'Oviposition strategies adopted by gravid Aedes aegypti (L.) (Diptera: Culicidae) as detected by ovitraps in Trinidad, West Indies (2002-2006)', *Acta Tropica*, 111(3), pp. 279–283. doi: 10.1016/j.actatropica.2009.05.012.

Chadee, D. D. (2013) 'Resting behaviour of Aedes aegypti in Trinidad: with evidence for the re-introduction of indoor residual spraying (IRS) for dengue control.', *Parasites & vectors*, 6(1), p. 255. doi: 10.1186/1756-3305-6-255.

Chadee, D. D., Doon, R. and Severson, D. W. (2007) 'Surveillance of dengue fever cases using a novel Aedes aegypti population sampling method in Trinidad, West Indies: the cardinal points approach', *Acta Tropica*, 104(1), pp. 1–7. doi: 10.1016/j.actatropica.2007.06.006.

Chadee, D. D., Mahabir, R. S. and Sutherland, J. . (2012) 'Dengue Fever Epidemiology in the Caribbean: A Status Report (2012)', *Caribbean Medical Journal Commentary*, (January). Available at: https://www.researchgate.net/publication/261000519_Dengue_Fever_Epidemiology_and_Control_in_the_Caribbean_A_Status_Report_2012.

Chadee, D. D. and Rahaman, A. (2000) 'Use of water drums by humans and Aedes aegypti in Trinidad.', *Journal of vector ecology journal of the Society for Vector Ecology*. United States, 25(1), pp. 28–35.

Chadee, D. D., Williams, F. L. R. and Kitron, U. D. (2005) 'Impact of vector control on a dengue fever outbreak in Trinidad, West Indies, in 1998', *Tropical Medicine and International Health*, 10(8), pp. 748–754. doi: 10.1111/j.1365-3156.2005.01449.x.

Chang, W. *et al.* (2015) 'shiny: Web Application Framework for R'. Available at: https://cran.r-project.org/package=shiny.

Chaves, L. F. *et al.* (2012) 'Nonlinear impacts of climatic variability on the density-dependent regulation of an insect vector of disease', *Global Change Biology*, 18(2), pp. 457–468. doi: 10.1111/j.1365-2486.2011.02522.x.

Cheng, Q. *et al.* (2016) 'Climate and the Timing of Imported Cases as Determinants of the Dengue Outbreak in Guangzhou, 2014: Evidence from a Mathematical Model', *PLoS Neglected Tropical Diseases*, 10(2). doi: 10.1371/journal.pntd.0004417.

Choi, Y.-K. *et al.* (2006) 'Bayesian Inferences for Receiver Operating Characteristic Curves in the Absence of a Gold Standard', *Journal of Agricultural, Biological, and Environmental Statistics*, 11(2), pp. 210–229. doi: 10.1198/108571106X110883.

Chouin-Carneiro, T. *et al.* (2016) 'Differential Susceptibilities of Aedes aegypti and Aedes albopictus from the Americas to Zika Virus', *PLoS Neglected Tropical Diseases*, 10(3). doi: 10.1371/journal.pntd.0004543.

Ciota, A. T. *et al.* (2017) 'Vertical transmission of zika virus by Aedes aegypti and Ae. Albopictus mosquitoes', *Emerging Infectious Diseases*, 23(5), pp. 880–882. doi: 10.3201/eid2305.162041.

Claeys, C. *et al.* (2016) 'Socioecological factors contributing to the exposure of human populations to mosquito bites that transmit dengue fever, chikungunya and zika viruses: a comparison between mainland France and the French Antilles. Sous presse.', *Environ Risque Sante*, xx(xx), pp. 1–8. doi: 10.1684/ers.2016.0870.

Cobelens, F. G. J. *et al.* (2002) 'Incidence and risk factors of probable dengue virus infection among Dutch travellers to Asia', *Tropical Medicine and International Health*, 7(4), pp. 331–338. doi: 10.1046/j.1365-3156.2002.00864.x.

Codeço, C. T. *et al.* (2015) 'Surveillance of Aedes aegypti: Comparison of House Index with Four Alternative Traps', *PLoS Neglected Tropical Diseases*, 9(2), pp. 1–23. doi: 10.1371/journal.pntd.0003475.

Coffey, L. L., Failloux, A. B. and Weaver, S. C. (2014) 'Chikungunya virus–vector interactions', *Viruses*, 6(11), pp. 4628–4663. doi: 10.3390/v6114628.

Comach, G. *et al.* (2009) 'Dengue Virus Infections in a Cohort of Schoolchildren from Maracay, Venezuela: A 2-Year Prospective Study', *Vector-Borne and Zoonotic Diseases*, 9(1), pp. 87–92. doi: 10.1089/vbz.2007.0213.

Conway, M. J., Colpitts, T. M. and Fikrig, E. (2014) 'Role of the Vector in Arbovirus Transmission', *Annual Review of Virology*, 1(1), pp. 71–88. doi: 10.1146/annurev-virology-031413-085513.

Council of the European Union (2018a) *OUTCOME OF PROCEEDINGS: The EU list of non-cooperative jurisdictions for tax purposes*.

Council of the European Union (2018b) ‘Taxation: 3 jurisdictions removed, 3 added to EU list of non- cooperative jurisdictions’, *Council of the EU Recommendation*, pp. 1–2.

Cox, D. R. (1972) ‘Regression Models and Life-Tables’, *Journal of the Royal Statistical Society. Series B: Methodological*, 34(2), pp. 187–220. doi: 10.1007/978-1-4612-4380-9_37.

Cox, D. R. and Oakes, D. (1984) *Analysis of survival data*. 1st Editio. New York, NY: Chapman & Hall/CRC. doi: 10.1016/S0167-7306(08)60305-1.

Cromwell, E. A. *et al.* (2017) ‘The relationship between entomological indicators of Aedes aegypti abundance and dengue virus infection’, *PLoS Neglected Tropical Diseases*, 11(3). doi: 10.1371/journal.pntd.0005429.

Crowther, J. R. (1995) ‘ELISA. Theory and practice.’, *Methods in molecular biology (Clifton, N.J.)*. United States, 42, pp. 1–218. doi: 10.1385/0-89603-279-5:1.

Cummings, D. A. T. *et al.* (2009) ‘The impact of the demographic transition on dengue in Thailand: Insights from a statistical analysis and mathematical modeling’, *PLoS Medicine*, 6(9). doi: 10.1371/journal.pmed.1000139.

Darsie, R. J. and Ward, R. (2005) *Identification and geographical distribution of the mosquitoes of North America, north of Mexico.* Second Ed., *Mosquito Systematics*. Second Ed. University Press of Florida.

Daudens-Vaysse, E. *et al.* (2016) ‘Zika emergence in the French territories of america and description of first confirmed cases of Zika virus infection on Martinique, November 2015 to February 2016’, *Eurosurveillance*, 21(28), pp. 1–6. doi: 10.2807/1560-7917.ES.2015.20.34.30002.

David, M. R., Lourenço-de-Oliveira, R. and de Freitas, R. M. (2009) ‘Container productivity, daily survival rates and dispersal of Aedes aegypti mosquitoes in a high income dengue epidemic neighbourhood of Rio de Janeiro: Presumed influence of differential urban structure on mosquito biology’, *Memorias do Instituto Oswaldo Cruz*, 104(6), pp. 927–932. doi: 10.1590/S0074-02762009000600019.

Dayan, G. *et al.* (2015) ‘Prospective Cohort Study with Active Surveillance for Fever in Four Dengue Endemic Countries in Latin America’, *American Journal of Tropical Medicine and Hygiene*, 93(1738), pp. 18–23. doi: 10.4269/ajtmh.13-0663.

Delisle, E. *et al.* (2015) ‘Chikungunya outbreak in Montpellier, France, september to october 2014’, *Eurosurveillance*, 20(17). doi: Euro Surveill. 2015;20(17):pii=21108.

Dendukuri, N. and Joseph, L. (2001) ‘Bayesian approaches to modeling the conditional dependence between multiple diagnostic tests.’, *Biometrics*, 57(1), pp. 158–167. doi: 10.1111/j.0006-341X.2001.00158.x.

Denwood, M. (2016) ‘runjags: Interface Utilities, Model Templates, Parallel Computing Methods and Additional Distributions for MCMC Models in JAGS’. Available at: https://cran.r-project.org/package=runjags.

Dohoo, I. R., Martin, S. W. and Stryhn, H. (2012) *Methods in epidemiologic research*. Edited by S. W. Martin, H. Stryhn, and I. R. Dohoo. Charlottetown, P.E.I.: VER Inc.

Dormann, C. F. *et al.* (2013) ‘Collinearity: A review of methods to deal with it and a simulation study evaluating their performance’, *Ecography*, 36(1), pp. 027–046. doi: 10.1111/j.1600-0587.2012.07348.x.

Downes, A. S. (2010) *Poverty and its Reduction in the Small Developing Countries of the*

*Caribbean *.*

Downes, A. S. and Downes, D. A. (2003) *The Millennium Development Goals in the Eastern Caribbean : A Progress Report * List of Acronyms*.

Duffy, et. al *et al.* (2009) 'Zika Virus Outbreak on Yap Island, Federated States of Micronesia — NEJM', *New England Journal of Medicine*, p. 360(24), 2536-2543. Available at: http://www.nejm.org/doi/full/10.1056/NEJMoa0805715.

Duong, V. *et al.* (2015) 'Asymptomatic humans transmit dengue virus to mosquitoes', *Proceedings of the National Academy of Sciences*. United States, 112(47), pp. 14688–14693. doi: 10.1073/pnas.1508114112.

Earnest, A., Tan, S. B. and Wilder-Smith, A. (2012) 'Meteorological factors and El Niño Southern Oscillation are independently associated with dengue infections', *Epidemiology & Infection*, 140(07), pp. 1244–1251. doi: 10.1017/S095026881100183X.

ECDC, E. C. for D. P. and C. (2017) *CDTR. Week 39*.

Edelman, R. *et al.* (1994) 'A live attenuated dengue-1 vaccine candidate (45AZ5) passaged in primary dog kidney cell culture is attenuated and immunogenic for humans.', *The Journal of infectious diseases*. United States, 170(6), pp. 1448–1455.

Effler, P. V. *et al.* (2005) 'Dengue fever, Hawaii, 2001-2002', *Emerging Infectious Diseases*, 11(5), pp. 742–749. doi: 10.3201/eid1105.041063.

Eisen, L. and Moore, C. G. (2013) 'Aedes (Stegomyia) aegypti in the continental United States: a vector at the cool margin of its geographic range', *Journal of Medical Entomology*, 50(3), pp. 467–478. doi: http://dx.doi.org/10.1603/ME12245.

Endy, T. P., Chunsuttiwat, S., *et al.* (2002) 'Epidemiology of inapparent and symptomatic acute dengue virus infection: A prospective study of primary school children in Kamphaeng Phet, Thailand', *American Journal of Epidemiology*, 156(1), pp. 40–51. doi: 10.1093/aje/kwf005.

Endy, T. P., Nisalak, A., *et al.* (2002) 'Spatial and temporal circulation of dengue virus serotypes: A prospective study of primary school children in Kamphaeng Phet, Thailand', *American Journal of Epidemiology*, 156(1), pp. 52–59. doi: 10.1093/aje/kwf006.

Endy, T. P. *et al.* (2011) 'Determinants of inapparent and symptomatic dengue infection in a prospective study of primary school children in Kamphaeng Phet, Thailand', *PLoS Neglected Tropical Diseases*, 5(3), p. e975. doi: 10.1371/journal.pntd.0000975.

Endy, T. P., Yoon, I.-K. and Mammen, M. P. (2010) 'Prospective Cohort Studies of Dengue Viral Transmission and Severity of Disease', *Curr Top Microbiol Immunol*, 338, pp. 1–13. doi: 10.1007/978-3-642-02215-9_1.

Enøe, C., Georgiadis, M. P. and Johnson, W. O. (2000) 'Estimation of sensitivity and specificity of diagnostic tests and disease prevalence when the true disease state is unknown', *Preventive Veterinary Medicine*, 45(1–2), pp. 61–81. doi: 10.1016/S0167-5877(00)00117-3.

Erlanger, T. E., Keiser, J. and Utzinger, J. (2008) 'Effect of dengue vector control interventions on entomological parameters in developing countries: A systematic review and meta-analysis', *Medical and Veterinary Entomology*, 22(3), pp. 203–221. doi: 10.1111/j.1365-2915.2008.00740.x.

European Centre for Disease Prevention and Control (2013) *Dengue outbreak in Madeira, Portugal, October-November 2012*. Edited by ECDC. Stockholm: doi: 10.2900/75830.

European Centre for Disease Prevention and Control (2017) 'Clusters of autochthonous chikungunya cases in Conclusions and options for response', *Stockholm: ECDC*, (October). Available at: https://ecdc.europa.eu/sites/portal/files/documents/RRA-chikungunya-Italy-update-9-Oct-2017.pdf.

Ezanno, P. *et al.* (2015) 'A generic weather-driven model to predict mosquito population dynamics applied to species of Anopheles, Culex and Aedes genera of southern France', *Preventive Veterinary Medicine*. Elsevier B.V., 120(1), pp. 39–50. doi: 10.1016/j.prevetmed.2014.12.018.

Fauci, A. S. and Morens, D. M. (2016) 'Zika Virus in the Americas — Yet Another Arbovirus Threat', *New England Journal of Medicine*, 374(7), pp. 601–604. doi: 10.1056/NEJMp1002530.

Fields, B., Knipe, D. and Howley, P. (2013) *Fields Virology*. Sixth Edit. Edited by D. M. Knipe and P. M. Howley. Philadelphia, PA: Lippincott Williams & Wilkins. doi: 9781451105636.

Fink, K. *et al.* (2009) 'Depletion of macrophages in mice results in higher dengue virus titers and highlights the role of macrophages for virus control', *European Journal of Immunology*, 39(10), pp. 2809–2821. doi: 10.1002/eji.200939389.

Fitzmaurice, G. M., Laird, N. M. and Ware, J. H. (2004) 'Applied longitudinal analysis', *Wiley series in probability and statistics*, p. xxv, 701 p. doi: 10.1198/jasa.2005.s24.

Flacio, E. *et al.* (2016) 'Spread and establishment of Aedes albopictus in southern Switzerland between 2003 and 2014: an analysis of oviposition data and weather conditions', *Parasites & Vectors*. Parasites & Vectors, 9(1), p. 304. doi: 10.1186/s13071-016-1577-3.

Focks, D. A. *et al.* (1993) 'Dynamic Life Table Model for Aedes aegypti (Diptera: Culicidae): Simulation Results and Validation', *Journal of Medical Entomology*, 30(6), pp. 1018–1028. doi: 10.1093/jmedent/30.6.1018.

Focks, D. A. *et al.* (2000) 'Transmission thresholds for dengue in terms of Aedes aegypti pupae per person with discussion of their utility in source reduction efforts', *American Journal of Tropical Medicine and Hygiene*, 62(1), pp. 11–18.

Focks, D. A. (2003) 'Review of Entomological Sampling Methods and Indicators for Dengue Vectors', *WHO Special Programme for Research and Training in Tropical Diseases*.

Focks, D. A. and Chadee, D. D. (1997) 'Pupal Survey: An Epidemiologically Significant Surveillance Method for Aedes aegypti: An Example Using Data from Trinidad', *The American Journal of Tropical Medicine and Hygiene*, 56(2), pp. 159–167. Available at: http://ajtmh.org/content/journals/10.4269/ajtmh.1997.56.159.

Forrester, N. L. *et al.* (2012) 'Vector-Borne Transmission Imposes a Severe Bottleneck on an RNA Virus Population', *PLoS Pathogens*, 8(9). doi: 10.1371/journal.ppat.1002897.

Fouque, F. *et al.* (2006) 'Aedes aegypti survival and dengue transmission patterns in French Guiana.', *Journal of vector ecology : journal of the Society for Vector Ecology*, 31(2), pp. 390–399. doi: 10.3376/1081-1710(2006)31[390:AASADT]2.0.CO;2.

Fouque, F., Garinci, R. and Gaborit, P. (2004) 'Epidemiological and entomological surveillance of the co-circulation of DENV-1, DENV-2 and DENV-4 viruses in Frech Guiana.', *Tropical Medicine and International Health*, 9(1), pp. 41–46.

Fox, J. *et al.* (2017) 'effects: Effect Displays for Linear, Generalized Linear, and Other Models'. Available at: https://cran.r-project.org/package=effects.

Fox, J. and Weisberg, S. (2011) 'Cox Proportional-Hazards Regression for Survival Data in R', in *Appendix to an R and S-PLUS Companion to Applied Regression*. Second Edi, pp. 1–20.

Gallian, P. *et al.* (2015) 'To the editor : Prospective detection of chikungunya virus in blood donors , Caribbean 2014', 123(23), pp. 3679–3682.

Gambel, J. M., Drabick, J. J. and Martinez-Lopez, L. (1999) 'Medical surveillance of multinational peacekeepers deployed in support of the United Nations Mission in Haiti, June-October 1995', *International Journal of Epidemiology*, 28(2), pp. 312–318. doi: 10.1093/ije/28.2.312.

Garcia-rejon, J. *et al.* (2008) 'Dengue Virus – Infected Aedes aegypti in the Home Environment', *The American Society of Tropical Medicine and Hygiene*, 79(6), pp. 940–950. doi: 79/6/940 [pii].

Gardner, J. *et al.* (2015) 'Infectious Chikungunya Virus in the Saliva of Mice, Monkeys and Humans', *Plos One*, 10, p. e0139481. doi: 10.1371/journal.pone.0139481.

Garrett-Jones, C. (1964) 'Prognosis for interruption of malaria transmission through assessment of the mosquito's vectorial capacity', *Nature*, pp. 1173–1175. doi: 10.1038/2041173a0.

Gelman, A. and Hill, J. (2007) *Data analysis using regression and multilevel/hierarchical models*, *Cambridge*. doi: 10.2277/0521867061.

Genosys Biotechnologies (2016) *Melting Temperature*. Available at: https://www.biophp.org/minitools/melting_temperature/demo.php?formula=basic (Accessed: 8 October 2016).

Georgiadis, M. P. *et al.* (2003) 'Correlation-adjusted estimation of sensitivity and specificity of two diagnostic tests', *Journal of the Royal Statistical Society. Series C: Applied Statistics*, 52(1), pp. 63–76. doi: 10.1111/1467-9876.00389.

Georgiadis, M. P. *et al.* (2010) 'Estimation of Sensitivity and Specificity of Two Diagnostic Tests', *Appl. Statist.*, 52(1), pp. 63–76. Available at: http://www.jstor.org/stable/3592632.

Gérardin, P. *et al.* (2008) 'Estimating Chikungunya prevalence in La Réunion Island outbreak by serosurveys: two methods for two critical times of the epidemic.', *BMC infectious diseases*, 8, p. 99. doi: 10.1186/1471-2334-8-99.

Gething, P. W. *et al.* (2011) 'Modelling the global constraints of temperature on transmission of Plasmodium falciparum and P. vivax', *Parasites & Vectors*, 4(1), p. 92. doi: 10.1186/1756-3305-4-92.

Getis, A. *et al.* (2003) 'Characteristics of the Spatial Pattern of the Dengue Vector , Aedes Aegypti , in Iquitos , Peru', 69(5), pp. 494–505.

Gjenero-Margan, I. *et al.* (2011) 'Autochthonous dengue fever in Croatia, August- September 2010', *Eurosurveillance*, 16(9), pp. 1–4. doi: 19805 [pii].

Le Goff, G. *et al.* (2011) 'Natural vertical transmission of dengue viruses by Aedes aegypti in Bolivia.', *Parasite (Paris, France)*, 18(3), pp. 277–80. doi: P3V183R11 [pii].

Goindin, D. *et al.* (2015) 'Parity and Longevity of Aedes aegypti According to Temperatures in Controlled Conditions and Consequences on Dengue Transmission Risks', *Plos One*, 10, p. e0135489. doi: 10.1371/journal.pone.0135489.

Gómez-Dantés, H. and Willoquet, J. R. (2009) 'Dengue in the Americas: challenges for

prevention and control', *Cadernos de Saúde Pública*, 25(suppl 1), pp. S19–S31. doi: 10.1590/S0102-311X2009001300003.

Gómez, G. *et al.* (2009) 'Tutorial on methods for interval-censored data and their implementation in R', *Statistical Modelling: An International Journal*, 9(4), pp. 259–297. doi: 10.1177/1471082X0900900402.

Google Maps (2017) *Map of Saint Kitts [online]*, *Google*. Available at: https://www.google.com/maps/place/Saint+Kitts/@17.3035727,-62.7407458,12z/data=!4m5!3m4!1s0x8c121c7cbb89c1b3:0xddc8340c0ae20e02!8m2!3d17.3433796!4d-62.7559043 (Accessed: 24 March 2017).

Google Maps (2018) *Map of Saint Kitts [online]*, *Google*. Available at: https://www.google.com/maps/place/Saint+Kitts/@17.3035727,-62.7407458,12z/data=!4m5!3m4!1s0x8c121c7cbb89c1b3:0xddc8340c0ae20e02!8m2!3d17.3433796!4d-62.7559043 (Accessed: 15 February 2018).

Government of St. Kitts and Nevis (2006) 'Adaptation Strategy in Response to The New EU Sugar Regime. 2006-2013'. Basseterre, St. Kitts: Government of St. Kitts and Nevis - Ministry of Sustainable Development, p. 153. Available at: http://extwprlegs1.fao.org/docs/pdf/stk143763.pdf (Accessed: 11 April 2017).

Government of St. Kitts and Nevis (2016) *Henley and Partners reacts to CBS show and says St Kitts and Nevis Citizenship by Investment Program is one of the best in the world.* Available at: http://www.ciu.gov.kn/henley-and-partners-reacts-to-cbs-show-and-says-st-kitts-and-nevis-citizenship-by-investment-program-is-one-of-the-best-in-the-world/ (Accessed: 28 January 2018).

Grange, L. *et al.* (2014) 'Epidemiological risk factors associated with high global frequency of inapparent dengue virus infections', *Frontiers in Immunology*, 5(JUN), pp. 1–10. doi: 10.3389/fimmu.2014.00280.

Greiner, M. and Gardner, I. A. (2000) 'Application of diagnostic tests in veterinary epidemiologic studies.', *Preventive veterinary medicine*. Netherlands, 45(1–2), pp. 43–59.

Greiner, M., Sohr, D. and Göbel, P. (1995) 'A modified ROC analysis for the selection of cut-off values and the definition of intermediate results of serodiagnostic tests', *Journal of Immunological Methods*, 185(1), pp. 123–132. doi: 10.1016/0022-1759(95)00121-P.

Gubler, D. J. (1997) 'Epidemic dengue/dengue haemorrhagic fever: a global public health problem in the 21st century', *Dengue Bulletin*. ASM Press, 21(1), pp. 1–14. Available at: http://www.embase.com/search/results?subaction=viewrecord%7B&%7Dfrom=export%7B&%7Did=L37168479.

Gubler, D. J. (1998a) 'Dengue and dengue hemorrhagic fever', *Clinical Microbiology Reviews*, 11(3), pp. 480–496. doi: 10.1016/S0140-6736(97)12483-7.

Gubler, D. J. (1998b) 'Resurgent vector-borne diseases as a global health problem.', *Emerging infectious diseases*, 4(3), pp. 442–50. doi: 10.3201/eid0403.980326.

Gubler, D. J. *et al.* (2001) 'Climate variability and change in the United States: potential impacts on vector- and rodent-borne diseases.', *Environmental health perspectives*, 109 Suppl(May), pp. 223–33. doi: 10.2307/3435012.

Gubler, D. J. (2002) 'Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century', *Trends in Microbiology*, 10(2), pp. 100–103. doi: 10.1016/S0966-842X(01)02288-0.

Gubler, D. J. (2011) ‘Dengue, Urbanization and Globalization: The Unholy Trinity of the 21(st) Century.’, *Tropical Medicine and health*, 39(4 Suppl), pp. 3–11. doi: 10.2149/tmh.2011-S05.

Gubler, D. J. (2014) ‘Dengue Viruses: Their Evolution, History and Emergence as a Global Public Health Problem’, in Gubler, D. J. et al. (eds) *Dengue and Dengue Hemorrhagic Fever*. 2nd edn. Wallingford, Oxforshire: CABI, pp. 1–29.

Guo, X. *et al.* (2016) ‘Culex pipiens quinquefasciatus: a potential vector to transmit Zika virus.’, *Emerging microbes & infections*. Nature Publishing Group, 5(9), p. e102. doi: 10.1038/emi.2016.102.

Guzmán, M. G. *et al.* (2004) ‘Laboratory Diagnosis Of Dengue Infection: Epidemiology And Field Studies’, in TDR & WHO (ed.) *Dengue diagnostics : proceedings of an international workshop*. Geneva, pp. 20–27.

Guzmán, M. G. and Kourí, G. (2004) ‘Dengue diagnosis, advances and challenges’, *International Journal of Infectious Diseases*, 8(2), pp. 69–80. doi: 10.1016/j.ijid.2003.03.003.

Guzmán, M. G. and Vázquez, S. (2010) ‘The complexity of antibody-dependent enhancement of dengue virus infection’, *Viruses*, 2(12), pp. 2649–2662. doi: 10.3390/v2122649.

Hales, S. *et al.* (2002) ‘Potential effect of population and climate changes on global distribution of dengue fever: an empirical model’, *The Lancet*, 360(9336), pp. 830–834. doi: http://dx.doi.org/10.1016/S0140-6736(02)09964-6.

Halstead, S. B. (1979) ‘In vivo enhancement of dengue virus infection in rhesus monkeys by passively transferred antibody.’, *The Journal of infectious diseases*. United States, 140(4), pp. 527–533.

Halstead, S. B. (1983) ‘Original Antigenic Sin in Dengue’, *The American Society of Tropical Medicine and Hygiene*, 32(1), pp. 154–156. doi: https://doi.org/10.4269/ajtmh.1983.32.154.

Halstead, S. B. (1997) ‘Epidemiology of dengue and dengue hemorrhagic fever’, in Gubler, D. J. and Kuno, G. (eds) *Dengue and Dengue Hemorrhagic Fever*. Oxon: CABI, pp. 23–44.

Halstead, S. B. (2004) ‘Goals of the workshop’, in TDR & WHO (ed.) *Dengue Diagnostics: proceedings of an international*. Geneva, pp. 11–14.

Halstead, S. B. *et al.* (2005) ‘Dengue virus: Molecular basis of cell entry and pathogenesis, 25-27 June 2003, Vienna, Austria’, *Vaccine*, 23(7), pp. 849–856. doi: 10.1016/j.vaccine.2004.03.069.

Halstead, S. B. (2006) ‘Dengue in the Americas and Southeast Asia: do they differ?’, *Revista panamericana de salud publica = Pan American journal of public health*, 20(6), pp. 407–415. doi: 10.1590/S1020-49892006001100007.

Halstead, S. B. (2014) ‘Dengue Antibody-Dependent Enhancement: Knowns and Unknowns’, *Microbiology Spectrum*, 2(6), pp. 1–18. doi: 10.1128/microbiolspec.AID-0022-2014.

Halstead, S. B., Nimmannitya, S. and Cohen, S. N. (1970) ‘Observations related to pathogenesis of dengue hemorrhagic fever. IV. Relation of desease severity to antibody response and virus recovered’, *Yale Journal of Biology and Medicine*, 42, pp. 311–328. doi: 10.1086/315215.

Hardin, J. W. and Hilbe, J. M. (2002) *Generalized Generalized Estimating Estimating Equations eneralized Generalized Estimatin Estimating Equations Equations*. Chapman & Hall/CRC.

Harrell, F. E., Lee, K. L. and Mark, D. B. (1996) 'Tutorials in Biostatistics. Multivariable Prognostic Models: Issues in Developing Models, Evaluating Assumptions and Adequacy, and Measuring and Reducing Errors', *, Statistical Methods in Clinical Studies*, 15, pp. 361–387. doi: 10.1002/0470023678.ch2b(i).

Harrell Jr, F. E. (2017) 'rms: Regression Modeling Strategies'. Available at: https://cran.r-project.org/package=rms.

Harrell Jr, F. E. (2018) 'Hmisc: Harrell Miscellaneous'. Available at: https://cran.r-project.org/package=Hmisc.

Harrington, L. C. *et al.* (2001) 'Analysis of Survival of Young and Old Aedes aegypti (Diptera: Culicidae) from Puerto Rico and Thailand', *Journal of Medical Entomology*, 38(4), pp. 537–547. doi: 10.1603/0022-2585-38.4.537.

Harrington, L. C. *et al.* (2005) 'Dispersal of the dengue vector Aedes aegypti within and between rural communities', *American Journal of Tropical Medicine and Hygiene*, 72(2), pp. 209–220. doi: 72/2/209 [pii].

Harrington, L. C., Edman, J. D. and Scott, T. W. (2001) 'Why do female Aedes aegypti (Diptera: Culicidae) feed preferentially and frequently on human blood?', *Journal of Medical Entomology*, 38(3), pp. 411–422. doi: 10.1603/0022-2585-38.3.411.

Hayes, E. B. (2009) 'Zika virus outside Africa', *Emerging Infectious Diseases*, 15(9), pp. 1347–1350. doi: 10.3201/eid1509.090442.

Hesse, E. M. *et al.* (2017) 'Dengue virus exposures among deployed U.S. Military personnel', *American Journal of Tropical Medicine and Hygiene*, 96(5), pp. 1222–1226. doi: 10.4269/ajtmh.16-0663.

Hii, Y. L. *et al.* (2009) 'Climate variability and increase in intensity and magnitude of dengue incidence in Singapore', *Global Health Action*, 2(1), pp. 1–9. doi: 10.3402/gha.v2i0.2036.

Hoenig, J. M. and Heisey, D. M. (2001) 'The abuse of power: The pervasive fallacy of power calculations for data analysis', *American Statistician*, 55(1), pp. 19–24. doi: 10.1198/000313001300339897.

Højsgaard, S., Halekoh, U. and Yan, J. (2016) 'geepack: Generalized Estimating Equation Package'. Available at: https://cran.r-project.org/package=geepack.

Honório, N. A. *et al.* (2003) 'Dispersal of Aedes aegypti and Aedes albopictus (Diptera: Culicidae) in an Urban Endemic Dengue Area in the State of Rio de Janeiro, Brazil', *Memorias do Instituto Oswaldo Cruz*. Brazil, 98(2), pp. 191–198. doi: 10.1590/S0074-02762003000200005.

Honório, N. A. *et al.* (2009) 'Spatial evaluation and modeling of dengue seroprevalence and vector density in Rio de Janeiro, Brazil', *PLoS Neglected Tropical Diseases*, 3(11), pp. 1–11. doi: 10.1371/journal.pntd.0000545.

Horstick, O. *et al.* (2014) 'Comparing the usefulness of the 1997 and 2009 WHO dengue case classification: A systematic literature review', *American Journal of Tropical Medicine and Hygiene*, 91(3), pp. 621–634. doi: 10.4269/ajtmh.13-0676.

Horstick, O., Tozan, Y. and Wilder-Smith, A. (2015) 'Reviewing Dengue: Still a Neglected Tropical Disease?', *PLoS Neglected Tropical Diseases*, 9(4), pp. 1–18. doi: 10.1371/journal.pntd.0003632.

Hosmer, D. W. and Lemeshow, S. (2000) 'Applied Logistic Regression', *Wiley series in*

probability and statistics, p. 373. doi: 10.2307/2074954.

Hotez, P. J. (2013) 'NTDs V.2.0: "Blue Marble Health"-Neglected Tropical Disease Control and Elimination in a Shifting Health Policy Landscape', *PLoS Neglected Tropical Diseases*, 7(11). doi: 10.1371/journal.pntd.0002570.

Hsieh, F. Y. and Lavori, P. W. (2000) 'Sample-size calculations for the cox proportional hazards regression model with nonbinary covariates', *Controlled Clinical Trials*, 21(6), pp. 552–560. doi: 10.1016/S0197-2456(00)00104-5.

Huang, C. *et al.* (2001) 'Detection of arboviral RNA directly from mosquito homogenates by reverse-transcription–polymerase chain reaction', *Journal of Virological Methods*, 94(1), pp. 121–128. doi: http://dx.doi.org/10.1016/S0166-0934(01)00279-8.

Hugo, L. E. *et al.* (2014) 'Adult Survivorship of the Dengue Mosquito Aedes aegypti Varies Seasonally in Central Vietnam', *PLoS Neglected Tropical Diseases*, 8(2). doi: 10.1371/journal.pntd.0002669.

Hui, S. L. and Walter, S. D. (1980) 'Estimating the Error Rates of Diagnostic Tests', *Biometrics*. United States, 36(1), p. 167. doi: 10.2307/2530508.

Hunsperger, E. A. (2012) 'Flavivirus Diagnostics', in *Molecular Virology and Control of Flaviviruses*. Norfolk, UK: Caister Academic Press, pp. 271–296.

Husson, F. *et al.* (2017) 'FactoMineR: Multivariate Exploratory Data Analysis and Data Mining'. Available at: https://cran.r-project.org/package=FactoMineR.

Hyndman, R. *et al.* (2017) 'forecast: Forecasting Functions for Time Series and Linear Models'. Available at: https://cran.r-project.org/package=forecast.

Iyaloo, D. P. *et al.* (2014) 'Guidelines to site selection for population surveillance and mosquito control trials: A case study from Mauritius', *Acta Tropica*, 132(October 2015), pp. S140–S149. doi: 10.1016/j.actatropica.2013.11.011.

Jain, J. *et al.* (2016) 'Evidence for natural vertical transmission of chikungunya viruses in field populations of Aedes aegypti in Delhi and Haryana states in India—a preliminary report', *Acta Tropica*. Elsevier B.V., 162, pp. 46–55. doi: 10.1016/j.actatropica.2016.06.004.

Jänisch, T. *et al.* (1997) 'Emerging viral pathogens in long-term expatriates (II): Dengue virus', *Tropical Medicine and International Health*, 2(10), pp. 934–940. doi: 10.1046/j.1365-3156.1997.d01-149.x.

Johansson, M. A. *et al.* (2014) 'Nowcasting the spread of Chikungunya Virus in the Americas', *PLoS ONE*, 9(8). doi: 10.1371/journal.pone.0104915.

Johnson, B. W. *et al.* (2005) 'Serotype-Specific Detection of Dengue Viruses in a Fourplex Real-Time Reverse Transcriptase PCR Assay', *New York*, 43(10), pp. 4977–4983. doi: 10.1128/JCM.43.10.4977.

Johnson, P. (2017) 'devEMF: EMF Graphics Output Device'. Available at: https://cran.r-project.org/package=devEMF.

Johnson, P. H., Spitzauer, V. and Ritchie, S. A. (2012) 'Field Sampling Rate of BG-Sentinel Traps for Aedes aegypti (Diptera: Culicidae) in Suburban Cairns, Australia', *Journal of Medical Entomology*, 49(1), pp. 29–34. doi: 10.1603/ME11116.

Jolliffe, I. T. (2002) 'Principal Component Analysis, Second Edition', *Encyclopedia of Statistics in Behavioral Science*, 30(3), p. 487. doi: 10.2307/1270093.

Jose, J., Snyder, J. and Kuhn, R. (2009) ‘A structural and functional perspective of alphavirus replication and assembly’, *Future Microbiol*, 4, p. 30. doi: 10.2217/fmb.09.59.A.

Joseph, L. and Bélisle, P. (2017) ‘beta.parms.from.quantiles: [R] Computing Beta distribution parameters’. Available at: http://www.medicine.mcgill.ca/epidemiology/Joseph/PBelisle/BetaParmsFromQuantiles.html (Accessed: 20 November 2017).

Joseph, L., Gyorkos, T. W. and Coupal, L. (1995) ‘Bayesian estimation of disease prevalence and the parameters of diagnostic tests in the absence of a gold standard.’, *American journal of epidemiology*, 141(3), pp. 263–72. doi: 10.1093/oxfordjournals.aje.a117428.

Juliano, S. A., Lounibos, L. P. and O’Meara, G. F. (2004) ‘A field test for competitive effects of Aedes albopictus on A. aegypti in South Florida: Differences between sites of coexistence and exclusion?’, *Oecologia*, 139(4), pp. 583–593. doi: 10.1007/s00442-004-1532-4.

Jupp, P. G. *et al.* (1981) ‘Laboratory vector studies on six mosquito and one tick species with chikungunya virus.’, *Transactions of the Royal Society of Tropical Medicine and Hygiene*. England, 75(1), pp. 15–19.

Kahle, D. and Wickham, H. (2016) ‘ggmap: Spatial Visualization with ggplot2’. Available at: https://cran.r-project.org/package=ggmap.

Kamtchum-Tatuene, J. *et al.* (2017) ‘The potential role of Wolbachia in controlling the transmission of emerging human arboviral infections’, *Current Opinion in Infectious Diseases*, 30(1), pp. 108–116. doi: 10.1097/QCO.0000000000000342.

Kaplan, D. and Pruim, R. (2018) ‘ggformula: Formula Interface to the Grammar of Graphics’. Available at: https://cran.r-project.org/package=ggformula.

Kaplan, E. L. and Meier, P. (1958) ‘Nonparametric Estimation from Incomplete Observations’, *Journal of the American Statistical Association*, 53(282), pp. 457–481. doi: 10.1080/01621459.1958.10501452.

Kassambara, A. (2016) ‘ggcorrplot: Visualization of a Correlation Matrix using “ggplot2”’. Available at: https://cran.r-project.org/package=ggcorrplot.

Kassambara, A. (2017a) ‘ggpubr: “ggplot2” Based Publication Ready Plots’. Available at: https://cran.r-project.org/package=ggpubr.

Kassambara, A. (2017b) *Practical Guide to Principal Component Analysis (PCA) in R & Python*. 1st edn. STHDA. Available at: https://www.analyticsvidhya.com/blog/2016/03/practical-guide-principal-component-analysis-python/.

Kassambara, A. and Kosinski, M. (2017) ‘survminer: Drawing Survival Curves using “ggplot2”’. Available at: https://cran.r-project.org/package=survminer.

Kassambara, A. and Mundt, F. (2017) ‘factoextra: Extract and Visualize the Results of Multivariate Data Analyses’. Available at: https://cran.r-project.org/package=factoextra.

Kearney, M. *et al.* (2009) ‘Integrating biophysical models and evolutionary theory to predict climatic impacts on species’ ranges: The dengue mosquito Aedes aegypti in Australia’, *Functional Ecology*, 23(3), pp. 528–538. doi: 10.1111/j.1365-2435.2008.01538.x.

Kelly, M. G. E. (2015) *Biopolitical imperialism*. Winchester, UK: Zero books. Available at: http://www.zero-books.net/books/biopolitical-imperialism.

Kenney, J. L. *et al.* (2017) ‘Entomological Investigations During Early Stages of A

Chikungunya Outbreak In the United States Virgin Islands, 2014', *Journal of the American Mosquito Control Association*, 33(1), pp. 8–15. doi: 10.2987/16-6619.1.

Khan, K. *et al.* (2014) 'Assessing the origin of and potential for international spread of chikungunya virus from the Caribbean.', *PLoS currents*, 6, pp. 1–11. doi: 10.1371/currents.outbreaks.2134a0a7bf37fd8d388181539fea2da5.

Kit Lam, S. *et al.* (2000) 'Evaluation of a capture screening enzyme-linked immunosorbent assay for combined determination of immunoglobulin M and G antibodies produced during Dengue infection.', *Clinical and diagnostic laboratory immunology*, 7(5), pp. 850–2. doi: 10.1128/CDLI.7.5.850-852.2000.

St. Kitts Statistics Office (2015) *Annual Tourism Data: St. Kitts and Nevis*.

Klase, Z. A. *et al.* (2016) 'Zika Fetal Neuropathogenesis: Etiology of a Viral Syndrome', *PLOS Neglected Tropical Diseases*. Public Library of Science, 10(8), pp. 1–32. doi: 10.1371/journal.pntd.0004877.

Kleinbaum, D. G. and Klein, M. (2012) *Survival Analyais. A Self-Learning Text*. Third Edit, *Statistics for Biology and Health*. Third Edit. Edited by W. W. M. Gail, K. Krickeberg, J.M. Samet, A. Tsiatis. New York, NY: Springer. doi: 10.1017/CBO9781107415324.004.

Klowden, M. J. (1995) 'Blood, Sex, and the MosquitoThe mechanisms that control mosquito blood-feeding behavior', *BioScience*, 45(5), pp. 326–331. Available at: http://dx.doi.org/10.2307/1312493.

Knight, K. L. and Stone, A. (1977) *A catalog of the mosquitoes of the world (Diptera: Culicidae)*, *The Thomas Say Foundation*. College Park, Maryland: Entomological Society of America.

Kosasih, H. *et al.* (2013) 'Evidence for Endemic Chikungunya Virus Infections in Bandung, Indonesia', *PLoS Neglected Tropical Diseases*, 7(10), pp. 1–9. doi: 10.1371/journal.pntd.0002483.

Kraemer, M. U. G., Sinka, M. E., Duda, K. A., Mylne, A., *et al.* (2015) 'The global compendium of Aedes aegypti and Ae. albopictus occurrence', *Scientific Data*, 2, p. 150035. doi: 10.1038/sdata.2015.35.

Kraemer, M. U. G., Sinka, M. E., Duda, K. A., Mylne, A. Q. N., *et al.* (2015) 'The global distribution of the arbovirus vectors Aedes aegypti and Ae. Albopictus', *eLife*, 4(JUNE2015), p. 18. doi: 10.7554/eLife.08347.

Kuan, G. *et al.* (2009) 'The nicaraguan pediatric dengue cohort study: Study design, methods, use of information technology, and extension to other infectious diseases', *American Journal of Epidemiology*, 170(1), pp. 120–129. doi: 10.1093/aje/kwp092.

Kuan, G. *et al.* (2016) 'Seroprevalence of Anti-Chikungunya Virus Antibodies in Children and Adults in Managua, Nicaragua, After the First Chikungunya Epidemic, 2014-2015.', *PLoS neglected tropical diseases*, 10(6), p. e0004773. doi: 10.1371/journal.pntd.0004773.

Kucharski, A. J. *et al.* (2016) 'Transmission Dynamics of Zika Virus in Island Populations: A Modelling Analysis of the 2013–14 French Polynesia Outbreak', *PLoS Neglected Tropical Diseases*, 10(5), p. e0004726. doi: 10.1371/journal.pntd.0004726.

Kumar, A., Gittens-St Hilaire, M. and Nielsen, A. L. (2013) 'Epidemiological trends and clinical manifestations of dengue among children in one of the english-speaking caribbean countries', *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 107(4), pp. 254–260. doi: 10.1093/trstmh/trt007.

Kumar, C. V. M. N., Johnson, A. M. A. and Gopal, D. V. R. S. (2007) 'Molecular characterization of chikungunya virus from Andhra Pradesh, India & phylogenetic relationship with Central African isolates', *Indian J Med Res*, 126(6), pp. 534–540. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18219080.

Kumar, N. P. *et al.* (2011) 'Chikungunya virus outbreak in Kerala, India, 2007: a seroprevalence study ', *Memórias do Instituto Oswaldo Cruz* . scielo , pp. 912–916.

Kuno, G. (1995) 'Review of the factors modulating dengue transmission.', *Epidemiologic reviews*, 17(2), pp. 321–335. doi: 10.1093/oxfordjournals.epirev.a036196.

Kuno, G. (2001) 'Persistence of arboviruses and antiviral antibodies in vertebrate hosts: its occurrence and impacts.', *Reviews in medical virology*. England, 11(3), pp. 165–190.

Kuno, G. (2003) 'Serodiagnosis of flaviviral infections and vaccinations in humans.', in Maramorosch, K. et al. (eds) *The Flaviviruses: Detection, Diagnosis and Vaccine Development*. United States: Elsevier Academic Press, pp. 3–65.

Kuno, G. (2009) 'Emergence of the severe syndrome and mortality associated with dengue and dengue-like illness: Historical records (1890 to 1950) and their compatibility with current hypotheses on the shift of disease manifestation', *Clinical Microbiology Reviews*, 22(2), pp. 186–201. doi: 10.1128/CMR.00052-08.

Kunze, U. (2016) 'Tick-borne encephalitis—still on the map: Report of the 18th annual meeting of the international scientific working group on tick-borne encephalitis (ISW-TBE)', *Ticks and Tick-borne Diseases*, 7(5), pp. 911–914. doi: 10.1016/j.ttbdis.2016.04.009.

L'Azou, M. *et al.* (2014) 'Recent Epidemiological Trends of Dengue in the French Territories of the Americas (2000–2012): A Systematic Literature Review', *PLoS Neglected Tropical Diseases*, 8(11). doi: 10.1371/journal.pntd.0003235.

L'Azou, M. *et al.* (2015) 'Dengue Seroprevalence in the French West Indies: A Prospective Study in Adult Blood Donors', *The American Journal of Tropical Medicine and Hygiene*, 92(6), p. ajtmh.14-0211-. doi: 10.4269/ajtmh.14-0211.

De La Cruz Hernández, S. I. *et al.* (2015) 'Dengue laboratory diagnosis: still some room for improvement', *Future Virology*, 10(7), pp. 845–857. doi: 10.2217/fvl.15.59.

LaCon, G. *et al.* (2014) 'Shifting Patterns of Aedes aegypti Fine Scale Spatial Clustering in Iquitos, Peru', *PLoS Neglected Tropical Diseases*, 8(8). doi: 10.1371/journal.pntd.0003038.

Lahariya, C. and Pradhan, S. K. (2006) 'Emergence of chikungunya virus in Indian subcontinent after 32 years: A review.', *Journal of vector borne diseases*. India, 43(4), pp. 151–160.

Lambrechts, L. *et al.* (2011) 'Impact of daily temperature fl uctuations on dengue virus transmission by Aedes aegypti', *Proceedings of the National Academy of Sciences of the United States of America*, 108(18), pp. 1–6. doi: 10.1073/pnas.1101377108/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1101377108.

Lana, R. M. *et al.* (2014) 'Seasonal and nonseasonal dynamics of Aedes aegypti in Rio de Janeiro, Brazil: Fitting mathematical models to trap data', *Acta Tropica*, 129(1), pp. 25–32. doi: 10.1016/j.actatropica.2013.07.025.

Lanciotti, R. S. *et al.* (2008) 'Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007', *Emerging Infectious Diseases*, 14(8), pp. 1232–1239. doi: 10.3201/eid1408.080287.

Ledermann, J. P. *et al.* (2014) 'Aedes hensilli as a Potential Vector of Chikungunya and Zika Viruses', *PLoS Neglected Tropical Diseases*, 8(10). doi: 10.1371/journal.pntd.0003188.

Lednicky, J. *et al.* (2016) 'Mayaro virus in child with acute febrile illness, Haiti, 2015', *Emerging Infectious Diseases*, 22(11), pp. 2000–2002. doi: 10.3201/eid2211.161015.

Leparc-Goffart, I. *et al.* (2014) 'Chikungunya in the Americas', *The Lancet*, p. 514. doi: 10.1016/S0140-6736(14)60185-9.

Leslie, T. *et al.* (2014) 'Dengue serosurvey in Sint Eustatius', *PLoS ONE*. Public Library of Science, 9(6), pp. 1–5. doi: 10.1371/journal.pone.0095002.

Lessler, J. *et al.* (2016) 'Times to Key Events in the Course of Zika Infection and their Implications for Surveillance: A Systematic Review and Pooled Analysis', *Igarss 2014*, (1), pp. 1–5. doi: 10.1101/041913.

Leung, G. H. Y. *et al.* (2015) 'Zika Virus infection in Australia following a monkey bite in Indonesia.', *The Southeast Asian journal of tropical medicine and public health*. Thailand, 46(3), pp. 460–464.

Likos, A. *et al.* (2016) 'Local Mosquito-Borne Transmission of Zika Virus — Miami-Dade and Broward Counties, Florida, June–August 2016', *MMWR. Morbidity and Mortality Weekly Report*, 65(38), pp. 1032–1038. doi: 10.15585/mmwr.mm6538e1.

Lin, L. I.-K. (1989) 'A Concordance Correlation Coefficient to Evaluate Reproducibility', *Biometrics*. United States, 45(1), p. 255. doi: 10.2307/2532051.

Lin, L. I.-K. (2000) 'A note on the concordance correlation coefficient', *Biometrics*, 56, pp. 324–325.

Little, R. J. (1988) 'A test of missing completely at random for multivariate data with missing values', *Journal of the American Statistical Association*, pp. 1198–1202. doi: 10.1002/(SICI)1097-0258(19970830)16:16<1859::AID-SIM593>3.0.CO;2-3.

Little, R. J. A. and Rubin, D. B. (2002) *Statistical Analysis with Missing Data*. 2nd edn. Hoboken, New Jersey: Wiley - Interscience. doi: 10.2307/1533221.

Lofstedt, J. (2003) 'Gender and veterinary medicine', *Can Vet J*, 44, pp. 533–535.

Lumsden, W. H. R. (1955) 'An epidemic of virus disease in Southern Province, Tanganyika territory, in 1952–1953 II. General description and epidemiology', *Transactions of The Royal Society of Tropical Medicine and Hygiene*, 49(1), pp. 33–57. Available at: http://dx.doi.org/10.1016/0035-9203(55)90081-X.

Maciel-de-Freitas, R., Eiras, Á. E. and Lourenço-de-Oliveira, R. (2006) 'Field evaluation of effectiveness of the BG-Sentinel, a new trap for capturing adult Aedes aegypti (Diptera: Culicidae)', *Memorias do Instituto Oswaldo Cruz*, 101(3), pp. 321–325. doi: 10.1590/S0074-02762006000300017.

Mackenzie, J. S., Gubler, D. J. and Petersen, L. R. (2004) 'Emerging flaviviruses: The spread and resurgence of japanese encephalitis, west nile and dengue viruses', *Nature Medicine*, 10(12S), pp. S98–S109. doi: 10.1038/nm1144.

Mammen, M. P. *et al.* (2008) 'Spatial and temporal clustering of dengue virus transmission in Thai villages.', *PLoS medicine*, 5(11), p. e205. doi: 10.1371/journal.pmed.0050205.

Manica, M. *et al.* (2016) 'Spatial and Temporal Hot Spots of Aedes albopictus Abundance inside and outside a South European Metropolitan Area', *PLOS Neglected Tropical Diseases*. Edited by P. J. McCall, 10(6), p. e0004758. doi: 10.1371/journal.pntd.0004758.

Marrero-Santos, K. M. *et al.* (2013) 'Optimization of the cutoff value for a commercial anti-dengue virus IgG immunoassay', *Clinical and Vaccine Immunology*, 20(3), pp. 358–362. doi: 10.1128/CVI.00429-12.

Martins, V. E. P. *et al.* (2012) 'Occurrence of natural vertical transmission of dengue-2 and dengue-3 viruses in Aedes aegypti and Aedes albopictus in Fortaleza, Ceará, Brazil', *PLoS ONE*, 7(7), pp. 1–9. doi: 10.1371/journal.pone.0041386.

Massad, E. *et al.* (2014) 'Risk of symptomatic dengue for foreign visitors to the 2014 FIFA world cup in Brazil', *Memorias do Instituto Oswaldo Cruz*, 109(3), pp. 394–397. doi: 10.1590/0074-0276140133.

Mattar, S. *et al.* (2017) 'Undifferentiated tropical febrile illness in Cordoba, Colombia: Not everything is dengue', *Journal of Infection and Public Health*. King Saud Bin Abdulaziz University for Health Sciences, pp. 6–11. doi: 10.1016/j.jiph.2016.09.014.

Mayo Clinic (2016) *CHIKV - Clinical: Chikungunya IgM and IgG, Antibody, Serum*. Available at: https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/64173 (Accessed: 2 April 2017).

McBride, W. J. H. *et al.* (1998) 'The 1993 dengue 2 epidemic in North Queensland: A serosurvey and comparison of hemagglutination inhibition with an ELISA', *American Journal of Tropical Medicine and Hygiene*, 59(3), pp. 457–461.

McDonald, P. T. (1977) 'Population characteristics of domestic Aedes aegypti (diptera: culicidae) in villages on the Kenya coast. I. Adult survivorship and population size', *Journal of Medical Entomology*, 14(1), pp. 42–48. doi: 10.1093/jmedent/14.1.42.

McMeniman, C. *et al.* (2009) 'Stable Introduction of a Life-Shortening Wolbachia Infection into the Mosquito Aedes aegypti', *Science*, 323(January), pp. 141–144. doi: 10.5061/dryad.9g182.

Mellor, P. S. and Leake, C. J. (2000) 'Climatic and geographic influences on arboviral infections and vectors.', *Revue scientifique et technique (International Office of Epizootics)*, 19(1), pp. 41–54. doi: 10.20506/rst.19.1.1211.

Mena, N. *et al.* (2011) 'Factores asociados con la incidencia de dengue en Costa Rica', *Revista Panamericana de Salud Pública*, 29(4), pp. 234–242. doi: 10.1590/S1020-49892011000400004.

Messina, J. P. *et al.* (2016) 'Mapping global environmental suitability for Zika virus.', *eLife*, 5, p. e15272. doi: 10.7554/eLife.15272.

Miagostovich, M. P. *et al.* (1999) 'Evaluation of an IgG enzyme-linked immunosorbent assay for dengue diagnosis', *Journal of Clinical Virology*, 14(3), pp. 183–189. doi: 10.1016/S1386-6532(99)00059-1.

Mieulet, E. and Claeys, C. (2014) 'The implementation and reception of policies for preventing dengue fever epidemics: a comparative study of Martinique and French Guyana', *Health, Risk and Society*. Taylor & Francis, 16(7–8), pp. 581–599. doi: 10.1080/13698575.2014.949224.

Mohammed, H. *et al.* (2012) *Prevalence of Dengue Virus Infection in a Campus Community in Saint Kitts and Nevis*.

Mohammed, H. *et al.* (2015) 'A mosquito survey of the twin-island Caribbean nation of Saint Kitts and Nevis, 2010', *Journal of the American Mosquito Control Association*, 31(4), pp. 360–3. doi: 10.2987/moco-31-04-360-363.1.

Mordecai, E. A. *et al.* (2017) 'Detecting the impact of temperature on transmission of Zika, dengue, and chikungunya using mechanistic models', *PLoS Neglected Tropical Diseases*, 11(4), pp. 1–18. doi: 10.1371/journal.pntd.0005568.

Moreira, L. A. *et al.* (2009) 'A Wolbachia Symbiont in Aedes aegypti Limits Infection with Dengue, Chikungunya, and Plasmodium', *Cell*, 139(7), pp. 1268–1278. doi: 10.1016/j.cell.2009.11.042.

Moro, M. L. *et al.* (2010) 'Chikungunya virus in North-Eastern Italy: A seroprevalence survey', *American Journal of Tropical Medicine and Hygiene*, 82(3), pp. 508–511. doi: 10.4269/ajtmh.2010.09-0322.

Morrison, A. C. *et al.* (2008) 'Defining challenges and proposing solutions for control of the virus vector Aedes aegypti', *PLoS Medicine*. Public Library of Science, 5(3), pp. 0362–0366. doi: 10.1371/journal.pmed.0050068.

Morrison, A. C. *et al.* (2010) 'Epidemiology of dengue virus in iquitos, Peru 1999 to 2005: Interepidemic and epidemic patterns of transmission', *PLoS Neglected Tropical Diseases*, 4(5). doi: 10.1371/journal.pntd.0000670.

Muir, L. E. and Kay, B. H. (1998) 'Aedes aegypti survival and dispersal estimated by mark-release-recapture in northern Australia.', *The American journal of tropical medicine and hygiene*, 58(3), pp. 277–282. doi: 1998.58.277.

Muñoz, Á. G. *et al.* (2016) 'Analyzing climate variations at multiple timescales can guide Zika virus response measures', *GigaScience*. GigaScience, 5(1), p. 41. doi: 10.1186/s13742-016-0146-1.

Murray, C. J. L. *et al.* (2012) 'Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010', *The Lancet*, 380(9859), pp. 2197–2223. doi: 10.1016/S0140-6736(12)61689-4.

Murray, K. O. *et al.* (2013) 'Identification of dengue fever cases in Houston, Texas, with evidence of autochthonous transmission between 2003 and 2005', *Vector Borne Zoonotic Dis*, 13(12), pp. 835–845. doi: 10.1089/vbz.2013.1413.

Murray, N. E. A., Quam, M. B. and Wilder-Smith, A. (2013) 'Epidemiology of dengue: Past, present and future prospects', *Clinical Epidemiology*, 5(1), pp. 299–309. doi: 10.2147/CLEP.S34440.

Musso, D. (2015) 'Zika virus transmission from French Polynesia to Brazil', *Emerging Infectious Diseases*, 21(10), pp. 1887–1889. doi: 10.3201/eid2110.151125.

Musso, D., Nilles, E. J. and Cao-Lormeau, V.-M. (2014) 'Rapid spread of emerging Zika virus in the Pacific area', *Clinical Microbiology and Infection*. European Society of Clinical Infectious Diseases, 20(10), pp. O595–O596. doi: 10.1111/1469-0691.12707.

Nalca, A., Fellows, P. F. and Whitehouse, C. A. (2003) 'Vaccines and animal models for arboviral encephalitides', *Antiviral Research*, 60(3), pp. 153–174. doi: 10.1016/j.antiviral.2003.08.001.

Namekar, M. *et al.* (2013) 'Evaluation of a new commercially available immunoglobulin M capture enzyme-linked immunosorbent assay for diagnosis of dengue virus infection', *Journal of Clinical Microbiology*, 51(9), pp. 3102–3106. doi: 10.1128/JCM.00351-13.

Nathan, M. B. (1993) 'Critical review of Aedes aegypti control programs in the Caribbean and selected neighboring countries', *J Am Mosq Control Assoc*. United States, 9(1), pp. 1–7.

Nature (2016) ‘Zika imbalance’, p. 7. doi: 10.1038/537007a.

Neuwirth, E. (2014) ‘RColorBrewer: ColorBrewer Palettes’. Available at: https://cran.r-project.org/package=RColorBrewer.

Ngwe Tun, M. M. *et al.* (2016) ‘Persistence of Neutralizing Antibody Against Dengue Virus 2 After 70 Years from Infection in Nagasaki’, *BioResearch Open Access*. 140 Huguenot Street, 3rd FloorNew Rochelle, NY 10801USA: Mary Ann Liebert, Inc., 5(1), pp. 188–191. doi: 10.1089/biores.2016.0016.

Nitatpattana, N. *et al.* (2014) ‘Long-term persistence of Chikungunya virus neutralizing antibodies in human populations of North Eastern Thailand’, *Virology Journal*, 11(1), pp. 1–5. doi: 10.1186/1743-422X-11-183.

Nkrumah, K. (1965) *Neo-Colonialism: The Last Stage of Imperialism*. New York: International Publishers.

Normile, D. (2013) ‘Surprising new dengue virus throws a spanner in disease control efforts’, *Science*, 342(6157), p. 415. doi: 10.1126/science.342.6157.415.

O’Leary, D. R. *et al.* (2002) ‘Assessment of dengue risk in relief workers in Puerto Rico after Hurricane Georges, 1998.’, *The American journal of tropical medicine and hygiene*, 66(1), pp. 35–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12135265.

O’meara, G. F. *et al.* (1995) ‘Spread of Aedes albopictus and Decline of Ae. aegypti (Diptera: Culicidae) in Florida’, *Journal of Medical Entomology*, 32(4), pp. 554–562. Available at: http://dx.doi.org/10.1093/jmedent/32.4.554.

OECD (2018) *DAC List of ODA Recipients*. Available at: http://www.oecd.org/dac/financing-sustainable-development/development-finance-standards/DAC_List_ODA_Recipients2018to2020_flows_En.pdf (Accessed: 4 March 2018).

Padmanabha, H. *et al.* (2012) ‘The Interactive Roles of Aedes aegypti Super-Production and Human Density in Dengue Transmission’, *PLoS Neglected Tropical Diseases*, 6(8), p. 11. doi: 10.1371/journal.pntd.0001799.

PAHO / WHO (2017a) *Zika-Epidemiological Report: Saint Kitts and Nevis - 25 September 2017*. Available at: http://www.cardi.org/country-offices/st-kitts-nevis/.

PAHO / WHO (2017b) *Zika - Epidemiological Update - 25 Aug 2017*. Available at: http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=41708&lang=pt.

PAHO / WHO (2018a) *Chagas disease*. Available at: http://www.paho.org/hq/index.php?option=com_topics&view=article&id=10&Itemid=40743&lang=en (Accessed: 11 February 2018).

PAHO / WHO (2018b) *Health in the Americas - Saint Kitts and Nevis*. Available at: https://www.paho.org/salud-en-las-americas-2017/?p=4298 (Accessed: 26 February 2018).

Pan-ngum, W. *et al.* (2013) ‘Estimating the True Accuracy of Diagnostic Tests for Dengue Infection Using Bayesian Latent Class Models’, *PLoS ONE*. Edited by S.-S. Hong, 8(1), p. e50765. doi: 10.1371/journal.pone.0050765.

Pan American Health Organization (2012) *Health in the Americas. Edition: Country Volume.*

Pan American Health Organization (2016) *Core Indicators. Health Situation in the Americas 2016*.

Pan American Health Organization (2018) *PLISA: Health information Platform for the Americas*. Available at: http://www.paho.org/data/index.php/en/mnu-topics/indicadores-dengue-en/dengue-nacional-en/252-dengue-pais-ano-en.html?showall=&limitstart= (Accessed: 23 February 2018).

Pan, W. (2001) 'Akaike' s Information Criterion in Generalized Estimating Equations', *Biometrics*, 57(1), pp. 120–125.

Panning, M. *et al.* (2009) 'Performance of the RealStar Chikungunya virus real-time reverse transcription-PCR kit', *Journal of Clinical Microbiology*, 47(9), pp. 3014–3016. doi: 10.1128/JCM.01024-09.

Papaevangelou, G. and Halstead, S. B. (1977) 'Infections with two dengue viruses in Greece in the 20th century. Did dengue hemorrhagic fever occur in the 1928 epidemic?', *The Journal of tropical medicine and hygiene*. England, 80(3), pp. 46–51.

Parkash, O. and Shueb, R. H. (2015) 'Diagnosis of dengue infection using conventional and biosensor based techniques', *Viruses*, 7(10), pp. 5410–5427. doi: 10.3390/v7102877.

Patz, J. A. and Reisen, W. K. (2001) 'Immunology, climate change and vector-borne diseases', *Trends in Immunology*, pp. 171–172. doi: 10.1016/S1471-4906(01)01867-1.

Paupy, C. *et al.* (2009) 'Aedes albopictus, an arbovirus vector: From the darkness to the light', *Microbes and Infection*, 11(14–15), pp. 1177–1185. doi: 10.1016/j.micinf.2009.05.005.

Paz-Bailey, G. *et al.* (2017) 'Persistence of Zika Virus in Body Fluids — Preliminary Report', *New England Journal of Medicine*, p. NEJMoa1613108. doi: 10.1056/NEJMoa1613108.

Pereira-Silva, J. *et al.* (2017) 'First evidence of Zika virus venereal transmission in Aedes aegypti mosquitoes', *Memorias do Instituto Oswaldo Cruz*. Brazil, (November), pp. 1–16. doi: 10.1590/0074-02760170329.Running.

Perkins, T. A. *et al.* (2014) 'A Review of Transmission Models of Dengue: A Quantitative and Qualitative Analysis of Model Features', in Gubler, D. J. et al. (eds) *Dengue and Dengue Hemorrhagic Fever*. 2nd edn. Wallingford, Oxforshire: CABI, pp. 99–114. Available at: http://www.cabi.org/cabebooks/ebook/20143309291.

Petersen, L. R. and Powers, A. M. (2016) 'Chikungunya: epidemiology', *F1000Research*, 5, pp. 1–8. doi: 10.12688/f1000research.7171.1.

Pinheiro, J. and Bates, D. (2000) *Mixed-Effects Models in S and S-PLUS*. 1st edn. New York, NY: Springer-Verlag.

Pinheiro, J., Bates, D. and R-core (2017) 'nlme: Linear and Nonlinear Mixed Effects Models'. Available at: https://cran.r-project.org/package=nlme.

Plummer, M. *et al.* (2016) 'coda: Output Analysis and Diagnostics for MCMC'. Available at: https://cran.r-project.org/package=coda.

Plummer, M. (2016) 'rjags: Bayesian Graphical Models using MCMC'. Available at: https://cran.r-project.org/package=rjags.

Porter, K. R. *et al.* (1999) 'Evaluation of a commercially available immunoglobulin M capture enzyme-linked immunosorbent assay kit for diagnosing acute dengue infections.', *Clinical and diagnostic laboratory immunology*, 6(5), pp. 741–4.

Porter, K. R. *et al.* (2005) 'Epidemiology Of Dengue And Dengue Hemorrhagic Fever In A Cohort Of Adults Living In Bandung, West Java, Indonesia', *The American Journal of Tropical Medicine and Hygiene*, 72(1), pp. 60–66. doi: 72/1/60 [pii].

Potasman, I., Srugo, I. and Schwartz, E. (1999) 'Dengue seroconversion among israeli travelers to tropical countries', *Emerging Infectious Diseases*, 5(6), pp. 824–827. doi: 10.3201/eid0506.990615.

Powers, A. M. (2010) 'Chikungunya', *Clinics in Laboratory Medicine*, 30(1), pp. 209–219. doi: 10.1016/j.cll.2009.10.003.

Prat, C. M. *et al.* (2014) 'Evaluation of Commercially Available Serologic Diagnostic Tests for Chikungunya Virus', *Emerging Infectious Diseases*, 20(12), pp. 2129–2132. doi: 10.3201/eid2012.141269.

Pruim, R., Kaplan, D. and Horton, N. (2016) 'mosaicData: Project MOSAIC Data Sets'. Available at: https://cran.r-project.org/package=mosaicData.

Pruim, R., Kaplan, D. T. and Horton, N. J. (2017) 'mosaic: Project MOSAIC Statistics and Mathematics Teaching Utilities'. Available at: https://cran.r-project.org/package=mosaic.

Qiu, W. *et al.* (2015) 'powerSurvEpi: Power and Sample Size Calculation for Survival Analysis of Epidemiological Studies'. Available at: https://cran.r-project.org/package=powerSurvEpi.

Quist-Adade, C. (2017) *From the Local to the Global: Theories and Key Issues in Global Justice.* Rotterdam: Sense Publishers.

R Core Team (2017) 'R: A language and environment for statistical computing. R Foundation for Statistical Computing'. Vienna, Austria. Available at: https://www.r-project.org/.

Radke, E. G. *et al.* (2012) 'Dengue outbreak in Key West, Florida, USA, 2009', *Emerging Infectious Diseases*, 18(1), pp. 135–137. doi: 10.3201/eid1801.110130.

Ramos, M. M. *et al.* (2008) 'Epidemic dengue and dengue hemorrhagic fever at the Texas-Mexico border: Results of a household-based seroepidemiologic survey, December 2005', *American Journal of Tropical Medicine and Hygiene*, 78(3), pp. 364–369. doi: 78/3/364 [pii].

Ratanawong, P. *et al.* (2016) 'Spatial variations in dengue transmission in schools in Thailand', *PLoS ONE*, 11(9). doi: 10.1371/journal.pone.0161895.

Reinert, J. F., Harbach, R. E. and Kitching, I. J. (2004) 'Phylogeny and classification of Aedini (Diptera: Culicidae), based on morphological characters of all life stages', *Zoological Journal of the Linnean Society*, 142(3), pp. 289–368. doi: 10.1111/j.1096-3642.2004.00144.x.

Reiter, P. *et al.* (1995) 'Short report: Dispersal of Aedes aegypti in an urban area after blood feeding as demonstrated by rubidium-marked eggs', *American Journal of Tropical Medicine and Hygiene*, 52, pp. 177–179.

Reiter, P. (2001) 'Climate change and mosquito-borne disease.', *Environmental health perspectives*, 109(Suppl 1), pp. 141–61. doi: 10.2307/3434853.

Reiter, P. *et al.* (2003) 'Texas lifestyle limits transmission of dengue virus', *Emerging Infectious Diseases*, 9(1), pp. 86–89. doi: 10.3201/eid0901.020220.

Reiter, P. (2007) 'Oviposition, Dispersal, and Survival in *Aedes aegypti:* Implications for the Efficacy of Control Strategies', *Vector-Borne and Zoonotic Diseases*, 7(2), pp. 261–273. doi: 10.1089/vbz.2006.0630.

Rezza, G. *et al.* (2007) 'Infection with chikungunya virus in Italy: an outbreak in a temperate region', *Lancet*, 370(9602), pp. 1840–1846. doi: 10.1016/S0140-6736(07)61779-6.

Rezza, G. (2014) 'Dengue and chikungunya: long-distance spread and outbreaks in naïve

areas', *Pathogens and Global Health*, 108, pp. 349–355. doi: 10.1179/2047773214Y.0000000163.

Riou, J., Poletto, C. and Boëlle, P. Y. (2017) 'A comparative analysis of Chikungunya and Zika transmission', *Epidemics*. The Author(s), 19, pp. 43–52. doi: 10.1016/j.epidem.2017.01.001.

Ripley, B. (2017) 'MASS: Support Functions and Datasets for Venables and Ripley's MASS'. Available at: https://cran.r-project.org/package=MASS.

Ritchie, S. A., Montgomery, B. L. and Hoffmann, A. A. (2013) 'Novel estimates of Aedes aegypti (Diptera: Culicidae) population size and adult survival based on Wolbachia releases.', *Journal of medical entomology*, 50(3), pp. 624–31. doi: 10.1603/me12201.

Robinson, M. C. (1955) 'An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53. I. Clinical features.', *Transactions of the Royal Society of Tropical Medicine and Hygiene*. England, 49(1), pp. 28–32.

Rodríguez-Morales, A. J. (2015) 'Zika: The new arbovirus threat for latin america', *Journal of Infection in Developing Countries*, 9(6), pp. 684–685. doi: 10.3855/jidc.7230.

Roehrig, J. T., Hombach, J. and Barrett, A. D. T. (2008) 'Guidelines for Plaque-Reduction Neutralization Testing of Human Antibodies to Dengue Viruses', *Viral Immunology*. United States, 21(2), pp. 123–132. doi: 10.1089/vim.2008.0007.

Rohani, A. *et al.* (2011) 'Aedes larval population dynamics and risk for dengue epidemics in Malaysia', *Tropical Biomedicine*, 28(2), pp. 237–248.

Roiz, D. *et al.* (2015) 'Autochthonous Chikungunya Transmission and Extreme Climate Events in Southern France', *PLOS Neglected Tropical Diseases*, 9(6), p. e0003854. doi: 10.1371/journal.pntd.0003854.

Rooks, K. *et al.* (2016) 'Mitigating the Risk of Transfusion-Transmitted Dengue in Australia', *Journal of Blood Transfusion*. Hindawi Publishing Corporation, 2016, p. 3059848. doi: 10.1155/2016/3059848.

Rowley, W. A. and Graham, C. L. (1968) 'The effect of age on the flight performance of female aedes aegypti mosquitos', *J Insect Physiol*, 14(5), pp. 719–728. Available at: http://www.ncbi.nlm.nih.gov/pubmed/5649625%5Cnhttp://www.sciencedirect.com/science/article/pii/0022191068902308.

Rubin, D. B. (1976) 'Inference and missing data', *Biometrika*, pp. 581–592. doi: 10.1093/biomet/63.3.581.

La Ruche, G. *et al.* (2010) 'First two autochthonous dengue virus infections in metropolitan France, september 2010', *Eurosurveillance*, 15(39), pp. 1–5. doi: 19676 [pii].

Rudolph, K. E. *et al.* (2014) 'Review article: Incubation periods of mosquito-borne viral infections: a systematic review', *American Journal of Tropical Medicine and Hygiene*, 90(5), pp. 882–891. doi: 10.4269/ajtmh.13-0403.

Rueda, L. M. (2004) *Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with Dengue Virus Transmission*, *Zootaxa*. doi: 10.11646/zootaxa.589.1.1.

Runge-Ranzinger, S. *et al.* (2008) 'What does dengue disease surveillance contribute to predicting and detecting outbreaks and describing trends?', *Tropical Medicine and International Health*. England, 13(8), pp. 1022–1041. doi: 10.1111/j.1365-3156.2008.02112.x.

Rushton, S. P., Ormerod, S. J. and Kerby, G. (2004) 'New paradigms for modelling species distributions?', *Journal of Applied Ecology*, 41(2), pp. 193–200. doi: 10.1111/j.0021-8901.2004.00903.x.

Sabchareon, A. *et al.* (2012) 'Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial', *The Lancet*, 380(9853), pp. 1559–1567. doi: http://dx.doi.org/10.1016/S0140-6736(12)61428-7.

Sahu, R. *et al.* (2014) 'Neurologic complications in dengue virus infection: a prospective cohort study', *Neurology*, 83(18), pp. 1601–1609. doi: 10.1212/wnl.0000000000000935.

Saifur, R. G. M. *et al.* (2012) 'Changing domesticity of Aedes aegypti in northern peninsular Malaysia: Reproductive consequences and potential epidemiological implications', *PLoS ONE*, 7(2). doi: 10.1371/journal.pone.0030919.

Salazar, M. I. *et al.* (2007) 'Dengue virus type 2: replication and tropisms in orally infected Aedes aegypti mosquitoes.', *BMC microbiology*, 7, p. 9. doi: 10.1186/1471-2180-7-9.

Salyer, S. J. *et al.* (2014) 'Dengue Virus Infections among Haitian and Expatriate Non-governmental Organization Workers — Léogane and Port-au-Prince, Haiti, 2012', *PLoS Neglected Tropical Diseases*, 8(10). doi: 10.1371/journal.pntd.0003269.

San Martín, J. L. *et al.* (2010) 'The Epidemiology of Dengue in the Americas Over the Last Three Decades: A Worrisome Reality', *American Journal of Tropical Medicine and Hygiene*, 82(1), pp. 128–135. doi: 10.4269/ajtmh.2010.09-0346.

Sanchez-Vegas, C. *et al.* (2013) 'Prevalence of dengue virus infection in US travelers who have lived in or traveled to dengue-endemic countries', *Journal of Travel Medicine*, 20(6), pp. 352–360. doi: 10.1111/jtm.12057.

Sang, C. T., Cuzzubbo, A. J. and Devine, P. L. (1998) 'Evaluation of a commercial capture enzyme-linked immunosorbent assay for detection of immunoglobulin M and G antibodies produced during dengue infection.', *Clinical and diagnostic laboratory immunology*, 5(1), pp. 7–10.

Sarkar, D. (2017) 'lattice: Trellis Graphics for R'. Available at: https://cran.r-project.org/package=lattice.

Schilte, C. *et al.* (2013) 'Chikungunya Virus-associated Long-term Arthralgia: A 36-month Prospective Longitudinal Study', *PLoS Neglected Tropical Diseases*, 7(3). doi: 10.1371/journal.pntd.0002137.

Schiøler, K. L. and Macpherson, C. N. (2009) 'Dengue Transmission in the Small-Island setting. Investigations from the Caribbean Island of Grenada', *American Journal of Tropical Medicine and Hygiene*, 81(2), pp. 280–286. doi: 81/2/280 [pii].

Schuffenecker, I. *et al.* (2006) 'Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak', *PLoS Medicine*, 3(7), pp. 1058–1070. doi: 10.1371/journal.pmed.0030263.

Scott, T. W., Chow, E., *et al.* (1993) 'Blood-feeding patterns of Aedes aegypti (Diptera: Culicidae) collected in a rural Thai village.', *Journal of medical entomology*. England, 30(5), pp. 922–927.

Scott, T. W., Clark, G. G., *et al.* (1993) 'Detection of multiple blood feeding in Aedes aegypti (Diptera: Culicidae) during a single gonotrophic cycle using a histologic technique.', *Journal of medical entomology*. England, 30(1), pp. 94–99.

Scott, T. W., Morrison, A. C., *et al.* (2000) 'Longitudinal Studies of Aedes aegypti (Diptera : Culicidae) in Thailand and Puerto Rico : Population Dynamics Longitudinal Studies of Aedes aegypti (Diptera : Culicidae) in Thailand and Puerto Rico : Population Dynamics', *J Med Entomol*, 37(1), pp. 77–88. doi: 10.1603/0022-2585-37.1.77.

Scott, T. W., Amerasinghe, P. H., *et al.* (2000) 'Longitudinal Studies of Aedes aegypti (Diptera: Culicidae) in Thailand and Puerto Rico: Blood Feeding Frequency', *Journal of Medical Entomology*, 37(1), pp. 77–88. doi: http://dx.doi.org/10.1603/0022-2585-37.1.89.

Sergon, K. *et al.* (2007) 'Seroprevalence of Chikungunya virus infection on Grande Comore Island, Union of the Comoros, 2005', *American Journal of Tropical Medicine and Hygiene*, 76(6), pp. 1189–1193. doi: 76/6/1189 [pii].

Service, M. (2012) *Medical Entomology for Students*. Fifth Ed. New York, NY: Cambridge University Press.

Shamala, D. S. (2015) 'Laboratory diagnosis of dengue: A review', *International Medical Journal Malaysia*, 14(1), pp. 17–28. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-84931302784&partnerID=40&md5=24deb9f93acac1bb7bde3aab00b6a4f7.

Sharp, T. W. *et al.* (1995) 'Dengue Fever in U.S. Troops during Operation Restore Hope, Somalia, 1992–1993', *The American Society of Tropical Medicine and Hygiene*. The American Society of Tropical Medicine and Hygiene, 53(1), pp. 89–94. doi: https://doi.org/10.4269/ajtmh.1995.53.89.

Shepard, D. S. *et al.* (2011) 'Economic impact of dengue illness in the Americas', *American Journal of Tropical Medicine and Hygiene*, 84(2), pp. 200–207. doi: 10.4269/ajtmh.2011.10-0503.

Sierra, B. D. L. C., Kourí, G. and Guzmán, M. G. (2007) 'Race: A risk factor for dengue hemorrhagic fever', *Archives of Virology*, 152(3), pp. 533–542. doi: 10.1007/s00705-006-0869-x.

Silver, J. B. (2013) *Mosquito Ecology*. Third Ed., *Journal of Chemical Information and Modeling*. Third Ed. New York, NY: Springer. doi: 10.1017/CBO9781107415324.004.

Simmons, G. *et al.* (2016) 'High incidence of chikungunya virus and frequency of viremic blood donations during epidemic, Puerto Rico, USA, 2014', *Emerging Infectious Diseases*, 22(7), pp. 1221–1228. doi: 10.3201/eid2207.160116.

Singh, G. (2006) 'Determination of Cutoff Score for a Diagnostic Test', *The Internet Journal of Laboratory Medicine*, 2(1), pp. 1–4.

Siqueira-Junior, J. B. *et al.* (2008) 'Spatial point analysis based on dengue surveys at household level in central Brazil', *BMC Public Health*, 8, pp. 1–9. doi: 10.1186/1471-2458-8-361.

Sissoko, D. *et al.* (2008) 'Seroprevalence and risk factors of Chikungunya virus infection in Mayotte, Indian Ocean, 2005-2006: A population- based survey', *PLoS ONE*, 3(8), pp. 2005–2006. doi: 10.1371/journal.pone.0003066.

Skaug, H. *et al.* (2016) 'glmmADMB: Generalized Linear Mixed Models using "AD Model Builder"'.

Smith, J. *et al.* (2011) 'Adult Mosquito Survey of Saint Kitts and Nevis , 2010', in.

Song, B.-H. *et al.* (2017) 'Zika virus: History, epidemiology, transmission, and clinical

presentation', *Journal of Neuroimmunology*. The Authors, 308, pp. 50–64. doi: 10.1016/j.jneuroim.2017.03.001.

Spinu, V., Grolemund, G. and Wickham, H. (2017) 'lubridate: Make Dealing with Dates a Little Easier'. Available at: https://cran.r-project.org/package=lubridate.

Srikiatkhachorn, A. *et al.* (2016) 'Resolution of a Chikungunya outbreak in a prospective Cohort, Cebu, Philippines, 2012–2014', *Emerging Infectious Diseases*, pp. 1852–1854. doi: 10.3201/eid2210.160729.

Staikowsky, F. *et al.* (2009) 'Prospective study of chikungunya virus acute infection in the Island of La Réunion during the 2005-2006 outbreak', *PLoS ONE*, 4(10). doi: 10.1371/journal.pone.0007603.

Statistics Department (2012) *Preliminary Report, Population and Housing 2011 Census*.

Stoddard, S. T. *et al.* (2013) 'House-to-house human movement drives dengue virus transmission', *Proceedings of the National Academy of Sciences*, 110(3), pp. 994–999. doi: 10.1073/pnas.1213349110.

Stramer, S. L. *et al.* (2012) 'Dengue viremia in blood donors identified by RNA and detection of dengue transfusion transmission during the 2007 dengue outbreak in Puerto Rico', *Transfusion*. United States, 52(8), pp. 1657–1666. doi: 10.1111/j.1537-2995.2012.03566.x.

Strauss, J. H. and Strauss, E. G. (1994) 'The alphaviruses: gene expression, replication, and evolution.', *Microbiological reviews*, 58(3), pp. 491–562.

Suaya, J. A. *et al.* (2010) 'Disease burden of dengue fever and dengue hemorrhagic fever', in Preedy, V. and Watson, R. (eds) *Handbook of Disease Burdens and Quality of Life Measures*. New York: Springer, pp. 1263–1279.

Suaya, J. A., Shepard, D. S. and Beatty, M. E. (2007) 'Dengue : Burden Of Disease And Costs Of Illness', *Report of the Scientific Working Group Meeting on Dengue*, (October 2006), pp. 1–5. Available at: http://www.researchgate.net/profile/Mark_Beatty2/publication/242567673_Dengue_Burden_Of_Disease_And_Costs_Of_Illness/links/5415f6fb0cf2bb7347db43dc.pdf.

Sudre, B. *et al.* (2014) 'Zika virus infecton outbreak French Polynesia', *Rapid Risk Assesment*, (February), pp. 1–12. Available at: http://ecdc.europa.eu/en/publications/Publications/Zika-virus-French-Polynesia-rapid-risk-assessment.pdf?time=0.

Suhrbier, A., Jaffar-Bandjee, M.-C. and Gasque, P. (2012) 'Arthritogenic alphaviruses—an overview', *Nature Reviews Rheumatology*. Nature Publishing Group, 8(7), pp. 420–429. doi: 10.1038/nrrheum.2012.64.

Tabachnick, W. J. and Powell, J. R. (1979) 'A world-wide survey of genetic variation in the yellow fever mosquito, Aedes aegypti', *Genetical Research*. England, 34(3), pp. 215–229. doi: 10.1017/S0016672300019467.

Talbalaghi, A. *et al.* (2010) 'Are Aedes albopictus or other mosquito species from northern Italy competent to sustain new arboviral outbreaks?', *Medical and Veterinary Entomology*, 24(1), pp. 83–87. doi: 10.1111/j.1365-2915.2009.00853.x.

Tatem, A. J. *et al.* (2012) 'Air travel and vector-borne disease movement', *Parasitology*, 139(14), pp. 1816–1830. doi: 10.1017/S0031182012000352.

Teets, F. D. *et al.* (2014) 'Origin of the dengue virus outbreak in Martin County, Florida,

USA 2013', *Virology Reports*, 1–2(C), pp. 2–8. doi: 10.1016/j.virep.2014.05.001.

Tesh, R. B., Gubler, D. J. and Rosen, L. (1976) 'Variation among goegraphic strains of Aedes albopictus in susceptibility to infection with chikungunya virus.', *The American journal of tropical medicine and hygiene*. United States, 25(2), pp. 326–335.

Teurlai, M. *et al.* (2015) 'Socio-economic and Climate Factors Associated with Dengue Fever Spatial Heterogeneity: A Worked Example in New Caledonia', *PLoS Neglected Tropical Diseases*, 9(12). doi: 10.1371/journal.pntd.0004211.

The World Bank (2018) *World Development Indicators | DataBank*, *World Development Indicators*. Available at: http://databank.worldbank.org/data/reports.aspx?source=2&country=KNA (Accessed: 4 April 2018).

Therneau, T. M. (2017) 'survival: Survival Analysis'. Available at: https://cran.r-project.org/package=survival.

Therneau, T. M. and Grambsch, P. M. (2000) *Modeling Survival Data: Extending the Cox Model*. New York, NY: Springer New York (Statistics for Biology and Health). doi: 10.1007/978-1-4757-3294-8.

Thiberville, S. D. *et al.* (2013) 'Chikungunya Fever: A Clinical and Virological Investigation of Outpatients on Reunion Island, South-West Indian Ocean', *PLoS Neglected Tropical Diseases*, 7(1). doi: 10.1371/journal.pntd.0002004.

Thiberville, S. D. *et al.* (2013) 'Chikungunya fever: epidemiology, clinical syndrome, pathogenesis and therapy', *Antiviral Res*, 99(3), pp. 345–370. doi: 10.1016/j.antiviral.2013.06.009.

Thomas, S. J. *et al.* (2010) 'Dengue Plaque Reduction Neutralization Test (PRNT) in Primary and Secondary Dengue Virus Infections: How Alterations in Assay Conditions Impact Performance', 81(5), pp. 825–833. doi: 10.4269/ajtmh.2009.08-0625.Dengue.

Thongyuan, S. and Kittayapong, P. (2017) 'First evidence of dengue infection in domestic dogs living in different ecological settings in Thailand', *Plos One*, 12(8), p. e0180013. doi: 10.1371/journal.pone.0180013.

Tidwell, M. A. *et al.* (1990) 'Baseline data on Aedes aegypti populations in Santo Domingo, Dominican Republic', *Journal of the American Mosquito Control Association*. United States, 6(3), pp. 514–522. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2230783.

Torres, J. R. *et al.* (2017) 'Epidemiological Characteristics of Dengue Disease in Latin America and in the Caribbean: A Systematic Review of the Literature', *Journal of Tropical Medicine*, 2017. doi: 10.1155/2017/8045435.

Trpis, M. and Hausermann, W. (1986) 'Dispersal and other population parameters of Aedes aegypti in an African village and their possible significance in epidemiology of vector-borne diseases.', *The American journal of tropical medicine and hygiene*. United States, 35(6), pp. 1263–1279.

Tsuda, Y. *et al.* (2001) 'Movement of Aedes aegypti (Diptera: Culicidae) released in a small isolated village on Hainan Island, China.', *Journal of medical entomology*, 38(1), pp. 93–98. Available at: http://dx.doi.org/10.1603/0022-2585-38.1.93.

Tun-Lin, W. *et al.* (1996) 'Critical Examination Correlations of Aedes Aegypti With Abundance Indices ':, *The American Journal of Tropical Medicine and Hygiene*. The American Society of Tropical Medicine and Hygiene, 54(5), pp. 543–547. doi:

10.4269/ajtmh.1996.54.543.

Tun-Lin, W., Kay, B. H. and Barnes, A. (1995) 'The Premise Condition Index: a tool for streamlining surveys of Aedes aegypti.', *The American journal of tropical medicine and hygiene*. United States, 53(6), pp. 591–594.

Turell, M. J. (2016) 'Vector Competence versus Vectorial Capacity', in *ASTMH Conference*.

U.S. Department of State (2018a) *FY 2018 Congressional Budget Justification*. Available at: https://www.state.gov/documents/organization/271013.pdf.

U.S. Department of State (2018b) *International Narcotics Control Strategy Report. Volume II: Money Laundering.* Available at: https://www.state.gov/documents/organization/278760.pdf (Accessed: 10 April 2018).

Vacek, P. M. (1985) 'The Effect of Conditional Dependence on the Evaluation of Diagnostic Tests', *Source: Biometrics*, 41(4), pp. 959–968. Available at: http://www.jstor.org/stable/2530967.

Vanwambeke, S. O. *et al.* (2006) 'Multi-level analyses of spatial and temporal determinants for dengue infection.', *International journal of health geographics*, 5, p. 5. doi: 10.1186/1476-072X-5-5.

Vasilakis, N. and Weaver, S. C. (2008) *Chapter 1 The History and Evolution of Human Dengue Emergence*, *Advances in Virus Research*. Elsevier Inc. doi: 10.1016/S0065-3527(08)00401-6.

Vasilakis, N. and Weaver, S. C. (2016) 'Flavivirus transmission focusing on Zika.', *Current opinion in virology*. Elsevier B.V., 22, pp. 30–35. doi: 10.1016/j.coviro.2016.11.007.

Vassell, L. (2014) *Country Gender Assessment. St. Kitts & Nevis (Vol. 1)*.

Vaughn, D. W. *et al.* (1999) 'Rapid serologic diagnosis of dengue virus infection using a commercial capture ELISA that distinguishes primary and secondary infections', *American Journal of Tropical Medicine and Hygiene*, 60(4), pp. 693–698.

Vazeille, M. *et al.* (2008) *Chikungunya: A risk for Mediterranean countries?*, *Acta tropica*. doi: 10.1016/j.actatropica.2007.09.009.

Vazquez-Prokopec, G. M. *et al.* (2009) 'A New, Cost-Effective, Battery-Powered Aspirator for Adult Mosquito Collections', *Journal of Medical Entomology*, 46(6), pp. 1256–1259. doi: 10.1603/033.046.0602.

Vazquez, S. *et al.* (2007) 'Evaluation of immunoglobulin M and G capture enzyme-linked immunosorbent assay Panbio kits for diagnostic dengue infections', *Journal of Clinical Virology*, 39(3), pp. 194–198. doi: 10.1016/j.jcv.2007.04.003.

Vorndam, V. and Kuno, G. (1997) 'Diagnosis of dengue virus infections', in Gubler, D. and Kuno, G. (eds) *Dengue and dengue hemorrhagic fever*. Wallingford, Oxforshire: CAB International, pp. 313–333.

Wagner, E. *et al.* (2009) *Basic virology*, *Vasa*. Blackwell Pub. Available at: http://books.google.com/books?hl=en&lr=&id=vYJBuebvgIcC&oi=fnd&pg=PR7&dq=Basic+virology&ots=BZqNdK2c9a&sig=ouzdp1Q55Nizqm_u9eA8IRgA0AA.

Walter, S. D. (2005) 'The problem of imperfect reference standards', *Journal of Clinical Epidemiology*, 58(7), pp. 649–650. doi: 10.1016/j.jclinepi.2004.11.026.

Wang, X., Chen, M. H. and Yan, J. (2013) 'Bayesian dynamic regression models for interval

censored survival data with application to children dental health', *Lifetime Data Analysis*, 19(3), pp. 297–316. doi: 10.1007/s10985-013-9246-8.

Watts, D. M. *et al.* (1987) 'Effect of temperature on the vector efficiency of Aedes aegypti for dengue 2 virus.', *The American journal of tropical medicine and hygiene*. United States, 36(1), pp. 143–152.

Wearing, H. J., Robert, M. A. and Christofferson, R. C. (2016) 'Dengue and chikungunya: modelling the expansion of mosquito-borne viruses into naïve populations', *Parasitology*, pp. 1–14. doi: 10.1017/S0031182016000421.

Wearing, H. J. and Rohani, P. (2006) 'Ecological and immunological determinants of dengue epidemics', *Proceedings of the National Academy of Sciences*, 103(31), pp. 11802–11807. doi: 10.1073/pnas.0602960103.

Weaver, S. C. *et al.* (2012) 'Chikungunya virus and prospects for a vaccine.', *Expert review of vaccines*, 11(9), pp. 1087–101. doi: 10.1586/erv.12.84.

Weaver, S. C. *et al.* (2016) 'Zika virus: History, emergence, biology, and prospects for control', *Antiviral Research*, 130, pp. 69–80. doi: 10.1016/j.antiviral.2016.03.010.

Weaver, S. C. and Forrester, N. L. (2015) 'Chikungunya: Evolutionary history and recent epidemic spread', *Antiviral Research*, 120, pp. 32–39. doi: 10.1016/j.antiviral.2015.04.016.

Weaver, S. C. and Lecuit, M. (2015) 'Chikungunya Virus and the Global Spread of a Mosquito-Borne Disease', *New England Journal of Medicine*, 372(13), pp. 1231–1239. doi: 10.1056/NEJMra1406035.

Weaver, S. C. and Reisen, W. K. (2010) 'Present and future arboviral threats', *Antiviral Research*, 85(2), pp. 328–345. doi: 10.1016/j.antiviral.2009.10.008.

Wichmann, O. *et al.* (2005) 'Dengue antibody prevalence in German travelers', *Emerging Infectious Diseases*, 11(5), pp. 762–765. doi: 10.3201/eid1105.050097.

Wickham, H. *et al.* (2017) 'dplyr: A Grammar of Data Manipulation'. Available at: https://cran.r-project.org/package=dplyr.

Wickham, H. (2017a) 'reshape: Flexibly Reshape Data'. Available at: https://cran.r-project.org/package=reshape.

Wickham, H. (2017b) 'reshape2: Flexibly Reshape Data: A Reboot of the Reshape Package'. Available at: https://cran.r-project.org/package=reshape2.

Wickham, H. and Chang, W. (2016) 'ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics'. Available at: https://cran.r-project.org/package=ggplot2.

Wickham, H. and Henry, L. (2017) 'tidyr: Easily Tidy Data with "spread()" and "gather()" Functions'. Available at: https://cran.r-project.org/package=tidyr.

Wielanek, A. C. *et al.* (2007) 'Guillain-Barré Syndrome Complicating a Chickungunya Virus Infection', *Neurology*, 69(22), p. 2105 LP-2107.

Wilder-Smith, A. *et al.* (2010) 'Update on Dengue: Epidemiology, Virus Evolution, Antiviral Drugs, and Vaccine Development', *Current Infectious Disease Reports*, 12(3), pp. 157–164. doi: 10.1007/s11908-010-0102-7.

Wilder-Smith, A. (2014) 'Dengue vaccines: Dawning at last?', *The Lancet*, 384(9951), pp. 1327–1329. doi: 10.1016/S0140-6736(14)61142-9.

Wilder-Smith, A. and Gubler, D. J. (2008) 'Geographic Expansion of Dengue: The Impact of International Travel', *Medical Clinics of North America*, 92(6), pp. 1377–1390. doi: 10.1016/j.mcna.2008.07.002.

Wilder-Smith, A. and Macary, P. (2014) 'Dengue: Challenges for policy makers and vaccine developers', *Current Infectious Disease Reports*, 16(5), pp. 1–8. doi: 10.1007/s11908-014-0404-2.

Wilder-Smith, A. and Schwartz, E. (2005) 'Dengue in Travelers', *New England Journal of Medicine*, 353(9), pp. 924–932. doi: 10.1056/NEJMra041927.

Wilkerson, R. C. *et al.* (2015) 'Making mosquito taxonomy useful: A stable classification of tribe Aedini that balances utility with current knowledge of evolutionary relationships', *PLoS ONE*, 10(7), pp. 1–26. doi: 10.1371/journal.pone.0133602.

Williams, C. R. *et al.* (2006) 'Field efficacy of the BG-Sentinel compared with CDC Backpack Aspirators and CO2-baited EVS traps for collection of adult Aedes aegypti in Cairns, Queensland, Australia.', *Journal of the American Mosquito Control Association*. United States, 22(2), pp. 296–300. doi: 10.2987/8756-971X(2006)22[296:FEOTBC]2.0.CO;2.

Williams, C. R. *et al.* (2007) 'Aedes aegypti population sampling using BG-Sentinel traps in north Queensland Australia: statistical considerations for trap deployment and sampling strategy.', *Journal of medical entomology*, 44(March), pp. 345–350.

Wong-McClure, R., Suárez-Pérez, M. and Badilla-Vargas, X. (1999) 'Study of the dengue's seasonality in the Pacific coast of Costa Rica (1999–2004)', *Acta Médica Costarricense*, 49(1), pp. 38–41.

Wong, M. C. *et al.* (2011) 'A climate model for predicting the abundance of Aedes mosquitoes in Hong Kong', *Meteorological Applications*, 18(1), pp. 105–110. doi: 10.1002/met.218.

Wong, P. S. J. *et al.* (2013) 'Aedes (Stegomyia) albopictus (Skuse): A Potential Vector of Zika Virus in Singapore', *PLoS Neglected Tropical Diseases*, 7(8), pp. 1–5. doi: 10.1371/journal.pntd.0002348.

Wood, H. *et al.* (2014) 'Short report: Seroprevalence of seven zoonotic pathogens in pregnant women from the Caribbean', *American Journal of Tropical Medicine and Hygiene*, 91(3), pp. 642–644. doi: 10.4269/ajtmh.14-0107.

World Health Organization (2003) *The world health report 2003: Shaping the future*, *Geneva: Wold Health Organization*. Geneva: World Health Organization. doi: 10.1146/annurev.ecolsys.35.021103.105711.

World Health Organization (2009) 'Dengue: guidelines for diagnosis, treatment, prevention, and control', *Special Programme for Research and Training in Tropical Diseases*, p. 147. doi: WHO/HTM/NTD/DEN/2009.1.

World Health Organization (2015) 'Investing to overcome the global impact of neglected tropical diseases: third WHO report on neglected diseases 2015.', *Investing to overcome the global impact of neglected tropical diseases: third WHO report on neglected diseases*, p. 191. doi: ISBN 978 92 4 156486 1.

World Health Organization (2016) 'WHO statement on the first meeting of the International Health Regulations (2005) (IHR 2005) Emergency Committee on Zika virus and observed increase in neurological disorders and neonatal malformations', *WHO*. World Health Organization, 37(2005), pp. 2–5. Available at:

http://www.who.int/mediacentre/news/statements/2016/1st-emergency-committee-zika/en/ (Accessed: 11 April 2017).

World Health Organization (2017a) *Chikungunya. Fact sheet 327*, *WHO*. World Health Organization. Available at: http://www.who.int/mediacentre/factsheets/fs327/en/ (Accessed: 20 December 2017).

World Health Organization (2017b) *Dengue and severe dengue. Fact sheet 117*, *WHO*. World Health Organization. Available at: http://www.who.int/mediacentre/factsheets/fs117/en/ (Accessed: 30 November 2017).

World Health Organization (2017c) *Dengue Control: Epidemiology*, *WHO*. World Health Organization. Available at: http://www.who.int/denguecontrol/epidemiology/en/ (Accessed: 10 February 2018).

World Health Organization (2017d) *Dengue control Dengue data application*, *WHO*. World Health Organization. Available at: http://www.who.int/denguecontrol/epidemiology/dengue_data_application/en/ (Accessed: 11 February 2018).

World Health Organization (2017e) *Neglected tropical diseases*, *World Health Organization*. World Health Organization. Available at: http://www.who.int/neglected_diseases/diseases/en/ (Accessed: 11 February 2018).

World Health Organization (2017f) *World Health Organization*, *WHO*. World Health Organization. Available at: http://www.who.int/neglected_diseases/diseases/en/ (Accessed: 10 February 2018).

Wu, P. C. *et al.* (2007) 'Weather as an effective predictor for occurrence of dengue fever in Taiwan', *Acta Tropica*, 103(1), pp. 50–57. doi: 10.1016/j.actatropica.2007.05.014.

Wu, S. J. *et al.* (2000) 'Human skin Langerhans cells are targets of dengue virus infection.', *Nature medicine*. United States, 6(7), pp. 816–820. doi: 10.1038/77553.

Xie, Y. (2017) 'knitr: A General-Purpose Package for Dynamic Report Generation in R'. Available at: https://cran.r-project.org/package=knitr.

Yamashiro, T. *et al.* (2004) 'Seroprevalence of IgG specific for dengue virus among adults and children in Santo Domingo, Dominican Republic', *American Journal of Tropical Medicine and Hygiene*, 71(2), pp. 138–143. doi: 71/2/138 [pii].

Yang, H. M. *et al.* (2009) 'Assessing the effects of temperature on the population of Aedes aegypti, the vector of dengue.', *Epidemiology and infection*, 137(8), pp. 1188–1202. doi: 10.1017/S0950268809002040.

Yoo, E.-H. (2014) 'Site-specific prediction of West Nile virus mosquito abundance in Greater Toronto Area using generalized linear mixed models', *International Journal of Geographical Information Science*, 28(2), pp. 296–313. doi: 10.1080/13658816.2013.837909.

Yoon, I. K. *et al.* (2012) 'Fine scale spatiotemporal clustering of dengue virus transmission in children and aedes aegypti in rural Thai villages', *PLoS Neglected Tropical Diseases*, 6(7). doi: 10.1371/journal.pntd.0001730.

Yoon, I. K. *et al.* (2015) 'High Rate of Subclinical Chikungunya Virus Infection and Association of Neutralizing Antibody with Protection in a Prospective Cohort in The Philippines', *PLoS Neglected Tropical Diseases*, 9(5), pp. 1–14. doi: 10.1371/journal.pntd.0003764.

Yun, S. *et al.* (2016) 'Complete Genome Sequences of Three Historically Important, Spatiotemporally Distinct, and Genetically Divergent Strains of Zika Virus: MR-766, P6-740 and PRVABC-59', *American Society for Microbiology*, 4(4), pp. 1–4.

Zambrano, L. I. *et al.* (2012) 'Potential impacts of climate variability on dengue hemorrhagic fever in Honduras, 2010.', *Tropical Biomedicine*, 29(4), pp. 499–507.

Zanluca, C. *et al.* (2015) 'First report of autochthonous transmission of Zika virus in Brazil', *Memorias do Instituto Oswaldo Cruz*, 110(4), pp. 569–572. doi: 10.1590/0074-02760150192.

Zeger, S. L. and Liang, K.-Y. (1986) 'Longitudinal Data Analysis for Discrete and Continuous Outcomes', *Source: Biometrics BIOMETRICS*, 42(42), pp. 121–130. doi: 10.2307/2531248.

Zeileis, A. and Croissant, Y. (2017) 'Formula: Extended Model Formulas'. Available at: https://cran.r-project.org/package=Formula.

Zuur, A. F. *et al.* (2009) *Mixed EffectsModels and Extensions in Ecology with R*, *Statistics for Biology and Health*. Edited by M. Gail et al. New York, NY: Springer Science + Business Media, LLC. doi: 10.1017/978-0-387-87458-6.