EXPLORING *the* LINKS *between* DENGUE EPIDEMIOLOGY, POPULATION IMMUNE RESPONSE, *and* VACCINATION



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Dành tặng gia đình thân yêu của tôi

Dedicated to my beloved family

ABSTRACT

Dengue is the most rapidly spreading mosquito-borne viral disease in humans, causing around 58.4 million symptomatic cases and leading to 10,000–20,000 deaths annually. The only licensed dengue vaccine to date—*Dengvaxia*®—has been found to increase the chance of severe illness among naive recipients, and thus is recommended to be used only in seropositive individuals. Therefore, assessment of the long-term risk and benefit of future routine vaccination (with a *Dengvaxia*®-like vaccine) is a complex task that requires an understanding of multiple aspects of dengue transmission and vaccination, such as: [1] the link between the dynamics of dengue and the immune response of the population, [2] how this link may affect the performance of a selected pre-vaccination sero-screening method (employed to identify seropositive individuals), and [3] how vaccination may alter transmission and population immunity. This thesis consists of 3 major analyses that were designed to tackle the research questions above. The first analysis provides a statistical method to quantify the transmission intensity of dengue and to estimate the distribution of immunoglobulin G (IgG) antibody levels, using serological samples from 3 populations across Vietnam. The results of this work suggest the potential of using a quantitative approach to IgG levels to identify individuals with only 1 past infection (the primary targets of dengue vaccination). The second analysis develops a mathematical model to reconstruct the transmission dynamics of dengue in Hồ Chí Minh City (the most populous city in Vietnam) from case-record data of 2 major paediatric hospitals. Via this inference, the effect of maternal immunity on infants' risk of hospitalisation was also estimated. The final analysis combines the findings of the 2 prior investigations in a simulation study to examine how routine vaccination may affect the number of dengue hospitalisations in Hồ Chí Minh City. Collectively, the methodology developed throughout this thesis provides a modelling framework for evaluating the future epidemiological impacts of dengue vaccination on a vaccine-free population, if a *Dengvaxia*®-like vaccine is to be used.

DECLARATION

I hereby declare that this thesis, entitled '*Exploring the Links between Dengue Epidemiology, Population Immune Response, and Vaccination*', is an original record of my own research. The original idea for this work was developed in discussions with my supervisors—Hannah E. Clapham, Maia A. Rabaa, and Maciej F. Boni. I performed the entirety of the analyses and the writing task of the thesis. I confirm that appropriate credit has been given within the thesis wherever information or work has been derived from other sources. This thesis is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Oxford. It has not been submitted for any other degree or professional qualification except as specified.

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ABBREVIATIONS & ACRONYMS

ADE	antibody-dependent enhancement
C	capsid protein/gene (of the dengue virus)
CH1	Children's Hospital One (a paediatric hospital)
CH2	Children's Hospital Two (another paediatric hospital)
CI	confidence interval
CR	credible interval
DENV	dengue virus
DNA	deoxyribonucleic acid
DHF	dengue haemorrhagic fever
DIC	deviance information criterion
DSS	dengue shock syndrome
E	envelope glycoprotein/gene (of the dengue virus)
ELISA	enzyme-linked immunosorbent assay
FOI	force of infection
HI	hemagglutination inhibition
iELISA	inhibition enzyme-linked immunosorbent assay
IgG	immunoglobulin G
IgM	immunoglobulin M
MCMC	Markov Chain Monte Carlo
NS	non-structural proteins/genes (of the dengue virus)
OD	optical density
OR	odd ratio
prM	precursor membrane protein/gene (of the dengue virus)
PRNT	plaque reduction neutralization test
RNA	ribonucleic acid
SD	standard deviation
UI	uncertainty interval
WHO	World Health Organisation

1

OVERVIEW

1.1 INTRODUCTION

Dengue is a common mosquito-borne disease in humans that is caused by infection with the dengue virus (DENV). While it is generally agreed that the disease was domesticated long ago, its geographical origin is still a subject of debate (Holmes and Twiddy 2003). On the one hand, the fact that the primary mosquito vector of dengue (*Aedes aegypti*) originated from Africa has suggested an African root of the disease. By reconstructing the geographical expansion of the mosquito vectors (*Aedes aegypti* and *Aedes albopictus*), Mousson *et al.* (2005) speculated that the disease spread from Africa to the Americas between the 15th and 19th centuries (during the slave trade era), and to Asia in the 18th and 19th centuries with the rise of trade exchanges. However, this speculation has been challenged by a phylogeographical analysis by Wang *et al.* (2000), which suggested a long-time existence of both the sylvatic and the domestic cycles of dengue in Asia, indicating a potential Asian origin of the virus.

In the past 70 years, dengue transmission has been fuelled by human population growth, urbanisation, and globalisation, and has become a worldwide threat, with rising incidence in all human-inhabited continents (Messina *et al.* 2014) and 3 major hot spots in South America, Africa, and Southeast Asia (Cattarino *et al.* 2020). In 2010 it was estimated there were 390 million (95% credible interval (CR): 284–528 million) dengue infections, a quarter of which led to symptomatic cases (Bhatt *et al.* 2013). However, when considering the fact that dengue is an immunising disease (by which individuals should not be infected more than 4 times), the estimated number of infections was reduced to 105 million (95% confidence interval (CI): 95–114 million) per year (Cattarino *et al.* 2020). According to an estimate from the *Global Burden of Disease Study 2013* (Stanaway *et al.* 2016), between 1990 and 2013, the annual number of symptomatic dengue cases (including those that

were undetected by reporting systems) increased seven-fold, from 8.3 million (95% uncertainty interval (UI): 3.3–17.2 million) to 58.4 million (95% UI: 23.6–121.9 million). In this study, multiple data sources were combined in a modelling approach to make adjustments for dengue occurrence rates and reporting rates; however, data missingness still yielded wide uncertainty ranges in the approximations (Stanaway *et al.* 2016). Contrasting the rising number of cases, the annual number of deaths due to dengue was estimated to be levelling off at around 10,000 across the years 1990-2013 (Stanaway *et al.* 2016). While this fall in the mortality rate may indicate improvement in diagnosis and treatment, it should be noted that fatal dengue episodes could have been misdiagnosed and under-reported, especially in countries with other predominant febrile illnesses (Stanaway *et al.* 2016).

1.2 DENGUE INFECTION

1.2.1 *The Virus*

The DENV belongs to the genus *Flavivirus* of the *Flaviviridae* family. The genus consists of viruses that are carried in arthropods (primarily ticks and mosquitoes). Other commonly known mosquito-borne viruses in the same group include yellow fever, Japanese encephalitis, West Nile, and Zika viruses. There are 4 serotypes of DENVs—numbered from 1 to 4 (DENV-1 to DENV-4)—each of which was thought to have become domesticated independently around 1000–1500 years ago (Wang *et al.* 2000). Before domestication, non-human primates were the principle reservoir of DENVs. Although these sylvatic cycles still exist, their impacts on the epidemiology of dengue are unclear. While the 4 serotypes of DENVs only share around 70% amino acid identity across the genome—making them no more genetically similar to each other than to other flaviviruses (Kuno *et al.* 1998)—immune response following a primary DENV infection (i.e.the first DENV infection in life) with a serotype may provide transient protection against the other serotypes (Sabin 1952). Although antigenically, DENVs cluster by serotype, a recent study has found that the antigenic distance between 2 DENVs within the same serotype may be comparable to that between viruses of different serotypes (Katzelnick *et al.* 2015).

A dengue virion is a spherical particle of 40–50 nm diameter. The linear non-segmented genome of the virus comprises around 11 kb of positive, single-stranded ribonucleic acid (RNA) that encodes 10 proteins, including the precursor membrane (prM), the envelope (E) glycoprotein, the capsid (C), and 7 non-structural (NS) proteins (i.e. NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (Deubel *et al.* 1988; Mackow *et al.* 1987; Mason *et al.* 1987; Osatomi and Sumiyoshi 1990). Important pathological properties of DENV are known to be associated with the E, the prM, and the NS1 proteins (Carbaugh and Lazear 2020; Glasner *et al.* 2018; Nasar *et al.* 2020; Uno and Ross 2018).

1.2.2 *The Vectors*

The 2 primary mosquito carriers of DENV are *Aedes* (*Stegomyia*) *aegypti* and *Aedes* (*Stegomyia*) *albopictus* (Reeves 1965).[1] The average lifetime of both *A. aegypti* and *A. albopictus* mosquitoes is about 19–20 days, with nearly half of this time (around 9 days) spent in preadult development (Maimusa *et al.* 2016). While *A. aegypti* emerged from Africa (Mattingly 1957) and *A. albopictus* from Asia (Knudsen 1995), both species have quickly spread throughout the world due to human trading activities.

Between the 2 mosquito species, *A. aegypti* plays a more important role in dengue transmission (Mattingly 1967). Specializing in warm and wet climates, the mosquito has colonized tropical and subtropical areas (Lambrechts *et al.* 2010). There are 2 variants of *A. aegypti*: the dark tree-dwelling form *A. aegypti formosus*, and the pale canopy-dwelling form *A. aegypti aegypti* (Mattingly 1957). *A. aegypti aegypti*—the main cause of urban dengue epidemics worldwide—has a tendency of staying close to its man-made breeding sites (Mattingly 1957). Meanwhile, the better-flyer—*A. aegypti formosus*—prefers natural breeding sites and is thought to contribute only to forest transmission cycles in Africa. Evidence has suggested that *A. aegypti aegypti* is more susceptible to dengue than *A. aegypti formosus* (Failloux *et al.* 2002).

The secondary vector—*A. albopictus*—can be found in peri-urban, rural, and forest zones in both tropical and temperate countries (Gratz 2004). The wide spectrum of ecological preference has made the species very successful in expanding its geographical occurrence, even in places that are uninhabitable for *A. aegypti* such as Europe and North America (Benedict *et al.* 2007).

[1] According to the most recent classification, the mosquito species are taxonomically named as *Stegomyia aegypti* and *Stegomyia albopictus* since the subgenus *Stegomyia* has been upgraded to genus level. However, for simplicity of reference, the names *Aedes aegypti* and *Aedes albopictus* are still used more commonly in the scientific literature (Polaszek 2006).

Although *A. albopictus* has a lower vector competence for DENV, its alarming rate of invasion in the last few decades has changed the epidemiological picture of dengue (Lambrechts *et al.* 2010). In fact, *A. albopictus* is currently the principle vector for dengue in South China and Europe (Kraemer *et al.* 2015; Reiter 2010).

1.2.3 *The Transmission Cycle*

An *Aedes* mosquito may acquire DENVs in 2 ways: either by taking a blood meal from a human host who is in the viraemic stage of the disease (Duong *et al.* 2015), or—less frequently—by vertical transmission from its parent (Ferreira-de-Lima and Lima-Camara 2018; Goncalves *et al.* 2020). Although human infectiousness to mosquitoes has been found to positively correlate to patients' viraemia levels (Duong *et al.* 2015; Nguyet *et al.* 2013), asymptomatic and presymptomatic patients may have a higher transmission potential compared to symptomatic cases of the same viraemia level (Duong *et al.* 2015). A study has shown that inapparent human cases may account for 84% of DENV transmission (Bosch *et al.* 2018). After a mosquito acquires the viruses, the viruses replicate in mosquito midgut cells, and from there, they spread to other tissues. The mosquito only becomes infective 2–15 days after an infectious bloodmeal (given an environmental temperature of 30 °C), when the viruses have reached the salivary glands (Chan and Johansson 2012). From this point on, the mosquito is capable of transmitting the virus to every individual it feeds on.

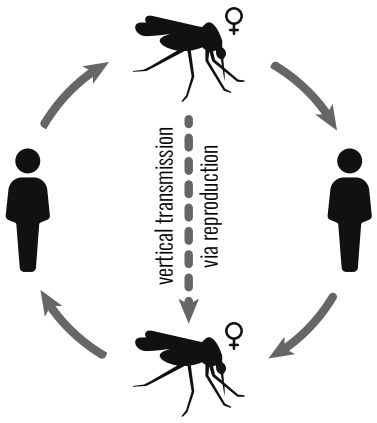


FIGURE 1.1 – The transmission cycle of dengue virus.

1.2.4 *Clinical Manifestation and Disease Classification*

Although DENV infections are often asymptomatic, infection can cause a wide range of clinical outcomes, from mild fever—normally followed by a

full recovery—to potentially deadly dengue shock syndrome (DSS) (C. P. Simmons *et al.* 2012). After the intrinsic incubation period of 3–7 days, the clinical manifestations of the disease in humans can be divided into 3 phases, in the following order: the febrile phase, the critical phase (occurring only in a small proportion of patients), and the recovery phase. The febrile stage often lasts for 3–7 days from illness onset, during which patients often present with high fever, severe headache, retro-orbital pain, muscle pain, joint pain, vomiting, and altered taste sensation. During this stage, mild disease may be mistaken for a flu-like illness; however, mild bleeding manifestation and rashes—occurring in some patients—may help to differentiate dengue from influenza.

While most patients proceed to the recovery phase directly from the febrile phase, under 5% of the symptomatic cases may experience clinical complications (Bhatt *et al.* 2013) around the time of defervescence, when the viral load is in steep decline (Yacoub and B. Wills 2014). This critical phase typically occurs on days 3–7 after symptoms onset, and is often characterised by capillary leakage, intravascular volume depletion, and haemorrhage (P. K. Lam *et al.* 2013; Rosenberger *et al.* 2016). Some individuals may develop deadly shock syndrome during the critical phase. However, with good supportive care, most patients—including those with complications—fully recover within 1–2 weeks.

Human challenge studies during World War II indicated that an infection by a DENV serotype provided long-term homotypic protection against symptomatic disease, even after 18 months (the longest tested interval) (Sabin 1952). Although a recent study on a cohort of 2,892 children in Nicaragua revealed 4 symptomatic cases of homotypic reinfection (Waggoner *et al.* 2016), this result also suggests that secondary homotypic DENV infections rarely lead to clinical symptoms. In contrast, secondary heterotypic infections are more likely to cause severe disease, as pre-existing heterologous antibody may mediate DENV pathologies.[2]

[2] This topic is discussed in more detail in §1.2.6 on page 9.

While dengue complications are often readily identifiable, milder disease can present in a very broad and subtle spectrum of clinical manifestations. Therefore, developing an effective classification system for disease severity—which is essential for clinical triage and management—has been

a great challenge. In 1997, the World Health Organisation (WHO) recommended the division of clinically diagnosed dengue cases into 2 groups: dengue fever and dengue haemorrhagic fever (DHF) (World Health Organization 1997). However, the usefulness of this scheme was questioned as many patients could develop haemorrhage and shock even they did not meet the criteria to be classified as DHF cases (Deen *et al.* 2006). For this reason, in 2009, the WHO proposed a new classification scheme (World Health Organization 2009), which has been adopted by many countries, including Vietnam. In this system, symptomatic patients are identified as having dengue or having severe dengue, depending on whether they present with severe complications in any of 3 categories: [1] severe plasma leakage, [2] severe bleeding, and [3] severe organ involvement. The group of dengue cases without severe complications is divided further into 2 subgroups, i.e. dengue with and without warning signs. Individuals with warning signs also require strict observation and medical intervention as they may be more likely to progress to severe dengue. While this revised classification system is more sensitive for the diagnosis of severe dengue and still in use to date, its effectiveness remains controversial (Farrar *et al.* 2013; S. R. S. Hadinegoro 2012; Halstead 2013).

1.2.5 *Human Immune Response*

A DENV infection starts at the site of the mosquito bite in the skin, where the viruses encounter various immune sentinel cells, such as macrophages, mast cells, and Langerhans cells (i.e. dendritic cells resident in the skin). Among these, macrophages and dendritic cells are known targets of DENV (St. John and Rathore 2019). As soon as the viruses are detected (by specialised proteins called pattern recognition receptors), the antiviral innate immune response is triggered with the activation of various transcription factors (e.g. interferon regulatory factors 3 and 7) and the degranulation of mast cells, which lead to the release of cytokines and chemokines (Ho *et al.* 2001; Libraty *et al.* 2001; St. John *et al.* 2011; Wathelet *et al.* 1998). As a result of these immune signals, cytotoxic cells (including $CD8^+$ T cells, natural killer cells, and natural killer T cells) and monocyte-derived dendritic cells are recruited to the site of infection (Rivino *et al.* 2015; Schmid and Harris 2014; St. John

et al. 2011). The cytotoxic cells can promote virus clearance by lysis of DENV-infected cells (Mathew *et al.* 1996) or by releasing cytokines (such as interferon gamma) that inhibit the replication of DENV (Costa *et al.* 2017; Gagnon *et al.* 1999). Meanwhile, the recruited dendritic cells may serve as additional targets for DENV infection and replication, facilitating the amplification of the virus in the skin. After successfully achieving viraemia in the skin, the viruses may infect skin-draining lymph nodes to establish a systemic infection (Kyle *et al.* 2007; Marchette *et al.* 1973). At this stage, dendritic cells and monocytes that travel through the infected lymph nodes may be susceptible to infection (Kyle *et al.* 2007; Marchette *et al.* 1973). Three to 7 days after the mosquito bite, the viraemia reaches its peak, which is often characterised by the onset of high fever in apparent cases (Nishiura and Halstead 2007; Tricou *et al.* 2011; Vaughn *et al.* 1997).

It has been speculated that the migration of infected dendritic cells from the skin to lymph nodes may contribute to the introduction of the viruses to the lymph nodes (St. John and Rathore 2019). Dendritic cells may also play a critical role in DENV-antigen presentation in lymph nodes, as studies have shown that infected dendritic cells may be able to upregulate the activation of resting T cells (Ho *et al.* 2001; Nightingale *et al.* 2008). Furthermore, an *in vitro* study has shown that DENV-exposed monocytes mediate the differentiation of B cells into plasmablasts and promote the production of immunoglobulin G (IgG) and immunoglobulin M (IgM) (Kwissa *et al.* 2014).

The immune clearance of DENV in the host involves multiple types of T cells and B cells (Rothman 2011). By comparing between asymptomatic and symptomatic cases, a study in Cambodia illustrated that asymptomatic infections were associated with increased activation of $CD4^+$ T cells (Simon-Lorière *et al.* 2017). Additionally, a study on Sri Lankan patients of known severity also suggested that a lower risk of hospitalised disease may link to a higher magnitude of T cell responses (Weiskopf *et al.* 2016). In patients with acute infection, a massive expansion of activated $CD8^+$ T cells have been observed during the febrile stage of illness (Friberg *et al.* 2011). In primary cases, this expansion reached its peak around the day of defervescence; meanwhile,

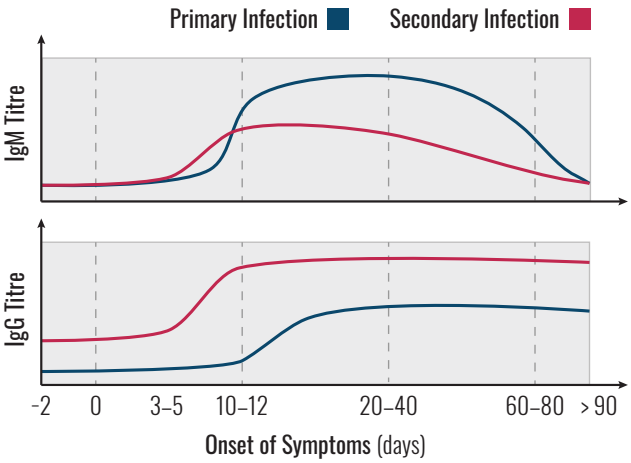


FIGURE 1.2
Approximate dynamics of IgM and IgG during and after primary and secondary infections of dengue (redrawn from Figure 4.1 of World Health Organization 2009).

in secondary cases, the peak occurred later (Friberg *et al.* 2011). Although evidence has suggested that the cytotoxic function of $CD4^+$ T cells may be protective (Tian *et al.* 2017; Weiskopf *et al.* 2015), the effectiveness of $CD8^+$ T cells in virus clearance is still under question (Chandele *et al.* 2016).

The dynamics of antibodies in primary and secondary infections are conceptually summarised in Figure 1.2. The start of the DENV-specific IgM and IgG production during a primary infection often coincides with the onset of fever (Vaughn *et al.* 1997). From this time point, both IgM and IgG titres increase substantially until early convalescence, i.e.15–25 days after symptoms onset (Balakrishnan *et al.* 2011). Although IgM titre rises much faster than that of IgG during the early phase of a primary infection (Clapham *et al.* 2016; Nguyen *et al.* 2018), by day 4–7 after the start of febrile symptoms, IgG already accounts for more than half of the antibody neutralisation (Balakrishnan *et al.* 2011). During a secondary infection, a significantly larger secretion of memory B cells and DENV-specific IgG is observed (Nguyen *et al.* 2018; Wrammert *et al.* 2012). while the kinetics of IgM in a secondary infection are similar to those of a primary infection, the levels of IgM are significantly lower (Nguyen *et al.* 2018). Antibodies, memory B cells, and T cells that are detected during the acute phase of a secondary infection may be cross-reactive to multiple DENV serotypes (Mongkolsapaya *et al.* 2003; Priyamvada *et al.* 2016; Xu *et al.* 2012), potentially leading to suboptimal immune responses (Sangkawibha *et al.* 1983; Zompi and Harris 2013). A key factor that distinguishes a secondary infection from a primary one is the presence of pre-existing antibodies, which are thought to enhance infection and cause more

severe disease (Halstead and O'Rourke 1977; Midgley *et al.* 2011; Rodenhuis-Zybert *et al.* 2011).

1.2.6 *Antibody-dependent Enhancement*

Cohort studies in Vietnam (Chau *et al.* 2008), Thailand (Sangkawibha *et al.* 1984), Cuba (Guzmán *et al.* 2000), and Nicaragua (Katzelnick *et al.* 2017) have shown that patients with prior homotypic immunity against a dengue serotype are more likely to develop severe disease upon infection with a different serotype. This observed phenomenon is often explained by the antibody-dependent enhancement (ADE) hypothesis, which theorises that pre-existing weakly-neutralising antibody may mediate virus infection and replication by promoting virus opsonisation (Halstead and O'Rourke 1977). Recent studies have illustrated that immature prM-containing virions produced during a DENV infection—which normally lack the ability to infect host cells—can become highly infectious when interacting with anti-prM antibodies (Alwis *et al.* 2014; Dejnirattisai *et al.* 2010). Similarly, antibodies that target the fusion loop on the DENV E protein can also render non-infectious immature viruses infectious (Rodenhuis-Zybert *et al.* 2011).

It is noteworthy that the enhancing properties of antibodies depend not only on their avidity but also on their titres. It has been demonstrated *in vitro* that strongly neutralising antibody may show enhancing characteristics when present at low concentration, while weakly neutralising antibody can effectively neutralise virus particles when present at high concentration (Pierson *et al.* 2007). Recent research has shown that ADE only occurs at a very specific range of pre-existing antibody titres. Results from a long-term paediatric cohort study in Nicaragua suggested that this opportunistic range may be between 1:21 and 1:80 when measured from serum samples using inhibition enzyme-linked immunosorbent assays (iELISAs),[3] as children with this level of pre-existing antibodies had 7.64-fold higher risk of developing severe disease (DHF/DSS) compared to naive individuals or those with a higher level of antibodies (Katzelnick *et al.* 2017). Consistently, a school-based cohort study in Thailand also showed that the risk of DHF in children with pre-existing serum antibodies of HI titres below 1:40 or plaque reduction neutralization test (PRNT) titres below 1:100 was 7.5 times

[3] Note that antibody titres produced by iELISAs are a twofold dilution higher than resulted from hemagglutination inhibition (HI) assays. More information on methods of antibody quantification is provided in §1.3.

higher than in naive individuals (Salje *et al.* 2018). Furthermore, Aguas *et al.* (2019) have suggested that serotype interaction may also play a role in ADE since the authors found that a moderate level of homotypic antibody against DENV-1 may enhance infection caused by DENV-2 and DENV-4, but may confer protection against DENV-3.

A NOTE ON TERMINOLOGIES: Infants born to dengue-immune mothers may experience ADE during their first infection in life, due to maternally transferred immunity.[4] Since maternal immunity and self-generated immunity may act differently in mediating the infection, the magnitude and the dynamics of the ADE are likely to be different between infants and the older age groups. Therefore, to clearly describe the context of ADE throughout this thesis, the terms *maternal ADE* and *non-maternal ADE* are used, respectively, to specify whether the phenomenon under consideration is occurring in infants or in older individuals.

[4] A more detailed discussion about ADE in infants is provided in §1.4.2 on page 14.

1.2.7 *Risk Factors of Severe Disease*

Secondary infection—during which ADE usually occurs—is the major risk factor of severe disease (L. Bravo *et al.* 2014; Burke *et al.* 1988; Graham *et al.* 1999; Guzmán and Kourí 2002; Thein *et al.* 1997). By examining 195 serologically confirmed paediatric dengue patients (who were below 10 years of age), a cohort study in Myanmar estimated that the risk of developing DSS (the most severe form of dengue infection) during a secondary infection is 82–103 times higher than in a primary infection (Thein *et al.* 1997). Since secondary cases often cluster within a specific age group in stable transmission settings, patient age is a strong predictor for the likelihood of severe disease (A. Kumar *et al.* 2020). Depending on the transmission intensity, the high-risk group can be children (Anders *et al.* 2011; G. L. Kumar *et al.* 2018; Sangkawibha *et al.* 1984) or young adults (Guzmán *et al.* 2000). During a secondary infection, bleeding is observed more often in adults than in children, while vascular permeability and shock occur more frequently in children (Trung *et al.* 2012). In highly endemic settings, infants are a special group that faces a high risk of severe disease during primary infections since maternally derived immunity may also lead to ADE[5] (Hung *et al.* 2004; C. P. Simmons *et al.* 2007).

[5] The mechanism of how ADE may occur in infants during their first dengue infection in life is discussed in detail in §1.4.2 on page 14.

Regardless of transmission setting, elderly dengue patients are at greater risk than younger age groups of developing severe complications (A. Kumar *et al.* 2020; Pang *et al.* 2017). A matched case-control study in Singapore has estimated that the risk of severe organ failure is 2.75 times higher in patients who are 60 years of age or older, as compared to those between 12–29 years of age (Pang *et al.* 2017). Obesity and pre-existing chronic conditions—such as diabetes, cardiac disorders, asthma, and sickle cell disease—may substantially increase the risk of severe episodes during a dengue infection (Diptyanusa *et al.* 2019; Elenga *et al.* 2020; Gallagher *et al.* 2020; Pang *et al.* 2017). Furthermore, patients' genetic backgrounds have also been suggested to affect the disease progression in many different ways (Boonnak *et al.* 2011; J. Bravo *et al.* 1987; Oliveira *et al.* 2018; Sierra *et al.* 2017; Xavier-Carvalho *et al.* 2017). While a few studies have shown evidence for the link between gender and disease outcomes, the mechanism of this association is still little understood. An epidemiological study on hospitalised paediatric and adult patients in Hồ Chí Minh City (southern Vietnam) between the years 1996–2009 found that compared to males, females faced higher risk of DSS (odd ratio (OR): 1.19; 95% CI: 1.14–1.24) and death (OR: 1.57; 95% CI: 1.14–2.17) (Anders *et al.* 2011). Meanwhile, a study on 1,484 hospitalised adult patients (≥18 years of age) in Bangkok (Thailand) observed that the rate of developing acute kidney injury during a dengue infection was 2.7 times higher on males than on females (Diptyanusa *et al.* 2019).

Studies have suggested that DENV serotype may be associated with disease severity. In both children and adults, primary infections of DENV-1 and -3 tend to cause more symptomatic cases than those of DENV-2 and -4 (Buchy *et al.* 2005; Clapham *et al.* 2015; Thai *et al.* 2010; Vaughn *et al.* 2000). Meanwhile, the severity of a secondary infection may be controlled by the serotypes of both the first and the second infection. High incidences of severe disease have been observed for infection sequences with serotypes 2-1, 1-2, 3-2, 4-2, and 1-3 (Gordon *et al.* 2013; Guzmán *et al.* 2012; Guzmán *et al.* 2002; Hubert 2009; Sangkawibha *et al.* 1984). However, it should be noted that our understanding about the association between dengue serotype and disease severity is still very limited since this subject is plagued by 2 major problems. First, serotyping has not often been incorporated in large-scale studies as it does not impact treatment and can rather be costly and labour-intensive,

especially in the case of sequential infections. Second, dengue epidemics are often dominated by 1 or 2 serotypes—which then can be replaced after a few years—making it difficult to discriminate whether the number of severe cases is driven by serotype's intrinsic characteristics or by time-dependent and/or population-specific elements. For example, changes in serotype-unrelated factors—such as the herd immunity profile, population age structure, climate, and effectiveness of vector control programmes—can also lead to alteration of the transmission intensity (Cummings *et al.* 2009; Katzelnick *et al.* 2018; Reiner *et al.* 2014), thus yielding variation in the number of dengue incidences and possibly in the number of severe cases.

1.3 MEASUREMENT OF DENGUE ANTIBODY

1.3.1 *Enzyme-linked Immunosorbent Assay*

The enzyme-linked immunosorbent assay (ELISA) is among the most common methods used to measure the concentration of certain types of antibodies, such as IgM, IgG, or antibodies against a specific part of the virus protein. In this process, tested samples (serum, plasma, or saliva) are incubated in antigen-coated microtitre wells to create antigen-antibody complexes (if the samples contain antibodies). A detection agent that is complementary to the measured antibody is then added to the wells. In direct ELISAs, this detection agent is a labelling enzyme, whereas in indirect ELISAs, it is a conjugate of a secondary antibody and a labelling enzyme. Finally, a substrate is added to the wells to be acted on by the labelling enzyme to produce colour changes or fluorescence emissions. These optical signals are often evaluated in optical densities to give a quantitative value to the amount of antibody in each ELISA well (Dittmar *et al.* 1979). Alternatively, serial dilutions of samples can be tested, and antibody titres are defined as the reciprocal of the dilution that yielded a pre-specified optical density (Chau *et al.* 2009).

1.3.2 *Plaque Reduction Neutralisation Test*

The plaque reduction neutralization test (PRNT) (Roehrig *et al.* 2008; Russell and Nisalak 1967) is the most specific and sensitive serological assay for

detecting and quantifying the neutralisation level of dengue antibodies. However, this method is low-throughput, more time-consuming, and more labour-intensive than the ELISA. The PRNT involves mixing serial dilutions of the tested serum with a constant amount of virus, followed by plating of the mixture onto appropriate host cells. After a certain incubation time, the number of infected host cells (plaques) is counted and divided by the original number of virions to calculate the percentage neutralisation. Interpretation of a dengue PRNT is often based on PRNT_{50}, which is defined as the highest dilution capable of inhibiting 50% of the total virions. Despite efforts to standardise the assay (Guzmán and Kourí 2004; Putnak *et al.* 2008; Roehrig *et al.* 2008), there is still a wide variety of PRNT methods being used across laboratories. This leads to great difficulty in comparing and interpreting the test results from different studies as research has shown that altering certain assay conditions (such as the incubation time, the cell line, and the DENV strain used for the reagents) may lead to considerable variation in the titre estimates (Salje *et al.* 2014; Thomas *et al.* 2009). For a long time, PRNTs have been employed in dengue vaccine trials to test the immunogenicity of the vaccines (Kirkpatrick *et al.* 2015; Sabchareon *et al.* 2012; Sirivichayakul *et al.* 2016). However, it has been found that the neutralisation levels produced by this assay are not good correlates of protection against DENV (Shrivastava *et al.* 2017).

1.3.3 *Hemagglutination Inhibition Assay*

In a hemagglutination inhibition (HI) assay, the titre of antibody is determined based on the maximum dilution of serum that effectively inhibits the virus binding to cells (World Health Organization 2009). Historically, this assay was commonly used; however, it is less frequently adopted in recent studies. Antibody titres measured from HI assays often correlate well with ELISA results (World Health Organization 2009).

1.4 DENGUE IN INFANTS

1.4.1 *Disease Burden*

In children and adults, severe dengue cases are generally associated with secondary infection (L. Bravo *et al.* 2014; Burke *et al.* 1988; Graham *et al.* 1999; Thein *et al.* 1997); however, in infants who are born to dengue-immune mothers, severe disease can also occur during their first dengue infection (Hung *et al.* 2004; C. P. Simmons *et al.* 2007). Data from a paediatric hospital in Thailand showed that between 1988 and 1995, infants (below 2 years old) represented 7.7% of all hospitalised dengue paediatric patients (Pancharoen and Thisyakorn 2001). During an outbreak between the years 2001 and 2002 in Chennai, India, nearly 20% of hospitalised and confirmed dengue infections at a children's hospital were under 1 year of age (Kabilan *et al.* 2003). A retrospective study on dengue hospitalised cases at a paediatric hospital in Bangkok, Thailand showed that, compared to older children, infants had a 3–6 times higher risk of developing DHF symptoms (e.g. upper respiratory tract infection symptoms, diarrhoea, convulsions and encephalopathy) while having a slightly lower chance of experiencing shock syndrome (i.e. 25% versus 30.9%) (Kalayanarooj and Nimmannitya 2003). It has been estimated that infants constitute around 5% of all children hospitalised due to DHF in Vietnam, Thailand, Myanmar, and Indonesia (Halstead *et al.* 2002). This high proportion of infant cases, however, has not been observed in places with shorter or non-endemic history of dengue circulation (Rahman *et al.* 2002; Souza *et al.* 1995), suggesting that the risk of developing severe symptoms on infants may link to the immune status of the population (Clapham *et al.* 2015) and to the ADE effect caused by maternally derived antibodies (Chau *et al.* 2008).

1.4.2 *Antibody-dependent Enhancement in Infants*

ADE mediated by maternally acquired DENV-specific IgG is among the primary hypotheses proposed to explain the increased severity of primary infection in infants (who have not been able to produce anti-DENV IgG). This hypothesis postulates that maternal antibodies have a biphasic effect, which

confers protection at birth, when IgG concentration is high. However, as maternal IgG antibodies break down over time, this concentration quickly declines to a sub-neutralising level, potentially leading to ADE upon infection (Halstead and Simasthien 1970; Kliks *et al.* 1988). As the occurrence of ADE may require a very specific level of cross-reactive antibodies (Katzelnick *et al.* 2017; Salje *et al.* 2018), there may also be a very specific time window in which ADE occurs in infants. This hypothesis has been supported by many studies in endemic settings. Kliks *et al.* (1988) found that the actual ages of DHF/DSS infant cases in Bangkok, Thailand (i.e. 5–9 months) matched the ages at which maximum enhancing activity was predicted by their *in vitro* experiment. In this study, by assuming that maternal IgG levels of newborns at birth were similar to the antibody levels of the mothers, the authors could then estimate the infection enhancement effect in infants by using mothers' sera at different dilutions; the enhancement activity was quantified by the magnitude of increase of virus production from cell cultures infected in the presence of test serum in comparison to that measured in the absence of test serum Kliks *et al.* (1988). Data from infant cases admitted to paediatric hospitals in Thailand, Vietnam, Indonesia, and Myanmar consistently showed that the incidence of DHF was clustered between 3 and 11 months of age, with the number of cases peaking at around the 7^{th} or the 8^{th} month (Chau *et al.* 2008; Halstead *et al.* 2002). A nested case-control study in the Philippines also found that all of the observed infant DHF cases were between 3 and 8 months of age, while the number of DHF cases fell to zero at the 9^{th} month (R. Z. Capeding *et al.* 2010; Libraty *et al.* 2009). Likewise, studies from a birth cohort in Northeast Brazil estimated that the enhancing activity peaked at 2 months of age and almost disappeared by the age of 4 months (Castanha *et al.* 2016); however, this conclusion was drawn from an *in vitro* analysis of paired sera of mothers and infants, as none of the dengue cases in the study developed severe symptoms (Castanha *et al.* 2017). Interestingly, while the estimated time window for ADE in Brazil was much shorter and earlier than that of the Philippines, the estimated incidence rates of infant dengue cases at the 2 study locations were only slightly different, i.e. 107 per 1,000 person-years for Brazil (Castanha *et al.* 2017) and 119 per 1,000 person-years for the Philippines (R. Z. Capeding *et al.* 2010).

Generally, the opportunistic time window for ADE to occur in a newborn depends on 3 factors: [1] the level of maternally derived antibodies the child receives, [2] the decaying speed of this maternal immunity, and [3] the level of antibodies required for ADE to occur. A study in 1980–1981 in Thailand showed a strong correlation between DENV-2 $PRNT_{50}$ titres in mothers' sera and the age of DHF/DSS infants (Kliks *et al.* 1988). A more recent nested case-control study in Thailand also found an association between DENV-3 $PRNT_{50}$ titres in maternal plasma (estimated at birth) and infant ages at the onset of symptomatic primary DENV-3 infections (Libraty *et al.* 2009). These findings suggest that the opportunistic time windows for ADE in newborns may depend on how much dengue-specific IgG antibody they receive from mothers, as the levels of maternally derived antibodies in newborns are proportional to the antibody titres of mothers (Castanha *et al.* 2016; Perret *et al.* 2005). However, there still remains the question of what factors control the amount of IgG antibody passed from mothers to infants. A cross-sectional study on Thai mothers indicated that cord antibody titres in infants (measured by HI assays) born to younger mothers ($\leq$20 years old) were statistically significantly higher than those born to older mothers (Perret *et al.* 2005). But it is unclear whether this phenomenon linked to the possibility that the $\leq$20 years old mothers might have higher levels of antibody than the older ones due to more recent exposure to infection (Perret *et al.* 2005) or it was caused by age-related biological factors. Likewise, a study in Brazil—with most mothers having experienced DENV-3 monotypic or DENV-3/DENV-4 multitypic infections before giving birth—showed that monotypic-immune mothers transferred greater levels of antibodies to their newborns during birth than mothers with multitypic immunity (Castanha *et al.* 2016). Studies in Bangkok, Thailand and Hồ Chí Minh City, Vietnam—where DENV-4 was the least prevalent among the 4 serotypes in the studied mothers—consistently observed that in both maternal blood and umbilical cord blood, the level of antibodies specific to DENV-4 (determined by $PRNT_{50}$) were much lower than those to other serotypes (Chau *et al.* 2009; Khamim *et al.* 2015; Pengsaa *et al.* 2006). These findings indicate that the infection history of the mother may play an important role in varying the amount of dengue-specific antibody passed to infants, and hence in altering the infection enhancing effect in infants. Another important factor that may alter not only the level but also the decaying speed

of maternally derived antibodies is the route through which maternal antibodies could be transferred to infants. Although many studies in Vietnam, Thailand, and Brazil conveyed that dengue-specific IgG was effectively transported via the placenta at very high concentrations (Castanha *et al.* 2016; Chau *et al.* 2009; Khamim *et al.* 2015; Pengsaa *et al.* 2006; Perret *et al.* 2005), it is not known whether maternal immunity could also be passed and sustained in a another effective way. Breastfeeding, for example, has been found to be a more efficient route than gestation for transferring dengue-specific IgG in a mouse model (P. X. Lee *et al.* 2016).

Although most studies on dengue maternal immunity have reported non-neutralising levels of antibody after 12 months of age (Castanha *et al.* 2016; Chau *et al.* 2009; Panhuis *et al.* 2011), there have been differences in the estimated decay speed of maternal antibody. Studies in Vietnam, Thailand, and the Philippines have estimated that the half-life of maternal anti-DENV IgG was between 36 and 42 days (R. Z. Capeding *et al.* 2010; Chau *et al.* 2009; Libraty *et al.* 2009; C. P. Simmons *et al.* 2007; Watanaveeradej *et al.* 2003). Meanwhile, another study in Thailand reported a biphasic half-life of maternal IgG, which was 24–29 days long from birth to 3 months of age, and was 44–150 days after 3 months of age (Panhuis *et al.* 2011). Despite the slight variation, these estimates consistently suggest that the decaying rate of maternal antibodies is much faster than the estimated 4-year half-life of self-generated anti-DENV IgG (Katzelnick *et al.* 2017). Interestingly, Panhuis *et al.* (2011) found that the waning rate of maternal antibody may be different across serotypes, with the order from the fastest to the slowest being DENV-4, DENV-2, DENV-3, and DENV-1. Furthermore, the more dengue-specific antibody infants received at birth, the quicker this maternal immunity decayed; therefore, the persistence time of PRNT titres could not be predicted based on its value at birth (Panhuis *et al.* 2011). These results suggest that the transferred amount and the decay speed of maternal antibody may be strongly linked to circulating serotypes and transmission intensity.

The high number of DHF/DSS cases in infants between 3 and 12 months old—observed in endemic settings when maternally derived antibodies may have decayed below neutralizing levels (Chau *et al.* 2008; Halstead *et al.* 2002; Kliks *et al.* 1988; Libraty *et al.* 2009; C. P. Simmons *et al.* 2007)—is consistent with the concept of a peak enhancement titre for DHF/DSS (Katzelnick

et al. 2017; Salje *et al.* 2018). However, few studies have estimated the suitable level of maternally derived antibodies for ADE to occur in newborns. A study in Vietnam reported that 65% of infants with DHF had a $PRNT_{50}$ titre below 1:20 measured in plasma (C. P. Simmons *et al.* 2007). Likewise, a cohort study in the Northeast Region of Brazil found that at the age of 2 months—when maximum enhancing activity was estimated via an *in vitro* experiment—the mean dengue-specific $\log_{10}$ IgG titre (measured by ELISAs) of infants was 3.84 (standard deviation (SD): 0.82), and the mean $\log_{10}$ DENV-3 $PRNT_{50}$ titre was 1.82 (SD: 0.58) (Castanha *et al.* 2016).

Although a study using a mouse model showed that ADE caused by maternally derived antibodies could lead to lethal disease in mouse pups (Martínez Gómez *et al.* 2016), the link between ADE and severity in human infants is not fully understood. On the one hand, increased rates of DHF and DSS in infants have been observed in highly endemic settings, where most newborns may have maternal immunity against dengue (Halstead *et al.* 2002; Kliks *et al.* 1988). Using an *in vitro* enhancement assay, Chau *et al.* (2008) showed that maternal antibodies in infant plasma could facilitate DENV-2 infection and lead to more K562 cells being infected by the virus. On the other hand, a nested case-control study in the Philippines found that, while symptomatic DENV-3 infant cases linked to low neutralising activity of antibodies ($PRNT_{50} \leq 50$) and measurable ADE activity (determined via an *in vitro* assay), infants who developed DHF did not have higher frequencies or levels of ADE activity during infection than symptomatic infants without DHF (Libraty *et al.* 2009). Furthermore, a cohort study in Thailand did not find supporting evidence for any harmful effect of maternal immunity; however, this conclusion was based on the observation of only 12 non-severe infant cases (Pengsaa *et al.* 2006).

1.4.3 *Cellular Immune Response in Infants*

Little is known about the role of T cells in DENV infection of infants. Although studies on sequential DENV infections suggest that memory T cells may amplify cytokine release and lead to DHF/DSS (Rothman and Ennis 1999; Rothman 2009), this model cannot be applied for a primary infection in infants,

whose memory T cells have not yet formed (due to the lack of prior exposure to dengue antigens). In infants and older children, cellular immune activation has been found to be associated with disease severity (Juffrie *et al.* 2001). Furthermore, an analysis of DHF infant cases has shown a positive correlation between severity and markers of T lymphocyte activation (Chau *et al.* 2008). Regulatory T cells may be another important factor affecting disease severity in infants. A study on macaques infected with simian immunodeficiency virus demonstrated that infant monkeys produced a large number of suppressor T cells, which then led to inhibition of $CD4^+$ T-cell response and limited antibody production (Hartigan-O'Connor *et al.* 2007). As increased frequencies of suppressor T cells have been observed in humans with acute dengue infection (Lühn *et al.* 2007), a similar suppressive mechanism may exist in human newborns and result in ineffective immune responses. It has been reported that substantial numbers of maternal cells can be transferred to foetal lymph nodes via the placenta, inducing the development of $CD4^+CD25^{high}FoxP3^+$ regulatory T cells that suppress foetal antimaternal immunity and persist at least until adulthood (Mold *et al.* 2008). However, the amount of maternally transferred T cells is unlikely to be sufficient (Opstelten *et al.* 2019) to cause any cell-mediated immune response in infants.

1.5 DENGUE PREVENTION

1.5.1 *Vector Control*

Historically, dengue control measures have mainly relied on targeting vectors, which consists of 3 major components: [1] indoor and perifocal spraying to kill adult mosquitoes (Pettit *et al.* 2010), [2] container removal and larvicide treatment to manage immature mosquitoes (Tun-Lin *et al.* 2009), and [3] use of mosquito repellents or bed nets (Sharp *et al.* 2015). While these methods successfully reduced the number of dengue cases in a few small-scale campaigns, they have failed to stop the spread of dengue (Messina *et al.* 2014) due to the lack of sustained management in complex large metropolitan areas (Achee *et al.* 2015). However, this picture may change in the next few years with the advent of novel biotech-based control strategies. Among these, the most promising approach is to infect mosquitoes with a strain of

Wolbachia (a type of microbe) to create mosquito populations that are largely incapable of DENV transmission (Chambers *et al.* 2011). Additionally, other tools that directly target mosquitoes' life span and fitness have also been investigated. For example, a United Kingdom company, Oxitec, has developed a technique to modify the genes of male *A. aegypti* mosquitoes so that their mating with wild-type females would lead to nearly 100% of the offspring dying during the late larval development stages (Phuc *et al.* 2007). Another conceptually similar method is to infect male *A. aegypti* with *Wolbachia* to make them reproductively incompatible with wild-type females; population reduction would then be acquired through the constant release of infected males (McGraw and O'Neill 2013). Furthermore, *Wolbachia* can also be used to reduce the longevity of female *A. aegypti*, hence, lowering their transmission capacity (McMeniman *et al.* 2009). In summary, despite the finding that vector control alone may not be enough to confine the spread of dengue globally, its necessity is indisputable even if an effective dengue vaccine becomes available, since it would be a valuable complement to enhance the effectiveness of vaccination (Scott and Morrison 2010).

1.5.2 *Dengue Vaccines*

In the last decade, considerable effort and resources have been invested in the development of an effective dengue vaccine. However, the task of designing dengue vaccines has been hampered by the absence of a suitable animal model and reliable immunological correlates of protection (Hombach *et al.* 2007; Na *et al.* 2017; Shrivastava *et al.* 2017). Furthermore, to avoid increasing the risk of ADE in vaccinees, any dengue vaccine must provide long-term efficacious protection against all 4 DENV serotypes (Guzmán *et al.* 2010). Despite the aforementioned hurdles, 1 dengue vaccine has been licensed, and others are likely to be approved in the near future.

1.5.2.1 *Dengvaxia®*

The only dengue vaccine that has been licensed to date is *Dengvaxia®* (also known as *CYD-TDV*), which was developed and commercialised by Sanofi-Pasteur. This live attenuated recombinant tetravalent vaccine has been

approved for use in individuals between 9 and 45 years of age with previous documented exposure to dengue. The efficacy of this vaccine varies by dengue serotype, age at vaccination, and serostatus prior to vaccination (S. R. Hadinegoro *et al.* 2015; Sridhar *et al.* 2018). While phase-3 trial data of the vaccine illustrated a pooled efficacy of 60% (95% CI: 56%–65%) over 25 months, a notably higher efficacy (up to 93.7%) was reported in individuals who were seropositive prior to vaccination (S. R. Hadinegoro *et al.* 2015). However, studies have shown that the vaccine increases the risk of developing severe illness for seronegative vaccinees (Halstead 2017; Sridhar *et al.* 2018), possibly due to the ADE effect caused by vaccine-derived antibodies. Therefore, the WHO has recommended a 'pre-vaccination screening strategy' for *Dengvaxia®*, in which only seropositive individuals are vaccinated (Wilder-Smith *et al.* 2018; World Health Organization 2018). A recent study has also suggested that a screen-and-vaccinate strategy would be more cost-effective than vaccinating without screening, except for very low transmission settings (Coudeville *et al.* 2020). However, implementing this strategy is not as simple as it may sound since the misclassification rate of screening—on which the cost-effectiveness of the vaccination programmes depends (España *et al.* 2019)—is affected not only by the sensitivity and the specificity of the screening assays but also by the transmission intensity (Rodríguez-Barraquer *et al.* 2019), let alone the prospect that pre-vaccination screening will incur noteworthy operational costs (Turner *et al.* 2018).

1.5.2.2 *Other Vaccines*

Many other dengue vaccines that are currently under clinical trials (Table 1.1) have shown potential in generating immune responses against dengue; however, it is not clear whether they would induce harmful effects on naive vaccinees, similarly to what has been observed with *Dengvaxia®*. Among these vaccine candidates, *DENVax* (also known as *TDV*, developed by Takeda Vaccines) and *TetraVax-DV* (also known as *TV003* or *LAVΔ30*, developed by the US National Institutes of Health) are the most advanced. Phase-1 and -2 trial data showed that both of these live attenuated vaccines were well-tolerated by recipients (Kirkpatrick *et al.* 2015; Osorio *et al.* 2014; Sirivichayakul *et al.* 2016; Whitehead *et al.* 2017). Two doses of *DENVax* led to a titre of above 10 in a $PRNT_{50}$ in more than 95% and 87.5% recipients for DENV-1–3 and

DENV-4, respectively (Sirivichayakul *et al.* 2016). Currently, *DENVax* is undergoing a large multicentre phase-3 clinical trial in Asia and Latin America. The primary efficacy data of this study—collected from over 20,000 children between 4 and 16 years of age—showed that 2 doses of *DENVax* could protect individuals from virologically confirmed dengue with an efficacy of 74.9% and 82.2% in seronegative and seropositive recipients, respectively (Biswal *et al.* 2019). Furthermore, the vaccine also reduced the risk of hospitalisation (due to virologically confirmed dengue) of seronegative and seropositive children respectively by 97.2% and 94.4% (Biswal *et al.* 2019). Nevertheless, since these primary results were only based on a 12-month follow-up, the long-term safety of *DENVax* remains under investigation. Meanwhile, trial data of *TetraVax-DV*—another vaccine that is undergoing a phase-3 trial—also illustrated high seroconversion rates (76% for DENV-2 and over 92% for other serotypes) (Kirkpatrick *et al.* 2015). Additionally, a human infection study that challenged 48 dengue-naive subject with a strain of DENV-2 (rDEN2 Δ30)

TABLE 1.1 — Vaccine candidates for dengue that are currently under phase-1–3 clinical trials.

Vaccine	Developers†	Type	Trial Phase	Formulation
DENVax (TAK003)	Takeda	Live attenuated	3	Attenuated DENV-2, and chimera of DENV-2 backbone with prM and E genes of DENV-1, -3, -4 (Sirivichayakul *et al.* 2016).
TetraVax-DV (TV003 or LAVΔ30)	NIAID	Live attenuated	3	Whole-genome DENV-1–4 lacking 30nt in 3'UTR (Kirkpatrick *et al.* 2015).
TDEN-LAV	WRAIR and GSK	Live attenuated	2	Attenuated DENV-1–4 (Thomas *et al.* 2013).
TDEN-PIV	WRAIR, GSK, and Fiocruz	Purified inactivated	2	Formalin-inactivated DENV-1–4 (M. Simmons *et al.* 2010).
TLAV prime / TPIV boost	NMRC and WRAIR	Heterologous prime/boost	1	Using TDEN-LAV as a prime dose and TDEN-PIV as a booster, or vice versa (M. Simmons *et al.* 2010).
V180 (DEN-80E)	Merck	Recombinant subunit	1	80% E protein of DENV-1–4 (Manoff *et al.* 2015).
$TVDV^{VAX}$ (TVDV)	NMRC	DNA-based	1	Plasmid DNA-expressing prM and E proteins of DENV-1–4 (Porter *et al.* 2012).

† *Abbreviations: GSK—GlaxoSmithKline; NIAID—National Institute of Allergy & Infectious Diseases (USA); NMRC—Naval Medical Research Center (USA); WRAIR—Walter Reed Army Institute of Research.*

showed that *TetraVax-DV* successfully blocked viraemia in all 21 vaccine recipients when challenged at 6 months after the immunisation (Kirkpatrick *et al.* 2016). However, the efficacy and the long-term safety of *TetraVax-DV* is still not known as its phase-3 trial is still in the data collection phase.

1.6 OVERVIEW OF DENGUE TRANSMISSION MODELS

Mathematical modelling has been used as the primary method to investigate the complex dynamics of dengue transmission and to evaluate intervention strategies. While different dengue models are built on different sets of assumptions, most of the published dengue models have the same backbone structure that divides the human population into groups of infection statuses, namely susceptible, infectious, and immune (Johansson *et al.* 2011). Depending on the objective of each model, this structure can be extended to include more infection categories to account for heterotypic infections (Bosch *et al.* 2016; Fischer and Halstead 1970; Wikramaratna *et al.* 2010), interserotype interaction (Lourenço and Recker 2013), cross-serotypic protection (induced by a primary infection) (Clapham *et al.* 2017; Reich *et al.* 2013), non-infant ADE (which may occur during a secondary infection) (Cummings *et al.* 2005; Ferguson, R. Anderson, *et al.* 1999; Recker *et al.* 2009), and maternal immunity (which may lead to short-term protection in infants and/or maternal ADE) (Hladish *et al.* 2016). Although the majority of the published works either omitted the vector population or allowed the vector dynamics to be absorbed into a few simple transmission parameters, some models also had mosquitoes explicitly simulated (Focks *et al.* 1995; Hladish *et al.* 2016; 2020). While reconstructing vector population dynamics and behaviour is critical for models that evaluate vector control strategies (Alphey *et al.* 2011; Luz *et al.* 2011; Yang and Ferreira 2008), the impact of having explicit vector components on the other types of models is unclear. However, a recent sensitivity analysis has suggested that higher complexity may negatively affect the reliability of the model if the aim is only to estimate basic epidemiological parameters (e.g. the transmission rate and the recovery rate) from cumulative case data (Lizarralde-Bejarano *et al.* 2020). A more detailed discussion on

modelling aspects that are directly related to this thesis is provided in the next few passages.

1.6.1 *Estimating Transmission Intensity*

The transmission intensity of dengue is often measured in force of infection (FOI)—the rate at which susceptible individuals are exposed to infection. While there has been an attempt to estimate the FOI for each dengue serotype (Castanha *et al.* 2013), the majority of the studies to date still assumed an equal transmission intensity for all 4 serotypes due to the lack of serotype-specific data. Although FOI can be estimated from case-notification data (Coelho and Carvalho 2016; Imai *et al.* 2016; O'Driscoll *et al.* 2020), this method suffers from a big unknown, namely the case reporting rate (i.e. the probability that an infection is reported as a case). Therefore, the more common and perhaps more reliable method to estimate the transmission intensity is still via serological data and by assuming that the likelihood that individuals are seropositive is correlated to how long they have been in the endemic environment (Ferguson, Donnelly, *et al.* 1999; Imai *et al.* 2015; Katzelnick *et al.* 2018; Mishra *et al.* 2018). While this method often leads to robust results, its correctness is sensitive to cross-reactivity caused by vaccination or infection with other flaviviruses. This cross-reactivity problem may be worsened due to the fact that serological assays—most of which are continuous quantitative measurements—are often treated as binary tests of perfect sensitivity and specificity when being used to estimate FOI.

1.6.2 *Modelling Maternal Immunity*

While epidemiological data and *in vitro* experiments have suggested that maternally derived immunity may have a strong effect on infants' risk of symptomatic disease,[6] no modelling studies have explicitly examined this relationship. At the time this thesis was written, a thorough PubMed[7] search yield only 1 epidemiological model that takes into account the effect of maternal immunity by assuming that infants below 3 months of age would be completely asymptomatic when infected, and those between 3 and 8 months would experience disease-enhancing effects when exposed to infection (as if it were

[6] See §1.4.2 on page 14 for more detail.

[7] https://pubmed.ncbi.nlm.nih.gov

a secondary infection) (Hladish *et al.* 2016). While these assumptions may be good enough for a vaccination simulation (Hladish *et al.* 2016), they are clearly an oversimplification as the increased risk of symptomatic disease caused by maternal ADE may not be the same as that caused by non-infant ADE.

1.7 MODELLING DENGUE VACCINATION

Modelling is a useful tool to predict the outcomes of different vaccination strategies (Andraud *et al.* 2012) in various transmission settings. However, due to our knowledge gap regarding the development of immunity to dengue and its effect against infection, modelling studies for dengue vaccination have suffered from multiple important unknowns (Lourenço and Recker 2016). For example, data from the phase-3 trials of *Dengvaxia®* showed that the vaccine efficacy varied across different countries and continents (M. R. Capeding *et al.* 2014; S. R. Hadinegoro *et al.* 2015; Sabchareon *et al.* 2012; Villar *et al.* 2015); however, the main reasons for this discrepancy are still undiscovered (although many hypotheses have been proposed). Furthermore, it is unclear whether the vaccine can confer protection from infection or only from clinical disease (i.e.vaccinees may still be infected but they are asymptomatic). Because of these uncertainties, models of dengue vaccination must be built on many untested assumptions.

1.7.1 *Major Assumptions in Dengue Vaccination Models*

Since the completion of the phase-3 trials of *Dengvaxia®*, multiple modelling studies have been carried out to investigate the risks and the benefits of this vaccine at a population level (Aguiar *et al.* 2016; Coudeville *et al.* 2018; Ferguson *et al.* 2016; Flasche *et al.* 2016).[8] While the mathematical models used in these analysis varied in terms of modelling techniques and structures, all of them they were either partly or fully calibrated based on the trial data of *Dengvaxia®*. Additionally, they consistently shared a few important assumptions about the vaccine and the infection of DENV:

[8] In fact, most of the major modelling works regarding this topic have been collectively presented in the paper of Flasche *et al.* (2016).

[1] Individuals can experience up to 4 distinct infections, each by a DENV serotype that they have not experienced. Co-infections with multiple serotypes are not allowed.

[2] The risk of symptomatic disease is moderate upon a primary infection and high during a secondary infection. Post-secondary infections either never occur or occur with a negligible risk of symptomatic illness.

[3] In most models (except Lourenço and Recker 2013) a recovery from an infection by any serotype would lead to a transient cross-serotypic protection that last around 1–2 years. During this period, individuals are not susceptible to either infection (as in Ferguson *et al.* 2016; Karl *et al.* 2014; Rodriguez-Barraquer *et al.* 2014) or symptomatic disease (as in Nagao and Koelle 2008).

[4] The immunological effect of vaccination is similar to an asymptomatic non-transmissible infection that moves individuals up the infection ladder.

[5] Regardless of the serostatus of recipients, vaccination was assumed to provide temporary heterotypic protection against either infection (e.g.Ferguson *et al.* 2016; Lourenço and Recker 2013) or symptoms only (e.g.Nagao and Koelle 2008).

[6] The protective effect of the vaccine either wanes exponentially over time (e.g.Hladish *et al.* 2016; Lourenço and Recker 2013) or only lasts for a fix duration of around 8 months to 1 year (e.g.Ferguson *et al.* 2016; Nagao and Koelle 2008; Perkins *et al.* 2016). While a majority number of models assumed that the rate of this waning does not to depend on the serostatus of vaccinees, other models assumed that the effect of the vaccine lasts much longer—even for a lifetime (as in the model of Karl *et al.* 2014)—in seropositive recipients.

1.7.2 *Major Vaccine-related Implications from Modelling Works*

Based on results from a simulation study, Lourenço and Recker (2016) suggested that the immune interaction between dengue serotypes should be taken into account when designing vaccination strategies since it may play an important role in the cycling of dengue epidemics (Lourenço and Recker 2013)

and may lead to differences in the prevalence of different serotypes (Clapham *et al.* 2015), against which vaccines may vary in efficacy. Other modelling studies have suggested that vaccination may potentially lead to short-term negative impacts at a population level. For example, a temporary growth in the number of dengue haemorrhagic fever cases may occur when transmission is reduced by vaccination (Nagao and Koelle 2008). Another possibility is that the infection age may be shifted toward pregnancy—similarly to what has been projected with rubella (Metcalf *et al.* 2013), thus changing the maternal immunity in newborns, and potentially resulting in harmful effects.

Due to the finding that *Dengvaxia*® elevates the risk of developing severe illness among seronegative recipients (Sridhar *et al.* 2018), many modelling efforts in recent years have focused on searching for an optimal deployment strategy to reduce the negative effects of this vaccine. Based on the assumption that *Dengvaxia*® acts like an asymptomatic natural infection in boosting immunity, Ferguson *et al.* (2016) and Flasche *et al.* (2016) suggested that the use of *Dengvaxia*® is less likely to cause increased hospitalisation risk in places where the transmission intensity is higher. Another modelling study has predicted that the disease burden could decrease considerably when vaccination programmes only include individuals that have been exposed to at least 1 dengue infection (Aguiar *et al.* 2016). If the vaccine only protects recipients from severe symptoms but does not suppress infection, vaccination may not reduce transmission in the long term (Ferguson *et al.* 2016); however, in scenarios where vaccines are effective at blocking infection—thus providing indirect protection for unvaccinated individuals—vaccination could lead to more favourable results (Coudeville *et al.* 2018).

1.8 DENGUE IN VIETNAM

In Vietnam, the first dengue cases were recorded in 1959 in the North, and in 1960 in the South (Ha and Huan 1997). In 1963, The first dengue outbreak occurred in the Southern region, leading to 331 hospitalised children, of whom 116 died (Ha and Huan 1997). Since then, the disease has spread across the country, with the number of cases significantly increasing over years. By

1996, dengue had been reported in 83% of the provinces and cities in the country, leaving only a few mountain provinces in the North and 1 in the Central Highland uninfected (Ha and Huan 1997). During the epidemic of 1996, only 77,692 dengue cases were reported, the majority of which came from the South (51,957 cases) and the Coastal Central (22,346 cases) (Ha and Huan 1997). Between the years 2004 and 2010, the annual number of case reports rose to beyond 91,000, making Vietnam the third country (only after Brazil and Indonesia) with the highest number of dengue cases (World Health Organization 2012). According to an estimate in 2013, the annual number of dengue infections in the country was 10.5 million, a quarter of which were symptomatic (Bhatt *et al.* 2013).

In contrast to the year-to-year increase of the incidence rate, the mortality rate of dengue in Vietnam was pushed down from 1.13 per 100,000 in 1980–1990 to 0.09 per 100,000 in 2000–2010, potentially due to improvements in treatment (Wartel *et al.* 2017). According to an epidemiological study of Anders *et al.* (2011) on 22,860 *clinically diagnosed* dengue cases in Hồ Chí Minh City (the South of Vietnam), who were hospitalised between 1996 and 2009, the average case fatality rate was 0.25%, with the annual estimates ranging between 0.10% and 0.64%. However, a more recent descriptive analysis on 2,922 *virologically confirmed* dengue adult cases at the 2 largest centres for infectious disease in Vietnam (i.e. the National Hospital for Tropical Diseases in Hà Nội and the Hospital for Tropical Disease in Hồ Chí Minh City) revealed a much higher case fatality rate of 0.8% (Huy *et al.* 2019). This discrepancy between the 2 studies may be partly stemmed from the possibility that many of the patients in the analysis of Anders *et al.* (2011) may have been misdiagnosed with dengue. In Hồ Chí Minh City, the mortality was found to be highest in young children below 6 years old and decreased with increasing age (the OR was 0.52 in 6- to 10-year-old children and 0.27 in 11- to 15-year-old children, as compared to the below-6-year age group); however, the risk of DSS was highest in patients between 6 and 10 years old (Anders *et al.* 2011). A large cohort study on southern Vietnamese children between 2 and 16 years old (who belonged to the control group of a vaccine trial in 2011–2013) estimated that the incidence of virologically confirmed dengue in this population was 3.2 (95% CI: 2.4–4.2) per 100 person-year with 5.9% (95% CI: 1.2%–16.2%) of the cases requiring hospitalisation (L'Azou *et al.* 2016). It

is noteworthy that although the majority of clinical cases in the South and the Central Coast of Vietnam occur in children below 15 years of age (World Health Organization 2008), above 80% of the reported cases in the North and the Central Highlands are young adults (World Health Organization 2008). This difference between the regions indicates that the risk of infection may be lower in the northern and the highlands areas than in the South and the Central Coast.

Maternal immunity may play an important role in shaping the epidemiological dynamics of dengue in Vietnam. Past epidemiological data showed that infants (under 1 year of age) constituted 4.6% of paediatric DHF/DSS hospitalisations in Hồ Chí Minh City (Halstead *et al.* 2002). Given that these infant cases are likely to be primary infections, this remarkably high number suggests that maternally derived immunity—which may cause ADE—may have contributed to the severity of the patients.[9] While this hypothesis is strongly backed by many other studies in Vietnam (Chau *et al.* 2009; Hung *et al.* 2004; C. P. Simmons *et al.* 2007), the effect of maternal immunity on the epidemiology of dengue in the country remains unquantified.

[9] The chance that a primary infection progress to a severe form of dengue (like DHF and DSS) without the involvement of ADE is generally low. See §1.2.7 on page 10.

Multiple studies has shown that the transmission intensity of dengue varies across Vietnam, with higher incidence observed in South and the Central Coast (Bett *et al.* 2019; Cuong *et al.* 2011; 2013). This may be attributed to the diverse climate in the country since the number of dengue case reports has been found to be strongly correlated with climatic variables (Bett *et al.* 2019; Cheng *et al.* 2020; H. S. Lee *et al.* 2017; Phung *et al.* 2015; Xuan *et al.* 2014). Climate may also be the driving force for the predominance of *A. albopictus* in the North and of *A. aegypti* in the South and the Central Coast (Higa *et al.* 2010), contributing to the difference in the transmission rate across the regions, as *A. aegypti* has higher vector competence than *A. albopictus* (Failloux *et al.* 2002). Additionally, socioeconomic and demographic factors may also add more variation to the transmission intensity. Studies have disclosed a strong association between population density and dengue case counts in Vietnam (Ashmore *et al.* 2020; Schmidt *et al.* 2011), probably due to the fact that dengue infections are likely to occur in close proximity to index cases (Anders *et al.* 2015; Mammen *et al.* 2008; Sabchareon *et al.* 2012). Additionally, urbanisation has been shown to influence the transmission as the lack of piped water supply can potentially increase the risk of dengue infection (Schmidt *et al.* 2011).

Despite the epidemiological variation across the country, case surveillance data—collected between 1994 and 2013 from Hà Nội (the Red River Delta region), Khánh Hòa (the South Central Coast region), Hồ Chí Minh City (the Southeast region), and An Giang (the Mekong River Delta region)—have suggested consistent dengue seasonality across all regions, with a peak occurring in the second half of each year, between May and December (H. S. Lee *et al.* 2017). Consistently with this finding, by examining the numbers of case notifications of 19 southern provinces during the years 2001–2010, Cuong *et al.* (2013) also found a strong signal of annual periodicity with an average of 82% (annual range: 74%–92%) of the cases reported in June–December (the rainy season) of each year; although evidence for multiannual cycle could not be detected. During the low-dengue season (i.e. January to April), infections still occur regularly in the southern and the central coastal regions; however, the incidence becomes very low in the North (H. S. Lee *et al.* 2017). Therefore, viral importation from the South has been suggested to be the main source for the year-to-year sustainability of the transmission in the northern area (Rabaa *et al.* 2013).

While all 4 dengue serotypes have been constantly circulating in Vietnam, surveillance data have suggested that each epidemic may be temporally dominated by only 1 or 2 serotypes (Ha *et al.* 2000; Hang *et al.* 2010; Recker *et al.* 2009; Wikramaratna *et al.* 2010). In Southern Vietnam, for instance, DENV-1 was the dominant serotype between 1990 and 1995 (Ha *et al.* 2000). Meanwhile, most of the cases observed in the region during the years 1996–2000 were caused by DENV-2 and -3 (Hang *et al.* 2010; Recker *et al.* 2009; Wikramaratna *et al.* 2010). Between 2001 and 2002, DENV-4 replaced DENV-3 to become a co-dominant serotype with DENV-2; after that, the cases were completely taken over by DENV-2 during 2003–2006, and DENV-1 during 2006–2008 (Hang *et al.* 2010; Recker *et al.* 2009; Wikramaratna *et al.* 2010). The phenomenon of serotype replacement is believed to be an important entity that may affect vaccination (Coudeville and Garnett 2012). However, the periodicity of this cycling behaviour is still unknown. Furthermore, it should be noted that most serotype data were only collected from clinically confirmed dengue cases. Therefore, if a link between serotype and severity exists, this method of data collection may be biased toward the serotypes that are more

likely to cause symptomatic disease and/or secondary infections (as they are more likely to be clinical than primary infections).

It has been long thought that the high transmission intensity in Vietnam (especially in the South) would mean that the country would benefit much from dengue vaccination. However, this prediction needs to be reviewed thoroughly after the finding that *Dengvaxia®* (the only licensed dengue vaccine to date) could harm naive vaccinees.[10] More specifically, the risk and the benefit of vaccination in Vietnam has to be evaluated with consideration of the diverse transmission intensity across the country since the risk may become higher in lower transmission settings (Ferguson *et al.* 2016; Flasche *et al.* 2016). Additionally, there remains a question of how the vaccine deployment should be designed (e.g. what age group to target, what coverage to aim for) to optimise its epidemiological and economic effectiveness (Clapham and B. A. Wills 2018; Turner *et al.* 2018). Furthermore, if dengue vaccines are to be added into the routine immunisation programme of Vietnam, their long-term impact also needs to be assessed as vaccination may alter the population immunity and yield indirect side-effects. For example, infants may acquire vaccine-derived immunity from their mothers and may a different magnitude of risk of ADE. In summary, it is likely that Vietnam will be among the very first countries where dengue vaccination becomes a norm; however, more research is still required to make sure of the safety and the benefit of vaccination, not only at an individual level but also at a population level.

[10] Future vaccines may be able to overcome this problem; however, it is still a big unknown. See §1.5.2 on page 20 for more details on *Dengvaxia®* and potential future vaccines.

1.9 THESIS AIMS & STRUCTURE

The primary aims of my DPhil study are: [1] to gain an insight into the complex link between dengue transmission and population immune response, and [2] to project the long-term impacts of routine vaccination on the epidemiology of dengue. As dengue vaccination will eventually become part of the immunisation programme of many endemic countries, these understandings are of great importance to evaluate and improve vaccination strategies. In this thesis, Vietnam was chosen to be the study context since its high and diverse endemicity is ideal for dengue epidemiology studies and vaccine trials. Furthermore, as the country shares common epidemiological

characteristics with many other places in the Southeast Asia region (Cuong *et al.* 2016; Halstead *et al.* 2002; Panhuis *et al.* 2015), findings from Vietnam may be generalisable to other countries.

Following this overview chapter, this thesis consists of 3 analysis chapters and a general discussion that are intended to explore the transmission dynamics of dengue, the population immune response, and the potential of vaccination in Vietnam. The analysis chapters are presented with their own methods section since each of the analyses employs a different set of methodologies. The subsequent chapters are structured as followed:

CHAPTER 2 uses statistical models to estimate the FOI of dengue in Vietnam from a serological data set. Furthermore, this chapter presents a novel classification method to identify individuals with a single previous dengue infection—the main targets of dengue vaccines—based on IgG levels in combination with individuals' ages and the measured FOI of dengue in the population.

CHAPTER 3 reconstructs the epidemiological dynamics of dengue in Hồ Chí Minh City (the capital city of the South of Vietnam) by fitting mathematical models to case-line data from 2 major paediatric hospitals of the city. Throughout the inference, I also estimate the magnitude of effect of maternally derived immunity on infants' risk of dengue hospitalisation.

CHAPTER 4 integrates the findings from the 2 previous chapters into a simulation analysis to assess the potential impacts of dengue vaccination on the number of hospitalisations in Hồ Chí Minh City.

CHAPTER 5 considers the combined results from chapters 2–4 to provide a critical assessment of whether Vietnam is a suitable target for routine dengue vaccination. The chapter also discusses the relevance of my work to our current understanding of dengue transmission and vaccination at a population level. Additionally, suggestions for potential future research are also presented.

Overall, I hope that the added knowledge from this thesis will contribute to the global initiative for controlling dengue, and that the modelling techniques developed throughout this study will be useful for future epidemiological studies on not only dengue but also other infectious disease systems.

2

SEROLOGICAL INFERENCE OF PAST PRIMARY AND SECONDARY DENGUE INFECTION[11]

2.1 INTRODUCTION

The study in this chapter was designed with 2 primary aims. The first aim is to estimate the FOI of dengue, and to understand how that FOI varies over different age/time periods and across different regions of Vietnam. To achieve this, I analysed serological data from 3 populations of Vietnam, i.e. An Giang, Hồ Chí Minh City, and Quảng Ngãi. The inferred transmission intensities from this study will help to evaluate the potential for routine vaccination in each location (Turner *et al.* 2018). The second aim of this analysis is to develop a statistical method to assess whether it is possible at a population level to use IgG antibody levels to differentiate past primary dengue virus infection from secondary infection. This research question was motivated by the observation that IgG levels tend to be higher in individuals who have experienced a secondary infection than in those with only 1 past infection (World Health Organization 1997), although the IgG-level difference between these 2 groups has not been quantified. A classification method of prior primary and secondary infections based on IgG levels could guide the use of sero-screening to target future vaccination programmes.

To generate the data for this analysis, an indirect IgG ELISA[12] was used. This type of serological tests has often been employed to infer individual infection histories, and to understand population-level exposure to dengue, through which the FOI of dengue can be estimated (Imai *et al.* 2015; Rodríguez-Barraquer *et al.* 2015; World Health Organization 2017). Conventionally, interpretation of this assay uses a single cut-off point with which ELISA antibody levels are binarily interpreted to be either seronegative or seropositive. The sensitivity and specificity of the test depend on the specific cut-off point used (Marrero-Santos *et al.* 2013). Since performance of the cut-off can be population-specific, re-calibration is recommended for each

[11] Parts of this chapter have been published in *the Journal of the Royal Society Interface* (16:20190207, 2019) under the title: '*Serological inference of past primary and secondary dengue infection: Implications for vaccination*'.

[12] See §1.3.1 on page 12 for a brief description of the ELISA technique.

new population (World Health Organization 2017). However, calibration can be impractical in some settings as it requires sets of samples with well characterized exposure history; therefore, the same cut-off is frequently used across different populations, potentially reducing the accuracy of interpretation. When put into context of dengue vaccination, this loss of accuracy may lead to seronegative individuals being vaccinated or seropositive cases not being vaccinated (Wilder-Smith *et al.* 2018) and hence, leading to loss in vaccination effectiveness and even increases in hospitalisation rates. Therefore, to overcome this problem of losing accuracy, this chapter introduces a novel quantitative approach of using IgG levels to improve the interpretation of the ELISA.

2.2 METHODS

2.2.1 *Serological Samples of Vietnamese Populations*

The Vietnamese serum samples tested in this analysis were collected as part of a serum bank established in 2009, which comprises residual serum samples from 10 hospital laboratories in 10 different provinces and cities of central and southern Vietnam. For this serum bank, 200 residual serum samples from across all age groups are randomly collected from each of the participating hospitals every 2 or 4 months. Samples were collected from both inpatients and outpatients (HIV patients are excluded) regardless of the reason for the patients' presentation at the hospitals. Samples were anonymised and delinked before being sent to the serum bank. Data on age, gender, date of sample collection, and hospital ward were preserved. The serum bank was approved by the Scientific and Ethical Committee of the Hospital for Tropical Diseases in Hồ Chí Minh City and the Oxford Tropical Research Ethics Committee at the University of Oxford, and has been used for past sero-epidemiology studies on avian influenza (Boni *et al.* 2013), human influenza (Nhat *et al.* 2017), hepatitis E virus (Berto *et al.* 2018), and chikungunya virus (Quan *et al.* 2018).

For the current analysis, serum samples collected between 2014 and 2015 were randomly selected from the following 3 hospitals (Figure 2.1): The Hospital for Tropical Diseases (in Hồ Chí Minh City, 356 samples), The An

Giang Central General Hospital (in Long Xuyên, An Giang Province, 276 samples), and The Quảng Ngãi General Hospital (in Quảng Ngãi City, Quảng Ngãi Province, 266 samples). These 3 hospitals were chosen (out of 10) since the cities/provinces they resided in are reasonably representative of major epidemiological regions of Vietnam[14]:

AN GIANG PROVINCE is located within the Mekong Delta, a highly endemic area with a fair mix of urban and rural localities.

HỒ CHÍ MINH CITY is the most densely populated[15] and highly urbanised city in Vietnam, located in the Southeast region, also known for it high dengue incidence.

QUẢNG NGÃI PROVINCE represents the Coastal Central region, an area with moderate dengue transmission rate.

For each location, the serum samples were selected based on their age groups (5-year increments) with an aim of having a uniform distribution of samples

[14] The distribution of dengue across Vietnam is described in detail in §1.8 on page 27.

[15] According to the census of 2014 (The General Statistics Office of Vietnam 2020), the population densities of An Giang, Hồ Chí Minh City, and Quảng Ngãi were 611, 4,097, and 237 per km^2, respectively.

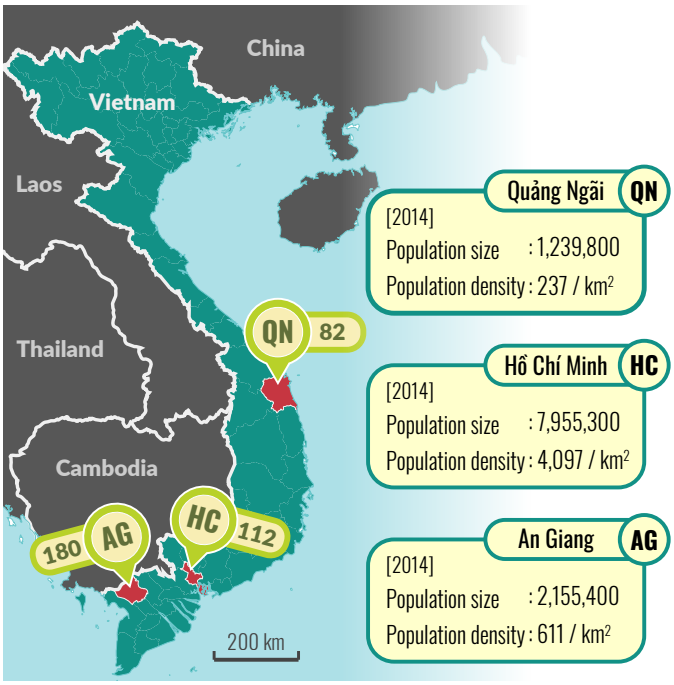


FIGURE 2.1 – Geographical locations and population demographics of An Giang (AG), Hồ Chí Minh City (HC), and Quảng Ngãi (QN). The demographic statistics were taken from the population census of 2014, published by The General Statistics Office of Vietnam (2020). The tagging numbers at the locations indicate the average annual reported dengue incidences per 100,000, calculated based on the number of clinically diagnosed cases reported between 2000 and 2010 (Panhuis *et al.* 2015) and the population sizes in 2009 (published by The General Statistics Office of Vietnam).

TABLE 2.1 — The number of samples used in this study. In total, 898 Vietnamese samples (collected from An Giang, Hồ Chí Minh City, and Quảng Ngãi) were used for the main analysis of this chapter. Additionally, 799 Indian samples—collected from Chennai for a previous study (Rodríguez-Barraquer *et al.* 2015)—were reused to explore the applicability of my statistical method (developed in this chapter) to a non-Vietnamese population.[13]

Age Group†	Vietnam			Chennai (India)
	An Giang	Hồ Chí Minh	Quảng Ngãi	
[00, 05)	40	43	34	0
[05, 10)	48	42	34	109
[10, 15)	47	74	30	112
[15, 20)	42	91	36	108
[20, 25)	43	52	29	99
[25, 30)	44	45	31	115
[30, 35)	12	9	38	118
[35, 40)	0	0	34	107
[40, 45)	0	0	0	31
All	276	356	266	799

† *Square opening brackets and round closing brackets indicate that the intervals are left-closed and right-open.*

[13] The use of the data from Chennai is described in more detail in §2.2.7 on page 44.

across age groups (Table 2.1). However, in Hồ Chí Minh City, more samples were collected for the age groups between 10 and 25 years old as they were part of a pilot analysis to assess the feasibility of using IgG levels for classifying primary and secondary exposures. Because I expected a lower dengue transmission intensity in Quảng Ngãi (Cuong *et al.* 2013; Quyen *et al.* 2018), the maximum targeted age groups in this location were increased up to 40 (instead of 35 as in An Giang and Hồ Chí Minh City) to capture more seropositive cases.

2.2.2 *Serological Assays for the Vietnamese Samples*

Each of the selected serum samples was tested for anti-DENV IgG, using a Panbio® Dengue IgG Indirect ELISA kit (Standard Diagnostics, Inc., Gyeonggi-do, Republic of Korea) according to the manufacturer's instructions (Alere Inc. 2018). The assays were performed by 2 laboratory technicians of the Oxford University Clinical Research Unit (Vietnam).

As per the manufacturer's instructions, the concentrations of IgG antibodies in each sample were quantified via measurement of optical density (OD). An OD *cut-off value* was calculated at the end of each experiment by multiplying the average OD of the triplicate of the calibrator (1 of the control samples of the ELISA kit) with the calibration factor (specified for each kit by the manufacturer). The IgG level, Y_i (measured in *Panbio® units*), of each individual, i, was calculated as follows:

$$Y_i = \frac{\text{OD of sample } i}{\text{OD cut-off value}} \times 10$$

2.2.3 *Statistical Models*

As my samples are all from 1 time point, it is not possible to separate the effects of age from that of time on the FOI of dengue (Ferguson, Donnelly, *et al.* 1999). In this analysis, I choose to present heterogeneous FOIs as if they were age-specific; however, it is also possible to interpret this heterogeneity[16] as time-dependent FOIs.

[16] The interpretation of the heterogeneity of FOIs is discussed in more detail in §2.4 on page 56.

2.2.3.1 *Binary Models*

I first estimated the FOI of dengue using a series of simple catalytic models (Ferguson, Donnelly, *et al.* 1999), which I refer to as binary models. In these models, I interpreted the IgG level of each individual as either seronegative or seropositive. Although the manufacturer of the ELISA kits suggested using a double-cut-off system to interpret the antibody levels (i.e. a sample, i, is considered seronegative when the IgG level $Y_i < 9$, and seropositive when $Y_i > 11$), I simplified the interpretation by setting a single cut-off at 10 (i.e. samples are seronegative when $Y_i < 10$, and seropositive when $Y_i \geq 10$). The effect of this simplification on my results should be minimal as few samples in my dataset fall into the recommended equivocal range between 9 and 11 (Figure 2.2).

Let E_i denote the number of dengue serotypes by which individual i has been challenged. The probability that the individual is still naive to dengue

is $\mathbb{P}\left(E_i = 0\right) = e^{-\hat{E}_i}$, in which $\hat{E}_i$ is calculated as follows (Ferguson, Donnelly, *et al.* 1999):

$$\hat{E}_i = \sum_{u=1}^{p} \left[\mathbb{1}_{\kappa_u < a_i} \times \left(\kappa_u - \kappa_{u-1} \right) \times \lambda_{\kappa_{u-1}, \kappa_u} \right] + \left[a_i - \max_{\kappa_v} \left(\kappa_v < a_i \right) \right] \times \lambda_{\kappa_v, \kappa_{v+1}}$$

where: a_i is the age (in years) of individual i;

$p \in \{1, 2, 3\}$ is the number of age-specific FOIs in the considered model;

κ_{u-1}, κ_u are age cut-offs ($\kappa_0 = 0$ and $\kappa_p = \infty$);

$\lambda_{\kappa_{u-1}, \kappa_u}$ is the total annual FOI of all the 4 serotypes of dengue that the age group between κ_{u-1} and κ_u years are exposed to;

$\mathbb{1}_{\kappa_u < a_i}$ is an indicator function, i.e. $\mathbb{1}_{\kappa_u < a_i} = \begin{cases} 1 & \text{if } \kappa_u < a_i, \\ 0 & \text{otherwise} \end{cases}$.

The binomial likelihoods of the binary models were computed as follows:

$$\mathcal{L}\left(\boldsymbol{\theta} \,\middle|\, Y\right) = \prod_{i=1}^{n} \left\{ \mathbb{1}_{Y_i < 10} \times e^{-\hat{E}_i} + \mathbb{1}_{Y_i \geq 10} \times \left(1 - e^{-\hat{E}_i} \right) \right\}$$

where: $\boldsymbol{\theta}$ denotes all the parameters in the models, including $\kappa_\theta, \ldots, \kappa_p$ and $\lambda_{\kappa_0, \kappa_1}, \ldots, \lambda_{\kappa_{p-1}, \kappa_p}$;

n is the number of samples in my dataset;

$\mathbb{1}_x$ is an indicator function, i.e. $\mathbb{1}_x = \begin{cases} 1 & \text{if } x \text{ is true}, \\ 0 & \text{otherwise} \end{cases}$.

2.2.3.2 *Continuous Models*

In continuous models, I assumed that the observed antibody levels of individuals (measured by the ELISAs) depend on the number of exposures to dengue (E_i). The antibody levels of seropositive individuals were modelled by gamma distributions. When individuals are seronegative, their IgG levels are expected to be close to zero; therefore, the IgG levels of this group were assumed to be exponentially distributed. Since prior knowledge—about how the measurements of IgG levels are distributed in a population—was not

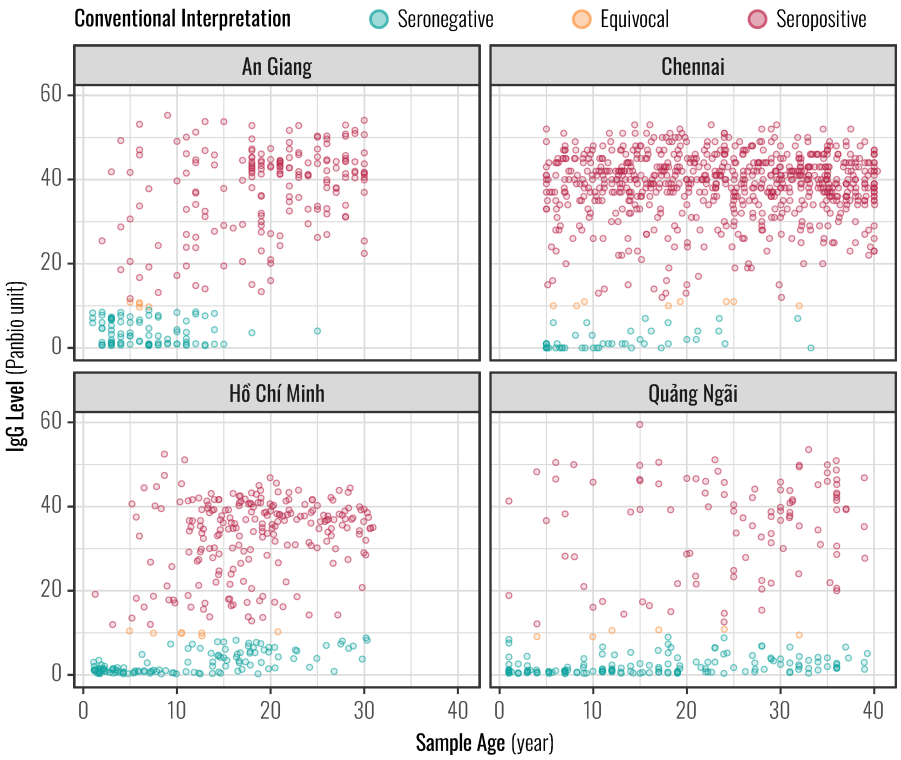


FIGURE 2.2 – The ages and the IgG levels (measured in Panbio® unit) of the serological samples used in this analysis. Teal, orange, and red points respectively represent samples with IgG levels below 9, between 9–11, and above 11. Conventionally, these 3 groups are often interpreted as seronegative, equivocal, and seropositive samples, respectively (Alere Inc. 2018).

available at the time of this analysis, the above choice of statistical distributions was only based on the fact that antibody quantities need not to be negative.

In continuous models with 2 distributions of IgG levels (i.e. $Y_i \mid E_i = 0$ and $Y_i \mid E_i \geq 1$), the binomial likelihoods were calculated as follows:

$$\mathcal{L}\left(\boldsymbol{\theta} \,\middle|\, \boldsymbol{Y}\right) = \prod_{i=1}^{n} \left\{ f\left(Y_i \,\middle|\, E_i = 0\right) \times e^{-\hat{E}_i} + f\left(Y_i \,\middle|\, E_i \geq 1\right) \times \left(1 - e^{-\hat{E}_i}\right) \right\}$$

where $f\left(Y_i \,\middle|\, E_i = 0\right)$ and $f\left(Y_i \,\middle|\, E_i \geq 1\right)$ are the probability density function of IgG levels of seronegative and seropositive individuals, respectively.

For continuous models with 3 distributions of IgG levels (i.e. $Y_i \mid E_i = 0$, $Y_i \mid E_i = 1$, and $Y_i \mid E_i \geq 2$), the chances that individuals have experienced 1 and more than 1 serotype need to be computed separately. These calculations require knowing the FOI of each serotype or realising how much each serotype contributed to the total FOI. However, such serotype-specific data were not

available. Therefore, in this analysis, I assumed that all 4 dengue serotypes had equal FOIs (i.e. $\frac{\lambda}{4}$). The probability that an individual of age a_i has been challenged by 1 of the 4 serotypes but is still naive to the other serotypes was calculated as follows:

$$\mathbb{P}\left(E_i = 1 \,\middle|\, a_i\right) = 4e^{-\frac{3}{4}\hat{E}_i}\left(1 - e^{-\frac{1}{4}\hat{E}_i}\right)$$

where: $1 - e^{-\frac{1}{4}\hat{E}_i}$ is the probability that the individual has experienced 1 of the 4 dengue serotypes;

$e^{-\frac{3}{4}\hat{E}_i}$ corresponds to the chance that the individual has escaped the other 3 serotypes.

The probability that an individual of age a_i has been exposed to more than 1 dengue serotype is:

$$\begin{aligned}\mathbb{P}\left(E_i \geq 2 \,\middle|\, a_i\right) &= 1 - \mathbb{P}\left(E_i = 0 \,\middle|\, a_i\right) - \mathbb{P}\left(E_i = 1 \,\middle|\, a_i\right) \\ &= 1 - e^{-\hat{E}_i} - 4e^{-\frac{3}{4}\hat{E}_i}\left(1 - e^{-\frac{1}{4}\hat{E}_i}\right)\end{aligned}$$

Therefore, in models with 3 IgG-level distributions (i.e. $Y_i \mid E_i = 0$, $Y_i \mid E_i = 1$, and $Y_i \mid E_i \geq 2$), the multinomial likelihoods were calculated as follows:

$$\begin{aligned}\mathcal{L}\left(\boldsymbol{\theta} \,\middle|\, \boldsymbol{Y}\right) = \prod_{i=1}^{n}\Bigg\{ & f\left(Y_i \,\middle|\, E_i = 0\right) \times e^{-\hat{E}_i} \\ & + f\left(Y_i \,\middle|\, E_i = 1\right) \times 4e^{-\frac{3}{4}\hat{E}_i}\left(1 - e^{-\frac{1}{4}\hat{E}_i}\right) \\ & + f\left(Y_i \,\middle|\, E_i \geq 2\right) \times \left[1 - e^{-\hat{E}_i} - 4e^{-\frac{3}{4}\hat{E}_i}\left(1 - e^{-\frac{1}{4}\hat{E}_i}\right)\right]\Bigg\}\end{aligned}$$

where $f\left(Y_i \,\middle|\, E_i = 0\right)$, $f\left(Y_i \,\middle|\, E_i = 1\right)$, and $f\left(Y_i \,\middle|\, E_i \geq 2\right)$ are the probability density functions of IgG levels of individuals given that they were estimated to be seronegative (naive to dengue), primary-seropositive (having experienced only primary infections), or secondary-seropositive (having experienced secondary infections), respectively. The likelihood function above assumes that homotypic re-exposure did not alter IgG antibody levels.

Although it is expected that IgG levels of secondary-seropositive individuals are higher than those of primary-seropositive cases[17] (World Health Organization 1997; 2009), I did not impose this restriction on my models.

[17] The differences in IgG levels between the first and the second dengue infection are conceptually illustrated in Figure 1.2 on page 8.

2.2.4 *Model Fitting and Model Selection*

This modelling analysis was divided into 2 major steps. In the first step, the data from An Giang, Hồ Chí Minh City, and Quảng Ngãi were analysed separately to estimate the FOIs of dengue and to assess the likelihood of age-varying FOIs in each of the locations. Five binary models and 10 continuous models, with either constant or age-varying FOI, were tested for each population. In age-varying-FOI models, the age cut-offs were either estimated or fixed at 6 years (for models with 2 or 3 age groups) and 18 years (for models with 3 age groups). In the continuous models, the number of distributions of IgG levels was either 2 or 3.

In the second step, I assessed whether the distributions of IgG levels were shared across populations by fitting 4 additional continuous models[18] to the whole Vietnamese dataset (which included all samples from An Giang, Hồ Chí Minh City, and Quảng Ngãi). In these models, the configuration of the FOI in each population and the number of IgG-level distributions were based on the best-fitting continuous models acquired from the first step of this analysis. While the baseline model of this group (`VnmCon01`) allowed all 3 IgG-level distributions to be population-specific, the other models tested 1 of the following 3 assumptions: [1] all the IgG-level distributions were shared across the populations, [2] only the IgG-level distribution of seronegative cases was population-specific, or [3] only those of seropositive cases were population-specific.

[18] The detailed configurations of the IgG-level distributions in these models are shown in Table 2.4 on page 48.

The models were fitted to the data using the Metropolis-Hastings algorithm (Hastings 1970). The parameters of each model were estimated through 4 independent Markov Chains Monte Carlo (MCMCs) of 800,000 iterations (with the first 300,000 iterations removed as burn-in). Model convergence was visually assessed based on the trace plots and the histograms of posterior samples. A model was considered converged if: [1] all of its 4 MCMC runs led to overlapping trace plots and histograms (after removing the burn-in iterations), and [2] the posterior histograms of all model

parameters resembled unimodal distributions.[19] The deviance information criterion (DIC) was employed for model comparison. Models were classified as *'best-fitting'* if their DICs were within a 10-unit difference compared to the lowest DIC of the corresponding model group. The likelihood function and chain iteration were implemented in a C++ application, whose source code is accessible at: https://gitlab.com/lamhm/igg-to-foi. Post-MCMC analyses were carried out in the R language (R Core Team 2020).

[19] See APPENDIX A on page 132 for example trace plots and histograms of converged and unconverged models.

2.2.5 *Estimating Seroprevalence*

The dengue seroprevalence, $Prev(a)$, of a group of individuals of a given age, a, was calculated using the relationship between age and probability of exposure, established in the catalytic model (Ferguson, Donnelly, *et al.* 1999):

$$Prev(a) = 1 - \exp\left\{-\sum_{u=1}^{p} \mathbb{1}_{Y_i<10} \times (\kappa_u - \kappa_{u-1}) \times \lambda_{\kappa_{u-1},\kappa_u} + \left[a - \max_{\kappa_v}(\kappa_v < a)\right] \times \lambda_{\kappa_v,\kappa_{v+1}}\right\}$$

where the values of the FOIs (λ's) were drawn from the posteriors of the tested models.

2.2.6 *Estimating Primary-seropositive Probability Based on IgG-level Distributions*

To demonstrate the potential of using the IgG-level distributions (inferred from the continuous models) in guiding who should be vaccinated, I built a table for each of the study populations to show the probability that individuals in particular age groups with certain IgG levels were primary-seropositive. Each probability was calculated as follows:

$$\begin{aligned}\mathbb{P}(E_i = 1 \mid Y_i) = & \left\{f(Y_i \mid E_i = 1) \times 4e^{-\frac{3}{4}\hat{E}_i}\left(1 - e^{-\frac{1}{4}\hat{E}_i}\right)\right\} \\ & \div \left\{f(Y_i \mid E_i = 0) \times e^{-\hat{E}_i} + f(Y_i \mid E_i = 1) \times 4e^{-\frac{3}{4}\hat{E}_i}\left(1 - e^{-\frac{1}{4}\hat{E}_i}\right)\right. \\ & \left. + f(Y_i \mid E_i \geq 2) \times \left[1 - e^{-\hat{E}_i} - 4e^{-\frac{3}{4}\hat{E}_i}\left(1 - e^{-\frac{1}{4}\hat{E}_i}\right)\right]\right\}\end{aligned}$$

where $f\left(Y_i \mid E_i = 0\right)$, $f\left(Y_i \mid E_i = 1\right)$, and $f\left(Y_i \mid E_i = 2\right)$ are, respectively, the probability density functions of the seronegative, the primary-seropositive, and the secondary-seropositive IgG-level distributions; and $\hat{E}_i$ (which can be calculated when the age of the sample and the FOI are given) is the expected number of dengue serotypes by which individual *i* has been challenged.

The FOIs and the IgG-level distributions used for this demonstration were based on the median estimates from the best-fitting continuous models.

2.2.7 *Testing Continuous Models on a Non-Vietnamese Population*

To assess the performance of my method on a different population, I tested my continuous models on a serological dataset of 799 samples collected from Chennai, India (Table 2.1). These samples were originally collected in 2011 for a household-based serosurvey among individuals between 5 and 40 years of age, who lived in 50 probabilistically selected spatial locations in Chennai (Rodríguez-Barraquer *et al.* 2015). As part of the original study, the level of anti-DENV IgG in each sample was quantified using a Panbio® IgG indirect ELISA (manufactured by Inverness Medical Innovations, Brisbane, Australia). The procedure of this assay is identical to that used on the Vietnamese samples.[20] For the current analysis, only the dengue IgG levels and the ages of the Chennai samples were reused; other data, such as gender and household information, were completely ignored.

[20] See §2.2.2 on page 37.

It is noteworthy that the lack of below-5-year-old samples in the Chennai dataset makes it impossible to infer age-specific FOIs for young children. Furthermore, since the population of Chennai was exposed to a very high annual FOI of dengue (0.23 between 2004 and 2011, and 0.10 before 2004) (Rodríguez-Barraquer *et al.* 2015), it is likely that the majority of the cases in this dataset had experienced a secondary exposure. This was presumably the main cause of the lack of samples with low or moderate IgG levels in the dataset (Figure 2.2), thus rendering it difficult to infer the IgG-level distributions for seronegative and primary cases. To overcome this issue, I used the posteriors of the IgG-level distributions of seronegative and primary-seropositive cases from a Vietnamese population (inferred from the previous

analysis) as strong priors for the corresponding IgG-level distributions of Chennai.

Six continuous models with 3 IgG-level distributions were fitted to the data from Chennai (as the analysis on the Vietnamese populations strongly supported 3-IgG-distribution models[21]). The FOI in Chennai was considered as being either constant or time-varying.[22] The time cut-offs were either estimated or set at 6, 7, and 18 years (before 2011). The 7-year cut-off was added into this analysis based on the findings of the original paper (Rodríguez-Barraquer *et al.* 2015). Each of the models was fitted through a MCMC of 800,000 iterations, with the first 300,000 iterations removed as burn-in.

[21] See §2.3.

[22] Note that I interpret the heterogeneity of the FOI in Chennai as being time-dependent as suggested in the original paper.

2.3 RESULTS

Eight of 15 binary models that were tested on the data obtained from the Vietnamese populations resulted in converged chains (Table 2.2). Six of the 7 unconverged models (`AgiBin03`, `AgiBin05`, `HcmBin03`, `HcmBin05`, `QngBin03`, and `QngBin05`) had age-varying FOI with estimated age cut-offs. In these unconverged chains, varying the age cut-offs had little effect on the likelihoods of the models, indicating that the information presented in the data may not be sufficient for identifying the cut-offs. The other unconverged model (`AgiBin04`), although having fixed age cut-offs, could not estimate the FOI for the age group of above 18 years in An Giang since the seroprevalence of this age group may have been saturated at nearly 100%.

Fitting the continuous models to the data from An Giang, Hồ Chí Minh City, and Quảng Ngãi separately led to 19 converged chains (Table 2.3). All of the 11 models that did not converge had issue estimating the age cut-offs for age-varying FOIs. In all of the unconverged continuous models, the FOIs of all the age groups were successfully estimated; however, the FOI estimates overlapped closely either among all the age groups (for Hồ Chí Minh City and Quảng Ngãi) or between the 2 oldest age groups (for An Giang). Therefore, well behaved posteriors of the age cut-offs could not be estimated as altering the cut-offs produced little effect on the likelihoods of the models. For all 3 populations, models with 3 distributions for IgG levels significantly

TABLE 2.2 — Fifteen binary models that were fitted to the data of An Giang, Hồ Chí Minh City, and Quảng Ngãi separately. Models are arranged by their DICs in ascending order. DIC scores and median estimates of the FOIs (with 95% CRs presented in brackets) are only shown for converged models.

	Model	Nº of Free Param.	Age Group		Force of Infection‡			DIC
			Count	Cut-offs†				
An Giang	AgiBin02	2	2	6	0.06 (0.04; 0.09)		0.17 (0.13; 0.21)	206
	AgiBin01	1	1	–	0.11 (0.09; 0.13)			218
	AgiBin03	3	2	est.	–			–
	AgiBin04	3	3	6 & 18	–			–
	AgiBin05	5	3	est.	–			–
Hồ Chí Minh	HcmBin01	1	1	–	0.07 (0.06; 0.09)			387
	HcmBin02	2	2	6	0.08 (0.05; 0.12)		0.07 (0.05; 0.10)	389
	HcmBin04	3	3	6 & 18	0.07 (0.04; 0.12)	0.08 (0.05; 0.12)	0.05 (0.00; 0.12)	390
	HcmBin03	3	2	est.	–			–
	HcmBin05	5	3	est.	–			–
Quảng Ngãi	QngBin01	1	1	–	0.03 (0.02; 0.04)			331
	QngBin02	2	2	6	0.04 (0.02; 0.07)		0.02 (0.01; 0.03)	331
	QngBin04	3	3	6 & 18	0.05 (0.03; 0.07)	0.02 (0.00; 0.04)	0.03 (0.01; 0.05)	332
	QngBin03	3	2	est.	–			–
	QngBin05	5	3	est.	–			–

† *'est.' indicates that the parameters were left to be estimated by the MCMC runs, but the chains did not converge.*

‡ *The number of FOI estimates in each model corresponds to the number of age groups in the model, with the first estimate corresponding to the youngest age group.*

TABLE 2.3 – Thirty continuous models that were fitted to the data of An Giang, Hồ Chí Minh City, and Quảng Ngãi separately. Models are arranged by their DIC in ascending order. DIC scores are only shown for converged models.

	Model	Nº of Free Param.	Age Group		DIC
			Count	Cut-offs†	
An Giang	AgiCon33	8	2	est.	1,936
	AgiCon32	7	2	6	1,941
	AgiCon34	8	3	6 & 18	1,943
	AgiCon31	6	1	–	1,960
	AgiCon24	6	3	6 & 18	1,977
	AgiCon22	5	2	6	1,983
	AgiCon21	4	1	–	2,009
	AgiCon23	6	2	est.	–
	AgiCon25	8	3	est.	–
	AgiCon35	10	3	est.	–
Hồ Chí Minh	HcmCon31	6	1	–	2,431
	HcmCon32	7	2	6	2,431
	HcmCon34	8	3	6 & 18	2,432
	HcmCon22	5	2	6	2,518
	HcmCon24	6	3	6 & 18	2,519
	HcmCon21	4	1	–	2,521
	HcmCon23	6	2	est.	–
	HcmCon25	8	3	est.	–
	HcmCon33	8	2	est.	–
	HcmCon35	10	3	est.	–
Quảng Ngãi	QngCon31	6	1	–	1,727
	QngCon32	7	2	6	1,727
	QngCon34	8	3	6 & 18	1,727
	QngCon21	4	1	–	1,757
	QngCon24	6	3	6 & 18	1,757
	QngCon22	5	2	6	1,758
	QngCon23	6	2	est.	–
	QngCon25	8	3	est.	–
	QngCon33	8	2	est.	–
	QngCon35	10	3	est.	–

† *'est.' indicates that the age cut-offs were left to be estimated by the MCMC runs; otherwise, the cut-offs were fixed at the presented values.*

improved the goodness-of-fit compared to models with only 2 IgG-level distributions. All of the 4 continuous models that were fitted to the whole Vietnamese dataset converged (Table 2.4).

2.3.1 *Force of Infection of Dengue in the Vietnamese Populations*

All the best-fitting models suggest that in the long term, individuals in An Giang and Hồ Chí Minh City experienced a significantly higher risk of infection compared to those in Quảng Ngãi (Table 2.2 and Table 2.5). In comparison to the binary models, the continuous models tended to result in slightly lower FOI estimates for An Giang, and higher approximations for Hồ Chí Minh City and Quảng Ngãi.

The best-fitting models for An Giang unequivocally suggest an age-varying FOI. In other words, individuals in An Giang showed a low FOI of 0.06 (95% CR: 0.04–0.09, in `AgiBin02`) or 0.04 (95% CR: 0.02–0.07, in `AgiCon33`) prior to the age of 6 years (`AgiBin02`) or 9.6 years (`AgiCon33`), and experienced a much higher FOI of 0.17 (95% CR: 0.13–0.21, in `AgiBin02`) or 0.20 (95% CR: 0.15–0.29, in `AgiCon33`) after that. One of the continuous models (`AgiCon34`) indicates that individuals in An Giang may have been exposed to the highest FOI after they reached 18 years of age; however, the difference between the FOI estimates of the above-18 age and that of the 6-to-18 age group is not statistically significant.

TABLE 2.4 – Configuration of the IgG-level distributions in the four continuous models that were fitted to the whole Vietnamese data set. In these models, constant FOIs were assumed for Hồ Chí Minh City and Quảng Ngãi, and an age-varying FOI was assumed for An Giang (with two age groups). Models are arranged by their DICs in ascending order.

Model	Configuration of the IgG-level Distributions		Nº of Free Param.	DIC
	Seronegative	Primary-seropositive & Secondary-seropositive		
VnmCon01	population-specific	population-specific	20	6,095
VnmCon02	shared across populations	population-specific	18	6,140
VnmCon03	population-specific	shared across populations	12	6,175
VnmCon04	shared across populations	shared across populations	10	6,196

TABLE 2.5 — Median estimates of the annual FOIs of dengue from the best-fitting continuous models that were fitted on the data of An Giang, Hồ Chí Minh City, or Quảng Ngãi separately. Numbers in brackets represent the 95% CRs of the estimates. All of the listed models support 3 IgG-level distributions.

	Model (DIC)	Age Groups		Force of Infection‡		
		Count	Cut-offs†	1st Age Group	2nd Age Group	3rd Age Group
An Giang	AgiCon33 (1,936)	2	9.60 (7.12; 14.08)	0.04 (0.02; 0.07)	0.20 (0.15; 0.29)	–
	AgiCon32 (1,941)	2	6	0.04 (0.02; 0.06)	0.15 (0.12; 0.18)	–
	AgiCon34 (1,943)	3	6 & 18	0.04 (0.02; 0.07)	0.14 (0.10; 0.18)	0.20 (0.09; 0.37)
Hồ Chí Minh	HcmCon31 (2,431)	1	–	0.12 (0.11; 0.14)	–	–
	HcmCon32 (2,431)	2	6	0.10 (0.06; 0.15)	0.14 (0.11; 0.17)	–
	HcmCon34 (2,432)	3	6 & 18	0.09 (0.05; 0.14)	0.15 (0.11; 0.20)	0.10 (0.02; 0.19)
Quảng Ngãi	QngCon31 (1,727)	1	–	0.05 (0.04; 0.06)	–	–
	QngCon32 (1,727)	2	6	0.08 (0.04; 0.12)	0.04 (0.03; 0.06)	–
	QngCon34 (1,727)	3	6 & 18	0.09 (0.05; 0.14)	0.03 (0.00; 0.06)	0.06 (0.03; 0.09)

† *The age cut-off in the model* `AgiCon33` *was estimated by the MCMC run. In the other models, the age cut-offs (if applicable) were fixed.*

‡ *In models with age-varying FOI, the first age group corresponds to the youngest age group.*

Meanwhile, the results for Hồ Chí Minh City and Quảng Ngãi suggest that the FOIs of dengue in these locations were likely to be constant. Although some of the best-fitting models for Hồ Chí Minh City and Quảng Ngãi also suggest age-varying FOIs, the FOI estimates in these models varied little (not statistically significant) across age groups. In Hồ Chí Minh City, the FOI was around 0.07 (95% CR: 0.06–0.09, in `HcmBin01`) or 0.12 (95% CR: 0.11–0.14, in `HcmCon31`). In Quảng Ngãi, the estimate was 0.03 (95% CR: 0.02–0.04, in `QngBin01`) or 0.05 (95% CR: 0.11–0.14, in `QngCon31`).

2.3.2 *Seroprevalence of Dengue in the Vietnamese Populations*

The seroprevalences of dengue in An Giang, Hồ Chí Minh City, and Quảng Ngãi were inferred based on the FOI estimates from the models with the lowest DIC (Table 2.6 and Figure 2.3). Compared to binary models, continuous models gave lower seroprevalence estimates for An Giang, but resulted in higher estimates for Hồ Chí Minh City and Quảng Ngãi. The binary models estimated that 57.2% (95% CR: 50.6%–64.2%, in `AgiBin02`) of 9-year-olds (the recommended age group for *Dengvaxia*®) in An Giang were dengue seropositive while only 49.0% (95% CR: 44.5%–53.6%, in `HcmBin01`) in Hồ Chí Minh City and 23.4% (95% CR: 19.6%–27.2%, in `QngBin01`) in Quảng Ngãi were seropositive. Nevertheless, results from the continuous models suggest that Hồ Chí Minh City had the highest seroprevalence among the populations with 66.8% (95% CR: 62.2%–70.9%, in `HcmCon31`) of 9-year-olds being seropositive; meanwhile, the number is 34.7% (95% CR: 21.4%–49.5%, in `AgiCon33`) for An Giang, and 37.2% (95% CR: 30.8%–43.5%, in `QngCon31`) for Quảng Ngãi. In general, the median ages of infection in An Giang (`AgiBin02`: 8.1 years; `AgiCon33`: 11.0 years) and Hồ Chí Minh City (`HcmBin01`: 9.3 years; `HcmCon31`: 5.7 years) are substantially lower than the estimate for Quảng Ngãi (`QngBin01`: 23.4 years; `QngCon31`: 13.4 years).

2.3.3 *IgG-level Distributions from Continuous Models*

All the best-fitting continuous models acquired from analysing the data of each population separately suggest 3 distributions for IgG levels (Table 2.7 and Figure 2.4). These distributions, in the order of increasing mean values, correspond to 3 exposure classes: dengue-naive individuals (seronegative), individuals with primary exposures (primary-seropositive), and individuals with secondary exposures (secondary-seropositive). In all 3 populations, the IgG levels of secondary-seropositive cases were well separated from those of seronegative cases. However, primary-IgG levels and seronegative-IgG levels are less distinguishable—especially in Hồ Chí Minh City and Quảng Ngãi—as the 2 distributions overlap. An Giang stands out with the mean IgG levels of seronegative cases (`AgiCon33`: 5.3 [95% CR: 4.1–7.1]) and primary cases (32.4 [95% CR: 28.4–36.2]) notably higher than those in Hồ Chí Minh

TABLE 2.6 – Estimates of the median age of infection and the proportion of 9-year-olds being seropositive. The numbers were approximated by the following models: `AgiBin02` and `AgiCon33` (for An Giang), `HcmBin01` and `HcmCon31` (for Hồ Chí Minh City), `QngBin01` and `QngCon31` (for Quảng Ngãi). Numbers in brackets represent the 95% CRs of the estimates. In these estimates, the FOI of Hồ Chí Minh City and Quảng Ngãi were assumed to be constant over age and time. Meanwhile, the FOI of An Giang was assumed to be age-dependent (rather than time-dependent) with an age cut-off fixed at 6 years (in the binary model, `AgiBin02`) or estimated at 9.6 years (in the continuous model, `AgiCon33`).

Population	Binary Model		Continuous Model	
	Median Age of Infection	Seroprevalence at 9 Years Old (%)	Median Age of Infection	Seroprevalence at 9 Years Old (%)
An Giang	8.1 (7.0; 8.9)	57.2 (50.6; 64.2)	11.0 (9.1; 12.9)	34.7 (21.4; 49.5)
Hồ Chí Minh	9.3 (8.1; 10.6)	49.0 (44.5; 53.6)	5.7 (5.1; 6.4)	66.8 (62.2; 70.9)
Quảng Ngãi	23.4 (19.7; 28.5)	23.4 (19.6; 27.2)	13.4 (10.9; 16.9)	37.2 (30.8; 43.5)

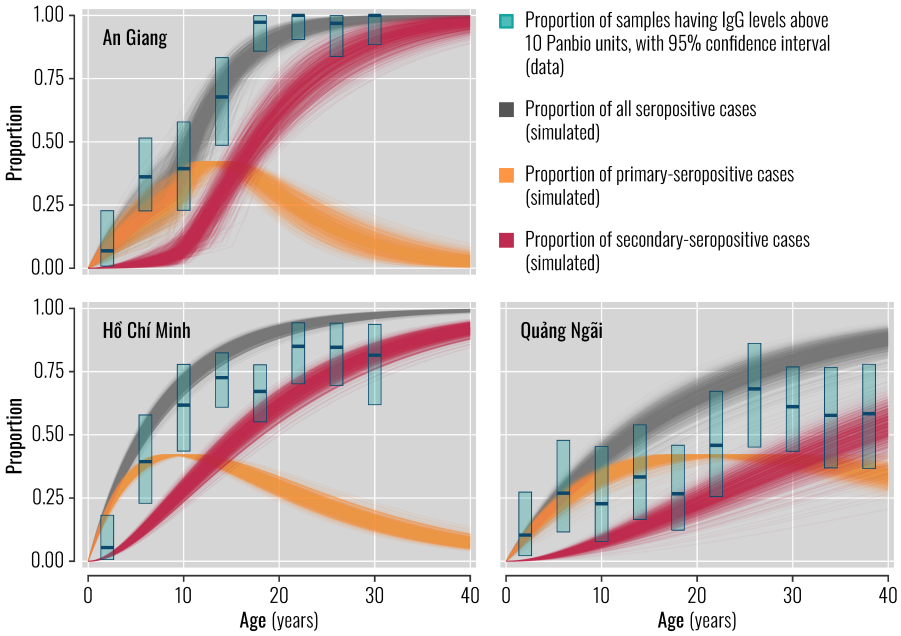


FIGURE 2.3 – The seroprevalence of dengue in the study populations. The bars represent the proportion (with 95% CR) of samples in each age group of four years that have IgG levels above 10 Panbio® units. The lines show the estimates of seroprevalence from 1,000 simulations, of which the parameters were drawn from the posteriors of the following models: `AgiCon33` (for An Giang), `HcmCon31` (for Hồ Chí Minh City), and `QngCon31` (for Quảng Ngãi).

TABLE 2.7 – Median estimates of the mean and the standard deviation (SD) of the IgG-level distributions inferred from the best-fitting continuous models that were fitted on the data of An Giang, Hồ Chí Minh City, or Quảng Ngãi separately. Numbers in brackets represent the 95% CR range of the estimates.

	Model (DIC)	Mean & SD of Sero-negative	Primary-seropositive		Secondary-seropositive	
			Mean	SD	Mean	SD
An Giang	AgiCon33 (1,936)	5.3 (4.1; 7.1)	32.4 (28.4; 36.2)	10.1 (7.9; 13.1)	43.8 (42.5; 45.0)	4.7 (3.7; 6.0)
	AgiCon32 (1,941)	5.3 (4.1; 7.1)	32.4 (28.5; 36.1)	10.0 (7.8; 12.8)	44.1 (42.8; 45.2)	4.5 (3.7; 5.7)
	AgiCon34 (1,943)	5.3 (4.0; 7.0)	32.3 (28.3; 36.1)	10.0 (7.8; 13.1)	43.9 (42.6; 45.1)	4.7 (3.7; 5.9)
Hồ Chí Minh	HcmCon31 (2,431)	1.3 (1.0; 1.8)	14.9 (11.9; 18.5)	10.8 (8.1; 14.0)	37.5 (36.7; 38.3)	4.5 (3.9; 5.3)
	HcmCon32 (2,431)	1.3 (1.0; 1.8)	15.0 (12.0; 18.6)	10.8 (8.1; 14.0)	37.5 (36.7; 38.3)	4.5 (3.9; 5.3)
	HcmCon34 (2,432)	1.3 (1.0; 1.8)	14.9 (11.9; 18.5)	10.7 (8.1; 13.9)	37.5 (36.7; 38.3)	4.6 (3.9; 5.3)
Quảng Ngãi	QngCon31 (1,727)	1.6 (1.2; 2.1)	15.1 (10.3; 21.2)	15.8 (10.6; 23.9)	42.4 (40.4; 44.3)	5.9 (4.4; 7.9)
	QngCon32 (1,727)	1.5 (1.1; 2.0)	14.5 (9.3; 22.0)	14.3 (9.2; 20.4)	42.4 (40.4; 44.3)	6.0 (4.5; 8.1)
	QngCon34 (1,727)	1.5 (1.1; 2.0)	14.3 (9.3; 21.4)	14.1 (9.1; 20.3)	42.3 (40.3; 44.2)	6.1 (4.6; 8.2)

City (`HcmCon31`: 1.3 for seronegative individuals, 14.9 for primary individuals) and Quảng Ngãi (`QngCon31`: 1.6 for seronegative individuals, 15.1 for primary individuals). The secondary-IgG distribution for An Giang (mean: 43.8; SD: 4.7), however, closely resembles that of Quảng Ngãi (mean: 42.4; SD: 5.9), and has a slightly higher mean than that of Hồ Chí Minh City (mean: 37.5; SD: 4.5).

Using the inferred IgG-level distributions from the best-fitting models (`AgiCon33`, `HcmCon31`, and `QngCon31`), I estimated that 12.6% (95% CR: 6.73%–21.37%) of dengue-naive individuals in An Giang would have a positive ELISA result if 11 Panbio® unit were chosen for the positive cut-off (as recommended by the manufacturer of the ELISA kits used in this study) (Table 2.8). Meanwhile, the false positive rate was estimated to be substantially lower for Hồ Chí Minh City (median estimate: 0.02%; 95% CR: 0.00%–0.24%) and Quảng Ngãi (median estimate: 0.10%; 95% CR: 0.01%–0.57%). Although 99.76% (95%

TABLE 2.8 – Estimated conditional probabilities (%) of having an IgG level of above 11 Panbio® unit (the recommended positive cut-off of the ELISA kits used in this study) of individuals in An Giang, Hồ Chí Minh City, and Quảng Ngãi, given their number of prior infections. The probabilities were approximated by the following models: `AgiCon33` (for An Giang), `HcmCon31` (for Hồ Chí Minh City), and `QngCon31` (for Quảng Ngãi). Numbers in brackets represent the 95% CRs of the estimates.

Nº of Prior Infections	Probability of Having IgG-levels Above 11 Panbio® Unit (%)		
	An Giang	Hồ Chí Minh	Quảng Ngãi
0	12.66 (6.73; 21.37)	0.02 (0.00; 0.24)	0.10 (0.01; 0.57)
1	99.76 (97.62; 99.99)	55.78 (44.05; 67.45)	50.54 (34.89; 72.24)
≥ 2	100 (100; 100)	100 (100; 100)	100 (100; 100)

CR: 97.62%–99.99%) of primary-seropositive individuals in An Giang would have an IgG level of above 11 Panbio® unit if tested, only 55.78% (95% CR: 44.05%–67.45%) of those in Hồ Chí Minh City and 50.54% (95% CR: 34.89%–72.24%) of those in Quảng Ngãi would have a similar result. In all the 3 locations, the positive rate of secondary-seropositive individuals was estimated to be close to 100%.

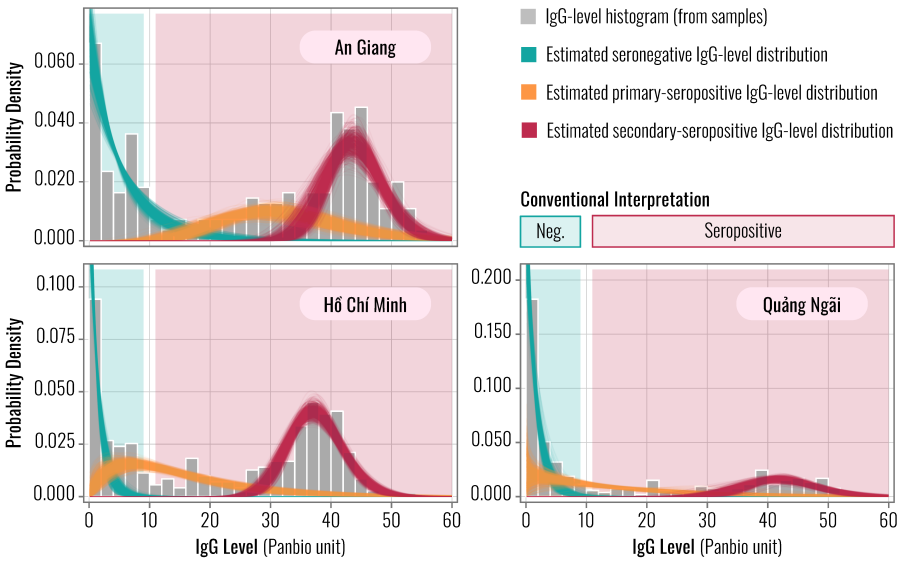


FIGURE 2.4 – The histograms[23] of the measured IgG levels (bars), and the inferred IgG-level distributions (lines) of the 3 exposure classes (i.e. seronegative, primary-seropositive, and secondary-seropositive) across the Vietnamese populations. The IgG-level distributions were simulated 1,000 times based on the posteriors of the following models: `AgiCon33` (for An Giang), `HcmCon31` (for Hồ Chí Minh City), and `QngCon31` (for Quảng Ngãi).

[23] Note that the scale of the y-axis is different for each figure panel.

The 4 additional continuous models designed to test the population-specificity of the IgG-level distributions were configured to have: [1] constant FOIs for Hồ Chí Minh City and Quảng Ngãi, [2] an age-varying FOI for An Giang (with 2 age groups), and [3] three distributions for IgG levels (this configuration was based on `AgiCon33`, `HcmCon31`, and `QngCon31`). The results from this group of models show that allowing the IgG-level distribution to be population-specific substantially improved the goodness-of-fit (Table 2.4).

2.3.4 *Estimating Primary-seropositive Probability Based on IgG Level and Patient Age*

To demonstrate the potential of incorporating sample age and dengue FOI into the interpretation of IgG levels, a probability table showing the likelihood of individuals being primary-seropositive was generated for each of the study populations (Figure 2.5). The FOI estimates and the IgG-level distributions used for the calculation were taken from the continuous models that had the lowest DICs: `AgiCon33` (for An Giang), `HcmCon31` (for Hồ Chí Minh City), and `QngCon31` (for Quảng Ngãi). In general, the tables suggest that using an ELISA cut-off of 11 Panbio® units (as suggested by the manufacturer of the kits) would lead to many seronegative cases in An Giang being misclassified as seropositive, and many primary-seropositive cases in Hồ Chí Minh City and Quảng Ngãi being misclassified as seronegative. Furthermore, within a population, children are more likely to be seronegative than adults if they have the same low or moderate IgG level.

2.3.5 *Testing Continuous Models on Chennai Data*

Using the posteriors of Hồ Chí Minh City or Quảng Ngãi as the priors for the seronegative and the primary-seropositive IgG-level distributions of Chennai always led to either non-convergence of the models or implausible results (e.g. the distribution mean of primary IgG levels falling below 10, or unrealistically high FOI estimates). Only the models that used the posteriors of An Giang as the priors led to converged chains, and hence are presented in this section. It should be noted that this finding fits well to the background

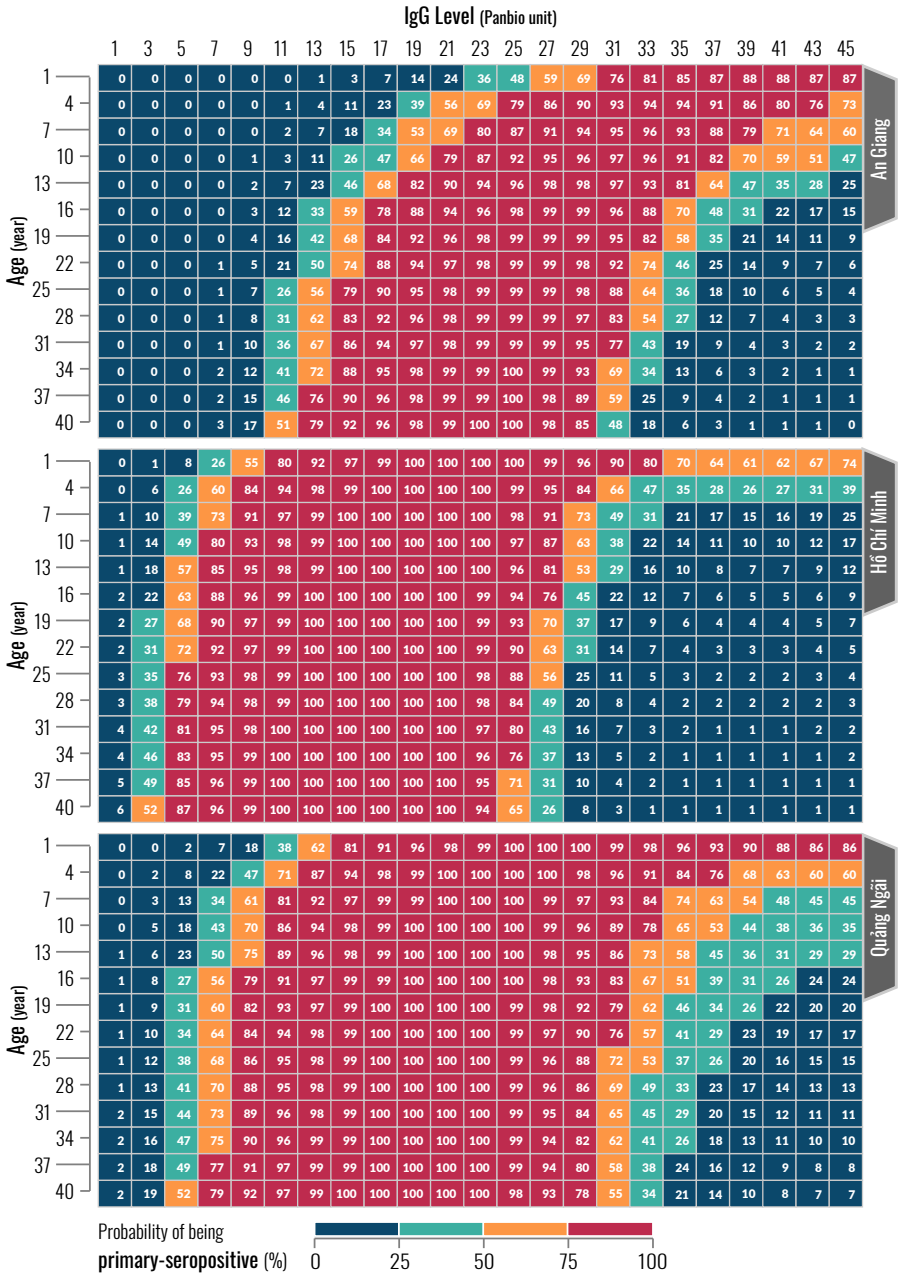


FIGURE 2.5 – Demonstration of using a continuous model to estimate the probability of a given sample being primary-seropositive. This estimation method makes use of not only the IgG levels (measured by ELISAs) but also the ages of patients and the FOI of dengue in the population. The probability tables (from the top to the bottom) were built based on the IgG-level distributions and the median FOI estimate of An Giang, Hồ Chí Minh City, and Quảng Ngãi (inferred from `AgiCon33`, `HcmCon31`, and `QngCon31`, respectively). The blue area on the left side of each table represents individuals who are likely to be seronegative. Meanwhile, individuals falling into the blue area on the right side of the tables are likely to be secondary-seropositive.

knowledge that among the 3 populations of Vietnam, An Giang was most similar to Chennai in terms of transmission intensity.

Five of 6 tested continuous models for Chennai—with the posteriors of the `AgiCon33` model (the lowest-DIC model for An Giang) used as the priors—resulted in converged chains (Table 2.9). All of the best-fitting models support a time-varying FOI in Chennai with a time cut-off at 6.4–13.3 years. According to the parameters estimated with these models, a recent period during which the FOI in Chennai was very high (`CheCon32e`: 0.28; 95% CR: 0.23–0.33) was preceded by a period with a much lower FOI (`CheCon32e`: 0.06; 95% CR: 0.04–0.08). The estimated seropositive-IgG and primary-IgG distributions of Chennai (Figure 2.6) closely resemble the corresponding distributions for An Giang, with the distribution means respectively being 4.8 (95% CR: 3.7–6.1, in `CheCon32e`) and 32.3 (95% CR: 30.3–34.4, in `CheCon32e`). Meanwhile, the distribution of secondary-IgG levels of Chennai has a slightly lower mean (`CheCon32e`: 41.1; 95% CR: 40.9–41.9) than that of An Giang (`AgiCon33`: 43.8; 95% CR: 42.5–45.0).

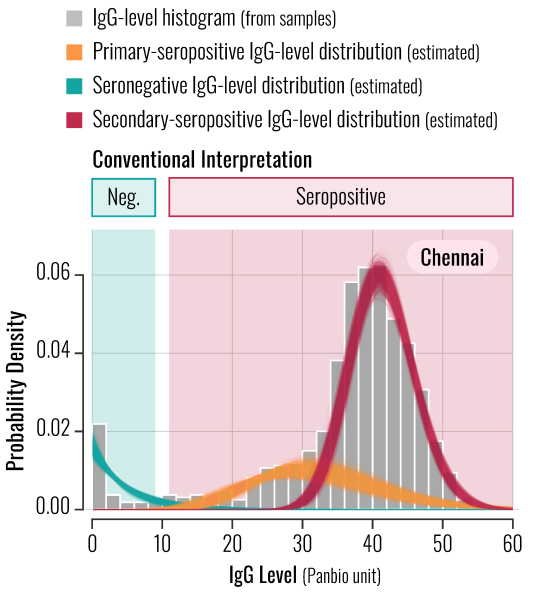


FIGURE 2.6
The histogram of the measured IgG levels (bars), and the inferred IgG-level distributions (lines) of the 3 exposure classes (seronegative, primary-seropositive, and secondary-seropositive) in Chennai. The IgG-level distributions were simulated 1,000 times based on the posteriors of the `CheCon32e` model.

2.4 DISCUSSION

In this analysis, I used a basic age-seroprevalence approach to infer the FOI of dengue virus at 3 sites in Vietnam. Multiple models of FOI and antibody acquisition were tested, with the majority of best-fitting models agreeing

TABLE 2.9 — Six continuous models that were fitted to the dataset of Chennai. The models are arranged by their DIC in ascending order; the last models in the list did not converge. Estimated parameters are shown by the median values and their 95% CR (in parentheses). Fixed time cut-offs are presented as a single number. The heterogeneity of the FOI (if it exists) is interpreted as being time-dependent as suggested by the original paper (Rodríguez-Barraquer *et al.* 2015). Estimates of the mean and the SD of the IgG-level distributions of Chennai are shown in the five rightmost columns. The posteriors of the IgG-level distributions of seronegative and primary-seropositive cases of An Giang (inferred from the `AgiCon33` model) were used as strong priors for the corresponding IgG-level distributions in these models of Chennai.

Model (DIC)	Nº of Free Param.	Time Periods		Force of Infection†			Mean & SD of Sero-negative	Primary-seropositive		Secondary-seropositive	
		Count	Cut-offs	1ST Time Period	2ND Time Period	3RD Time Period		Mean	SD	Mean	SD
CheCon32e (5,565)	7	2	7	0.28 (0.23; 0.33)	0.06 (0.04; 0.08)	–	4.8 (3.7; 6.1)	32.3 (30.0; 34.4)	9.2 (7.9; 10.8)	41.4 (40.9; 41.9)	4.6 (4.3; 5.0)
CheCon33 (5,565)	8	2	9.4 (6.4; 13.3)	0.24 (0.19; 0.31)	0.05 (0.02; 0.07)	–	4.7 (3.7; 6.1)	32.3 (30.0; 34.3)	9.2 (7.9; 10.8)	41.4 (40.9; 41.9)	4.6 (4.3; 5.0)
CheCon32 (5,568)	7	2	6	0.30 (0.25; 0.36)	0.06 (0.04; 0.08)	–	4.8 (3.7; 6.1)	32.4 (30.0; 34.4)	9.3 (8.0; 10.8)	41.4 (40.9; 41.9)	4.6 (4.3; 5.0)
CheCon34 (5,568)	8	3	6 &18	0.28 (0.22; 0.35)	0.09 (0.04; 0.14)	0.05 (0.01; 0.08)	4.7 (3.7; 6.1)	32.3 (29.9; 34.3)	9.3 (7.9; 10.8)	41.4 (40.9; 41.9)	4.7 (4.3; 5.0)
CheCon31 (5,621)	6	1	–	0.14 (0.12; 0.15)	–	–	5.0 (3.9; 6.5)	33.5 (31.2; 35.3)	9.5 (8.2; 11.0)	41.2 (40.7; 41.7)	4.8 (4.4; 5.3)
CheCon35	10	3	est.‡	–	–	–	–	–	–	–	–

† *The number of FOI estimates in each model corresponds to the number of time periods in the model, with the first estimate corresponding to the most recent time period before the year of sample collection (i.e. 2011).*

‡ *'est.' indicates that the time cut-offs were left to be estimated by the MCMC runs, but the chains did not converge.*

that Quảng Ngãi had a lower overall FOI than Hồ Chí Minh City or An Giang, and that the FOI in An Giang was likely to be age-varying while the FOIs in Hồ Chí Minh City and Quảng Ngãi were generally constant. In addition, I show that using a quantitative approach to IgG levels (measured by indirect ELISAs) can help to distinguish primary-seropositive samples from secondary-seropositive samples, by using the relationship between age and serostatus (i.e. older individuals are more likely to be secondary-seropositive, in long-term endemic settings) to calibrate the relationship between IgG level and serostatus.

Although I cannot perform a direct statistical comparison between the continuous models and the binary models, it is likely that the continuous models would generally lead to more robust results. Firstly, the continuous models do not suffer from the loss of information due to the categorisation of IgG levels and, hence, may yield more precise information (e.g. the age cut-offs of An Giang could only be estimated in the continuous models). Secondly, the histograms of the IgG-level data from each of the populations visually show better fit to a mixture of 3 gamma distributions (as in the continuous models) rather than to a categorical distribution. Finally, while the sensitivity and the specificity of the ELISA are mathematically incorporated into the continuous models (via the IgG-level distributions), these factors are not considered in the binary models; this seems to be the major reason for the discrepancy in the FOI and the seroprevalence estimates between the 2 categories of models.

The non-constant FOI of An Giang can be interpreted in 2 ways. When interpreted as time-dependent, this heterogeneity would mean that the FOI of dengue in An Giang quickly decreased from 0.17–0.20 before 2005 to 0.04–0.06 after 2005 (the time cut-off is around 10 years prior to the sample collection). On the other hand, interpreting the heterogeneous FOI of An Giang as age-specific would mean that individuals greater than 10 years old in this population were constantly exposed to very high transmission while the below-ten-years age group experienced a low risk of infection, thus substantially delaying the median age of infection. Although a time-varying FOI could be caused by demographic changes or control efforts over time (Cummings *et al.* 2009; Katzelnick *et al.* 2018), it is more likely that the FOI of An Giang was age-dependent due to 2 reasons. Firstly, according to a

national surveillance, the annual number of reported dengue cases in An Giang was generally stable across the years between 1994 and 2010 (Panhuis *et al.* 2015; University of Pittsburgh 2019). Secondly, interpreting the FOI as age-varying in my continuous models led to seroprevalence estimates (Table 2.6) that are consistent with the seroprevalence of 22%–30% among 9-year-olds, approximated by a cohort study in Long Xuyen (the capital city of An Giang) between 2003 and 2007 (Tien *et al.* 2010). However, further study is needed to confirm whether the FOI of An Giang is age- or time-specific as this will affect vaccination strategies. An age-varying FOI (inferred from past data) could be used directly for vaccine recommendations in the near future. Meanwhile, a time-varying FOI would only be useful if we have adequate understanding of why and how transmission intensity changes over time.

While the binary models led to lower FOI estimates for Hồ Chí Minh City and Quảng Ngãi, compared to the continuous models, this may be an artefact of many primary-seropositive individuals being misclassified as seronegative in the binary models due to the overlapping IgG-level distributions of primary-seropositive and seronegative cases (shown by the continuous models). Despite the difficulty of reliably comparing FOI estimates inferred from serological and case data (Imai *et al.* 2016), the constant FOI of Hồ Chí Minh City (around 0.12) estimated from my best-fitting continuous models generally agrees with previous findings, which used case data to infer the city's annual attack rate of dengue to be 11.6% (Cuong *et al.* 2016). Since this is the first study to measure the FOI of dengue in Quảng Ngãi, I cannot validate my estimates in this population. However, the low transmission intensity inferred by my models fits well to the low incidence rate in Quảng Ngãi[24] (Panhuis *et al.* 2015).

Following a common practice in dengue modelling, this analysis assumed that homotypic reinfections did not alter the IgG levels of individuals. However, this assumption is possibly wrong. Given that homotypic DENV reinfections are able to yield symptomatic disease (Waggoner *et al.* 2016), it is likely that this type of infections may also boost antibody levels. On the one hand, most of the findings from my models would remain valid if the rise of IgG attributed to homotypic re-exposures was significantly smaller than that caused by heterotypic infections. In this immunological scenario, the models could still correctly distinguish individuals with heterotypic infections from

[24] As shown in Figure 2.1 on page 36, Quảng Ngãi had the lowest reported incidence rate among the 3 locations of Vietnam.

those who had experienced only 1 serotype even though the means and the variances of both the primary-seropositive and the secondary-seropositive IgG-level distributions may have been increased due to the boosting effect. On the other hand, if homotypic re-exposures and heterotypic infections led to comparable IgG stimulus, the dengue FOIs returned from my models would be overestimates. This is because the models assumed that individuals' IgG levels could only shift from the primary-seropositive class to the secondary-positive one through an infection with any of the 3 serotypes that are different from the one causing the primary infection. To compensate for the (unmodelled) possibility that the primary serotype could also cause that shift, my models may increase the overall FOI estimates to fit to the high number of individuals with secondary-positive IgG levels.

An obvious weakness of my continuous models lies in the assumption that all 4 serotypes always contributed equally to the overall FOIs. This oversimplification may lead to inaccuracy in the estimation of the FOIs and the IgG-level distributions when put into the context that dengue epidemics in Vietnam are often temporally dominated by only 1 or 2 serotypes (Ha *et al.* 2000; Hang *et al.* 2010; Recker *et al.* 2009; Wikramaratna *et al.* 2010). For example, in a system where 1 serotype dominates most of the transmission, the majority number of primary infections would be attributed to that dominant serotype. If secondary infections could only be caused by the much weaker heterotypic serotypes, the secondary incidence rate would be markedly lower than one from the modelled scenario of the same overall FOI (where the serotypes evenly contribute). Therefore, to resolve this discrepancy, my models would have to misclassify some primary-seropositive individuals as secondary-seropositive cases. Consequently, the estimated IgG-level distribution of the secondary-seropositive class would be shifted toward the primary-seropositive. Additionally, to compensate for the wrong reduction in the number of primary-seropositive cases (resulted from the misclassification above), the models may also lower their overall FOI estimates.

Nevertheless, the fact that my continuous models led to estimates that are consistent with the findings of other studies (which used a different type of data, or a different method of sample collection) indicates the insignificance of the aforementioned limitations as well as the negligibility

of sampling bias (potentially caused by the sera used in this study being residual samples collected from hospitals). Furthermore, testing the continuous models on the population of Chennai resulted in mostly similar outcomes compared to the original study from which the data were collected (Rodríguez-Barraquer *et al.* 2015), i.e.the FOI of Chennai was extremely high (above 0.20) between the years 2004–2011 and was much lower (around 0.10) before that period. This consistency is another indication for the robustness of my method.

The high distribution mean of primary-IgG levels in An Giang and likely in Chennai (although the analysis of Chennai was based on strong priors) may result from the very high transmission rates in the 2 locations. A possible explanation for this finding is that a primary infection would increase IgG to a moderately high level, which eventually wanes if infection does not reoccur (World Health Organization 1997). In other words, high FOIs in An Giang and Chennai could reflect high proportions individuals with homotypic boosting or recent primary infections (so that their IgG levels had not yet waned). An alternative hypothesis that could explain the differences—not only in the primary-IgG levels but also in the seronegative-IgG levels—between An Giang and the other Vietnamese populations is the possibility that the distributions of IgG levels may be age-dependent (even for individuals in the same exposure class). As the age structures of seronegative and primary-seropositive cases were very different across the populations, this would lead to differences in the IgG-level distributions.

The results from my models indicate that compared to secondary-seropositive cases, primary-seropositive individuals have a higher chance of being misclassified as seronegative. The inferred IgG-level distributions from the continuous models suggest that a secondary infection generally leads to a higher IgG level compared to a primary infection. In fact, this IgG-boosting phenomenon has been observed in dengue patients for many years (World Health Organization 1997); however, only 1 study has quantified this at a population level (Kucharski *et al.* 2018). Additionally, my findings are consistent with the reported sensitivities in the manual of the ELISA kits, with the sensitivities of primary and secondary cases, respectively, being 33.3% (95% CR: 23.4–44.5) and 97.9% (95% CR: 92.5–99.7) in a test, and 87.0%

(95% CR: 66.4–97.2) and 100% (95% CR: 96.1–100) in another test[25] (Alere Inc. 2018).

[25] I cannot specify why the sensitivities varied greatly between these tests as the manual descriptions are vague.

The low sensitivity of the ELISA on samples from primary-seropositive individuals (as observed in practice and confirmed in my continuous models) is particularly problematic when put into context of the recommended dengue vaccination strategy. On the one hand, we could take a conservative approach to avoid vaccinating seronegative individuals, probably by increasing the cut-off for the assay. In this case, most people with primary exposures—the main targets of the vaccine—would be misclassified as being seronegative, and hence, would not receive the vaccine. Conversely, if the ELISA cut-off was reduced to increase the number of primary-seropositive vaccinees, the risk of vaccinating a large number of seronegative individuals would also increase. Although using double cut-offs (with an equivocal buffer between the seronegative and seropositive ranges, as suggested by the manufacturer of the ELISA kits used in this study) may reduce the misclassification rate, it is not helpful in the context of dengue vaccination since we still have to decide whether individuals with equivocal results should receive the vaccine. It has been shown that the severity of this misclassification problem strongly depends on dengue transmission intensity, hence the need to take local FOI into account when carrying out pre-vaccination screening (Rodríguez-Barraquer *et al.* 2019; Wilder-Smith *et al.* 2018). In this work, I push this idea a step further by showing that patient age is another factor that should be incorporated into the interpretation of ELISA results. In other words, by combining age, IgG level, and dengue FOI, one can calculate a probability table (Figure 2.5) to assess the likelihood of each sample being primary-seropositive. Although more studies are needed to apply this method at individual levels, there is potential to use this probability table at population scales. For instance, one should consider having different ELISA cut-offs for different age groups in population sero-surveillance. More specifically, higher ELISA cut-offs should be set for lower age groups to compensate for the lower likelihood of younger age groups being seropositive. Additionally, in a routine vaccination campaign, my method can be employed to estimate the number of vaccine doses not given to primary-seropositive individuals and, thus, to calculate the effectiveness of the campaign.

This work shows the potential of using IgG-titre data quantitatively for refining estimates of FOIs, and for enhancing the accuracy of ELISA result interpretation. My models show robust performance across different transmission settings. These results suggest that Hồ Chí Minh City may be a suitable target for routine testing and vaccination campaigns against dengue, but that Quảng Ngãi and An Giang may not be or may require testing and vaccination at much higher ages.

3

ESTIMATING THE EFFECT OF MATERNAL IMMUNITY AGAINST DENGUE ON INFANTS' RISK OF HOSPITALISATION

3.1 INTRODUCTION

As discussed in §1.4 and §1.8,[26] anti-DENV maternal immunity may play an important role in contributing to the large number of severe infant hospitalisations observed in Hồ Chí Minh City and other highly endemic populations (Halstead *et al.* 2002). While multiple *in vitro* studies have suggested a strong immunological effect of maternally transferred immunity on the risk of severe illness in infants,[27] the epidemiological impacts of maternal immunity has not been quantified. Therefore, in the study of this chapter, I aimed to examine the epidemiological link between maternal immunity and infants' risk of severe disease, with hospitalisation being the proxy for severity. To achieve this, I introduced a epidemiological model that incorporates not only transmission-related but also maternal-immunity-related parameters. This model was then fitted to case-record data from 2 major paediatric hospitals in Hồ Chí Minh City to estimate the effect of maternal immunity on the hospitalisation risk of infants. Additionally, via this inference, the dynamics of dengue transmission in Hồ Chí Minh City was also reconstructed.

[26] See page 14 and page 27.

[27] See §1.4.2 on page 14.

3.2 METHODS

3.2.1 *Dengue Case Data*

3.2.1.1 *Data Collection*

The data used in this analysis were hospital admission records that were extracted from the electronic database of Children's Hospital One (CH1) and

Children's Hospital Two (CH2) in central Hồ Chí Minh City, Vietnam. These data consisted of all hospitalised children (below 16 years of age) who were admitted to the hospitals between January 2005 and December 2015 due to suspected dengue infection (based on the discharge diagnosis). Although CH1 and CH2 also served as referral hospitals for other provincial hospitals, only patients who were residents of Hồ Chí Minh City were included in this analysis. Patients' identities were anonymised by the hospitals during data extraction. The following fields of the patient records were preserved (if available) for analysis: gender, age (rounded to months for children under 2 years old, and rounded to years for the others), date of birth, date of admission, date of discharge, and discharge diagnosis. The majority of the patients in this data set were only clinically diagnosed. While it is known that dengue was confirmed in some patients by laboratory tests, this information was not available in the extracted data set.

3.2.1.2 *Data Exclusion*

Although the data from both CH1 and CH2 contained cases whose ages were up to 16 years, the age groups of 15- and 16-year-olds were excluded from this study due to the abnormally low number of cases in these groups, which may be caused by the fact that children of these ages are more likely to be admitted to a non-paediatric hospital than the younger kids. In the subsequent analyses, patients were often divided into 2 main groups based on their ages, i.e. *infants* (below 2 years of age) and *non-infants* (2 to 14 years of age).

While the data for CH1 contain a very low rate of missingness, with only 1.57% of the cases not having their date of birth recorded (all the other information was available), there were some abnormalities in the data. Firstly, between 1st January 2011 and 31st March 2011, there were only 9 recorded admissions for CH1. I suspect that this extremely low number of cases may be caused by mistakes during the data extraction process, given that in the lowest dengue season of the other years, CH1 still admitted more than 50 dengue patients per month. Therefore, the data of CH1 during the first 3 months of 2011 were treated as if they were completely missing. Secondly, while the patients' dates of birth were available for all the cases admitted to CH1 during the years 2005–2010, the proportion of the cases that were hospitalised on their birthdays were extremely high during this period, i.e. 17.6%–42.6% each

year. This high proportion is likely to be an artefact of the dates of birth being automatically filled in based on the patients' years of age and their admission dates. In subsequent analyses, where infants (patients that are below 2 years of age) are the focal subject, these likely false dates of birth may distort the inferences since the infant cases would be grouped by their months of age. Therefore, infant data of CH1 for 2005–2006 were treated as if they were completely missing since a third of the infant cases of CH1 in this period were indicated to have been hospitalised on their birthdays. For the years 2007–2010, all infant patients falling into this same category were simply ignored from the counts as they only contributed to less than 6% of the total infant cases of each year.

At CH2, 10.4%–45.4% of the total number of patients of each year between 2007 and 2009 did not have their ages nor their years of birth recorded. Therefore, in analyses where age served as an input variable, the data of CH2 between 2007 and 2009 were excluded.

3.2.2 *Descriptive Analysis*

A descriptive analysis was carried out for these case data, using the R language (R Core Team 2020).

3.2.3 *The Epidemiological Model*

3.2.3.1 *Modelling Disease Transmission*

In order to reconstruct the epidemiology of dengue in Hồ Chí Minh City across the years 2005–2015, I developed a mechanistic compartmental model for dengue transmission (3.1). In this model, individuals are classified into compartments—denoted by $S_a^{(0)}$, $I_{a,i}^{(1)}$, $R_{a,i}^{(1)}$, $I_{a,k}^{(\mathcal{P})}$, $S_{a,i}^{(1)}$, $I_{a,k}^{(2)}$, and $R_a^{(2)}$—based on their age group (a) and their infection status (i.e. susceptible, infected, and recovered—respectively denoted by S, I, and R). The $S_a^{(0)}$ classes represent individuals of the age group a that are immunologically naive to dengue. These individuals are moved to the $I_{a,i}^{(1)}$ classes as soon as they are exposed to an infection by serotype i. After recovering from this primary infection, individuals are moved to the $R_{a,i}^{(1)}$ classes, where they gain a life-long immunity

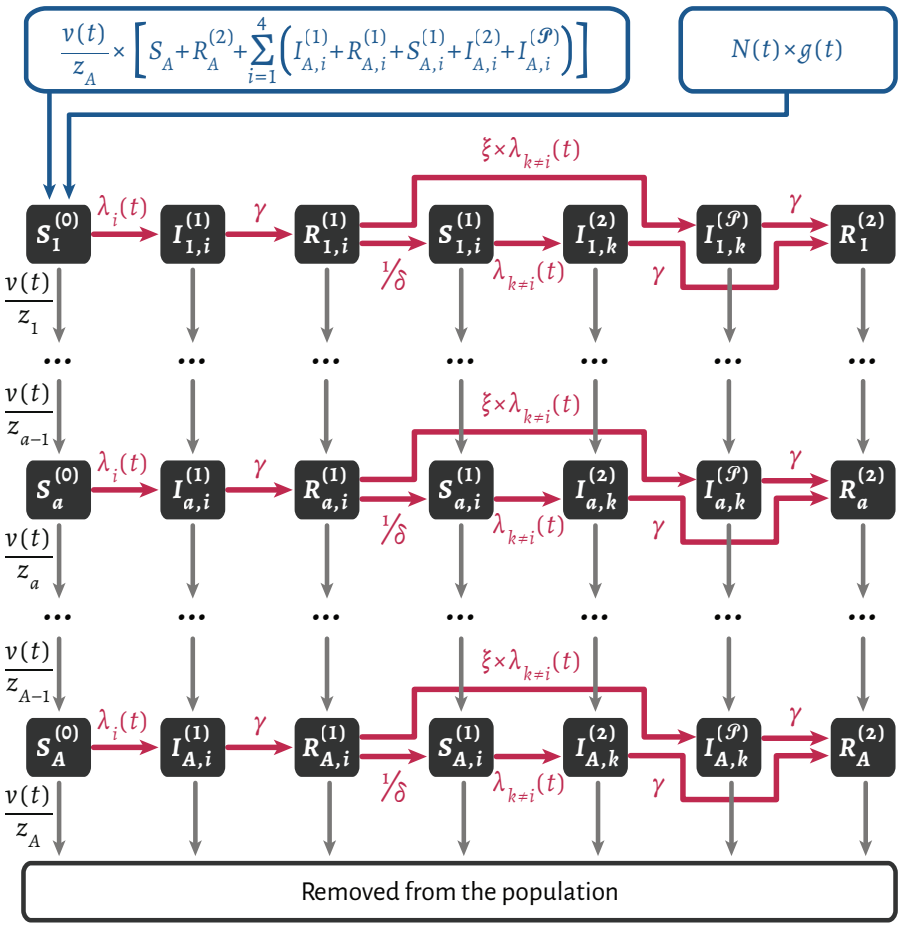


FIGURE 3.1 – The structure of the epidemiological model used in this analysis. The S, I, and R boxes in this diagram, respectively, represent the classes of susceptible, infected, and recovered individuals. The superscript in the notation of each model compartment either indicates the number of infections individuals have experienced (i.e. 0, 1, or 2) or shows that individuals are experiencing a secondary infection during the primary protection period (denoted by $\mathcal{P}$). The first subscripts of the compartments represent the age group of individuals while the second subscripts (if exist) indicate the last infecting serotype. The descriptions of the other notations are presented throughout the text of this chapter.[29] The red arrows represent individual transitions due to infections (with the transition rates shown in red text). Meanwhile, the grey arrows indicate transitions due to ageing (with the transition rates shown in black text). The two blue boxes on the top indicate the number of new births added into the model at time t. The amount in the blue box on the left equals the number of individuals in the oldest age groups that are removed from the system due to ageing. Meanwhile, the blue box on the right corresponds to the number of additional births caused by natural population growth. Population change due to migration is not presented in this graph.

[29] See Table B.1 and Table B.2 (pages 136, 137) for the summary descriptions of these notations.

against serotype i, and short-term cross-serotypic protection of length δ days. This cross-serotypic protection—here, also referred to as the *primary protection*—is assumed to protect individuals from developing symptoms if they are infected during this protected period. Furthermore, the protection is assumed to have an effect on the relative infection risk of individuals, which is modelled by the ξ parameter. If individuals are infected by a serotype k (where $k \neq i$) while under this protection, they are moved to the $I_{a,k}^{(\mathcal{P})}$ classes. Individuals in the $I_{a,k}^{(\mathcal{P})}$ classes were assumed to be completely asymptomatic, and hence not to contribute to the number of hospitalisations; however, their infectivity was assumed to be the same as that of other infected cases. After the short-term cross-protection period ends, individuals are transferred to the $S_{a,i}^{(1)}$ classes, where they become fully susceptible to a secondary infection by any serotype k that is different from the serotype of the first infection ($k \neq i$). Individuals are moved from the $S_{a,i}^{(1)}$ to the $I_{a,k}^{(2)}$ classes if they are infected again (by a serotype that is different from that of the primary infection). Individuals that have recovered from secondary infections (the $I_{a,k}^{(\mathcal{P})}$ and the $I_{a,k}^{(2)}$ classes) are moved to the $R_a^{(2)}$ classes, where they are assumed to have a life-long immunity against all the 4 serotypes of dengue, and thus cannot be infected again. Here, the superscripts in the notations of the compartments either indicate the number of infections that individuals have experienced (i.e. 0, 1, or 2) or imply that individuals are infected while still in the primary protection period, i.e. $I_{a,k}^{(\mathcal{P})}$. Meanwhile, the subscripts in the notations represent the age group (a) and—in case the second subscript exists—denotes the last infecting serotype (i or k) of individuals.

The epidemiological model above is formalised by the following system of time-dependent ordinary differential equations:

$$
\begin{aligned}
\frac{d}{dt}S_a^{(0)} &= -S_a^{(0)}\sum_{i=1}^{4}\lambda_i(t) - \frac{\nu(t)}{z_a}S_a^{(0)} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}S_{a-1}^{(0)} + \mathbb{1}_{a=1}\times B(t) \\
&\quad + m(t)\times S_a^{(0)} \\
\frac{d}{dt}I_{a,i}^{(1)} &= \lambda_i(t)\,S_a^{(0)} - \gamma I_{a,i}^{(1)} - \frac{\nu(t)}{z_a}I_{a,i}^{(1)} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}I_{a-1,i}^{(1)} + m(t)\times I_{a,i}^{(1)} \\
\frac{d}{dt}R_{a,i}^{(1)} &= \gamma I_{a,i}^{(1)} - R_{a,i}^{(1)}\times\left(\frac{1}{\delta} + \xi\sum_{k\neq i}\lambda_k(t)\right) - \frac{\nu(t)}{z_a}R_{a,i}^{(1)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}R_{a-1,i}^{(1)} + m(t)\times R_{a,i}^{(1)}
\end{aligned}
$$

$$
\begin{aligned}
\frac{d}{dt} I_{a,k}^{(\mathcal{P})} &= \xi \lambda_k(t) \sum_{i \neq k} R_{a,i}^{(1)} - \gamma I_{a,k}^{(\mathcal{P})} - \frac{\nu(t)}{z_a} I_{a,k}^{(\mathcal{P})} + \mathbb{1}_{a>1} \times \frac{\nu(t)}{z_{a-1}} I_{a-1,k}^{(\mathcal{P})} \\
&\quad + m(t) \times I_{a,k}^{(\mathcal{P})} \\
\frac{d}{dt} S_{a,i}^{(1)} &= \frac{1}{\delta} R_{a,i}^{(1)} - S_{a,i}^{(1)} \sum_{k \neq i} \lambda_k(t) - \frac{\nu(t)}{z_a} S_{a,i}^{(1)} + \mathbb{1}_{a>1} \times \frac{\nu(t)}{z_{a-1}} S_{a-1,i}^{(1)} \\
&\quad + m(t) \times S_{a,i}^{(1)} \\
\frac{d}{dt} I_{a,k}^{(2)} &= \lambda_k(t) \sum_{i \neq k} S_{a,i}^{(1)} - \gamma I_{a,k}^{(2)} - \frac{\nu(t)}{z_a} I_{a,k}^{(2)} + \mathbb{1}_{a>1} \times \frac{\nu(t)}{z_{a-1}} I_{a-1,k}^{(2)} + m(t) \times I_{a,k}^{(2)} \\
\frac{d}{dt} R_a^{(2)} &= \gamma \sum_{i=1}^{4} \left(I_{a,i}^{(2)} + I_{a,i}^{(\mathcal{P})} \right) - \frac{\nu(t)}{z_a} R_a^{(2)} + \mathbb{1}_{a>1} \times \frac{\nu(t)}{z_{a-1}} R_{a-1}^{(2)} + m(t) \times R_a^{(2)}
\end{aligned}
$$

where:

- t denotes time;
- a is the age group index;
- i, k denote dengue serotypes;
- $\lambda_i(t)$ is the FOI of serotype i at time t;
- $\nu(t)$ is the seasonal relative birth rate of the population at time t;
- z_a is the age-span of the age group a, measured in the number of days (in this model, $\sum_a z_a = 21,900$ days ≈ 60 years);
- $m(t)$ is the daily net migration rate of the population at time t;
- γ is the recovery rate of infected individuals;
- δ is the average duration of the primary protection period;
- ξ is the relative risk of infection for individuals that are in the primary protection period, as compared to unprotected susceptibles;
- $B(t)$ is the total number of new births at time t;
- $\mathbb{1}_x$ is an indicator function, i.e. $\mathbb{1}_x = \begin{cases} 1 & \text{if } x \text{ is true,} \\ 0 & \text{otherwise} \end{cases}$.

In this model, the risk that a susceptible individual is infected by serotype i (i.e. the FOI of serotype i) is calculated as follows:

$$
\lambda_i(t) = \beta_i(t) \times \frac{\sum_a \left(I_{a,i}^{(1)} + I_{a,i}^{(2)} + I_{a,i}^{(\mathcal{P})} \right)}{N(t)}
$$

where: $\beta_i(t)$ represents the transmission rate of serotype i at time t;

$N(t)$ is the total population size;

and the sum $\sum_a \left(I_{a,i}^{(1)} + I_{a,i}^{(2)} + I_{a,i}^{(\mathcal{P})} \right)$ corresponds to the total number of infectious individuals of serotype i at the time.

With the calculation of the FOI above, the infectivity is assumed to be the same for primary and secondary infected individuals, including those who are in the primary protection period. Furthermore, this model also assumes that the risk of infection by a serotype is the same for primary and secondary susceptible individuals. Since dengue epidemics in Hồ Chí Minh City are known to be strongly seasonal (Cuong *et al.* 2013; H. S. Lee *et al.* 2017), the transmission rate, $\beta_i(t)$, was modelled as a stepwise periodic function, of which the cycle length is a year. For the purpose of simplicity, all 4 dengue serotypes were assumed to have an identical transmission rate—denoted by β—at any given time t, i.e. $\beta_1(t) = \beta_2(t) = \beta_3(t) = \beta_4(t) = \beta(t)$. In this exercise, mosquito vectors are ignored and transmission is modelled as if it occurred from person to person. However, any changes in the vector population that affect the transmission intensity should be absorbed into the transmission rate function, $\beta(t)$.

The effect of the primary protection was modelled via 2 parameters, i.e. the length of the protection period (δ) and the relative risk of infection (ξ) while still in the protection period. The primary protection was assumed to be either *complete* or *partial*. *Complete protection* would block infection totally ($\xi = 0$), while *partial protection* would prevent symptoms but not alter the risk of infection ($\xi = 1$). The scenario in which the primary protection partly reduces the total risk of infection (i.e. $0 < \xi < 1$) was not investigated as the effects of ξ and δ on the number of observed hospitalisations are inseparable and hence cannot be estimated simultaneously. In other words, a raise in hospitalisation can be matched by either increasing ξ (i.e.weaken the primary protection) or by lowering δ (i.e.shorten the primary protection period) as both would lead to an increase in the number of secondary infections. Conversely, both reducing ξ and raising δ would lessen the number of secondary infection and thus bring down the number of hospitalisations.

3.2.3.2 *Modelling Demographic Changes*

In the model above, disease-related deaths are completely ignored and individuals are only removed from the population when they reach the maximum allowed age (i.e. 60 years). The ageing process was modelled via the ratio $\frac{v(t)}{z_a}$, which represents the number of individuals of an age group (a) being moved to the next older age group ($a+1$). Here, z_a is the size of age group a measured in number of days (e.g. the size of the groups of all individuals between 2 and 3 years old is 365 days). Meanwhile, $v(t)$—a periodic function of mean 1.0 and cycle length of 365 days—depicts the relative birth rate at time t, as compared to the other time of the year.

It is known that births are seasonal in Hồ Chí Minh City, with the number of births dropping to a minimum around February–March and peaking around October. While population monthly birth data were not available for this analysis, I was able to reconstruct this seasonal pattern in births using

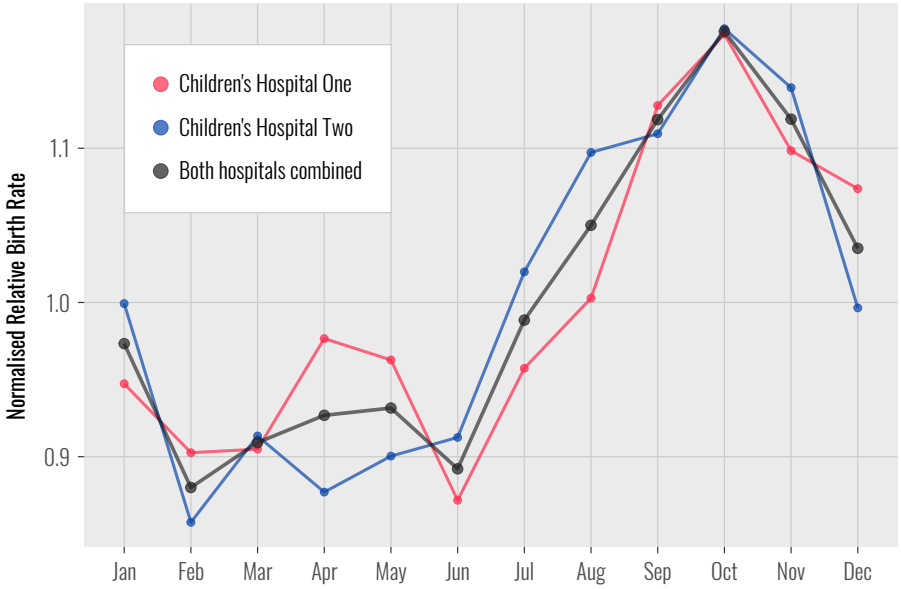


FIGURE 3.2 — Monthly relative birth rates of the Hồ Chí Minh City population, inferred from the available months of birth of the non-infant cases admitted to CH1 (red line), CH2 (blue line), and both hospitals combined (black line). These numbers were estimated by grouping the patients by their calendar months of birth and calculating the proportion of patients that each month of birth contributed to the total number of cases. The final relative birth rates were normalised by dividing these proportions by their mean value. The relative birth rates calculated from the data of both hospitals combined were used for subsequent analyses.

the months of birth of the non-infant cases in the CH1 and CH2 data set (Figure 3.2). The relative monthly birth rates were estimated by grouping the patients by their calendar months of birth and calculating the proportion of patients that each month of birth contributed to the total number of cases. The final normalised relative birth rates were computed by dividing these proportions by their mean value. These normalised relative birth rates were used as the $v(t)$ function in the system of ordinary differential equations above.

The number of new births, $B(t)$, that are added into the population at a given time t is calculated as follows:

$$B(t) = \frac{v(t)}{z_A} \times \left[S_A^{(0)} + R_A^{(2)} + \sum_{i=1}^{4} \left(I_{A,i}^{(1)} + R_{A,i}^{(1)} + S_{A,i}^{(1)} + I_{A,i}^{(\mathcal{P})} + I_{A,i}^{(2)} \right) \right] + N(t) \times g(t)$$

where:
- A is the index of the oldest age group in the model;
- z_A is the age-span of the oldest age group, measured in the number of days;
- $v(t)$ is a periodic function (of mean 1.0 and cycle length of 365 days) that represents the relative birth rate at time t;
- $N(t)$ is the total population size at time t;
- $g(t)$ is the daily natural population growth rate of the population at the time.

Here, the number of new births was modelled as a sum of 2 quantities. The first quantity, $\frac{v(t)}{z_A} \times [\cdots]$, is equal to the number of individuals that reach the maximum allowed age (i.e. 60 years) and are removed from the system. Meanwhile, the second quantity, $N(t) \times g(t)$, is the number of additional births that are needed to match the natural growth rate of the population. This method of modelling the number of new births was chosen for convenience as the population size would stay constant when the natural growth rate, $g(t)$, is set to zero.

As immigration contributes a large proportion to the total population of Hồ Chí Minh City, it is also taken into account in this exercise through the variable $m(t)$, which represents the daily net migration rate at time t. For simplicity's sake, immigrants were assumed to come from a population that has epidemiological characteristics and an age structure identical to Hồ Chí

Minh City. In other words, immigration is assumed not to alter the transmission intensity nor the age distribution.[30] This restriction was imposed within the model by forcing the number of immigrants added to each model class to be proportional to the number of pre-existing individuals in that class.

[30] In this model, immigration does not alter the FOI of dengue; however, it increases the population size, and hence raises the absolute number of infections.

The natural population growth rate and the net migration rate used for this modelling exercise (Table 3.1) were downloaded from the website of Hồ Chí Minh City Statistical Office (2020). Since the original data were annual estimates, they were converted to daily rates using the following approximations:

$$g(t) = \frac{\ln\left[1 + G(t)\right]}{365} \times v(t)$$

$$m(t) = \frac{\ln\left[1 + M(t)\right]}{365}$$

where: $v(t)$ is the relative birth rate at time t;

$g(t)$, $G(t)$ are, respectively, the daily and the annual rates of natural population growth at the time;

$m(t)$, $M(t)$ are, respectively, the daily and the annual rates of net migration.

For the purpose of simplicity, the age distribution of the Hồ Chí Minh City population prior to 2005 was assumed to be uniformly distributed across all age groups between 0 and 60 years.

3.2.3.3 *Modelling Maternal Immunity*

In this modelling study, I assume that maternally derived immunity has a biphasic effect on infants' risk of developing symptoms. In the first few months of life, this immunity is assumed to protect newborns from developing symptoms if they experience a primary infection. After this protection diminishes, infants are assumed to enter a period of increased risk of symptomatic disease if they get infected. From here on, the protection and the disease-enhancing effect that may be caused by maternal immunity will be referred to as *maternal protection* and *maternal ADE* to distinguish them from the similar effects that may occur after a primary infection.

TABLE 3.1 — Annual natural population growth rates, annual net migration rates, and population sizes of Hồ Chí Minh City across the years 2005–2015. The data were downloaded from the website of Hồ Chí Minh City Statistical Office (2020).

Year	Annual Natural Increase Rate (per thousand)	Annual Net Migration Rate (per thousand)	Population Size
2005	11.50	28.07	6,291,055
2006	10.75	27.95	6,541,508
2007	10.96	24.44	6,778,867
2008	10.29	21.73	7,000,746
2009	10.37	17.80	7,201,550
2010	10.35	16.27	7,396,446
2011	9.79	15.98	7,590,138
2012	10.07	16.07	7,791,789
2013	9.08	9.72	7,939,752
2014	8.26	8.27	8,072,129
2015	8.17	13.31	8,247,829

The risk ratio of having a symptomatic primary infection for infants that have maternal immunity—as compared to those who do not—is modelled by the following age-dependent function:

$$\phi(a) = \begin{cases} 0 & \text{if } a \leq \kappa_{\text{protect}}, \\ \left(\phi_{\max} - 1\right) \times \exp\left[-\left(\frac{a-\kappa_{\text{peak}}}{\kappa_{\text{sd}}}\right)^2\right] & \text{otherwise} \end{cases}$$

$$\left(\text{for } 0 < a,\, \kappa_{\text{sd}}\,;\;\; 1 \leq \phi_{\max}\,;\;\; 0 < \kappa_{\text{protect}} < \kappa_{\text{peak}} < 2\,\text{years}\right)$$

where: a represents the age of infants;

κ_{protect} is the maximum age at which maternal immunity is still protective;

κ_{peak} is the age at which the disease-enhancing effect of maternal ADE is the most prominent;

κ_{sd} is relative to the duration of maternal ADE;

$\phi_{\max}$ corresponds to the maximum possible risk ratio, which would occur if infants get infected at the age $a = \kappa_{\text{peak}}$.

Here, the effect of maternal ADE is conveniently formalised to have a functional form similar to that of a Gaussian distribution, so that κ_{peak} and κ_{sd} can also be, respectively, interpreted as the mean and the SD of the ages of infants who are affected by maternal ADE. In this model, by forcing $\phi_{\max}$ not to be less than 1, I assumed that infants' risk of symptomatic primary infection during the maternal ADE period is either equal to that of non-infants (resulting in $\phi_{\max} = 1$) or greater (resulting in $\phi_{\max} > 1$).

According to the reported statistics of Vietnam's Ministry of Health (2018), between the years 2009 and 2015, more than 92% of children in Vietnam were born to mothers of above 20 years of age.[31] At 20 years old, 91.31% (95% CR: 88.36%–93.63%) of women in Hồ Chí Minh City would have experienced at least 1 dengue infection, as estimated by the `HcmCon31` model in CHAPTER 2 (Figure 2.3). Since the age of pregnancy in the city is higher than the country's average,[32] the chance that the infants in this study were born to dengue-immune mothers may be higher than the seroprevalence estimate above. Therefore, for simplicity's sake, I assumed that 100% of babies in this modelling study were born with maternally derived immunity against dengue.

[31] While this percentage varied across the years, it was consistently above 92%.

[32] Although this is a well-known fact, I could not find a citable source for the age distribution of pregnant women in Hồ Chí Minh City.

3.2.3.4 *Modelling Age-dependent Hospitalisation Rates*

In this analysis, *hospitalisation rate* is defined as the quotient of the total number of suspected dengue cases observed at CH1 and CH2 divided by the estimated number of symptomatic infections in the population. For this exercise, the hospitalisation rate (η) was modelled by the following age-dependent stepwise function:

$$\eta(a) = \begin{cases} \eta_{\text{inf}} & \text{if } a < 2 \text{ years}, \\ \eta_{\text{noninf}} & \text{otherwise} \end{cases}$$

where: a represents individuals' age;

$\eta_{\text{inf}}, \eta_{\text{noninf}}$ are, respectively, the hospitalisation rates of infants and non-infants.

Although little is known about the link between patients' age and hospitalisation rate, clinicians at both CH1 and CH2 have communicated that infants are more likely to be hospitalised than non-infants—if they present the same severity of disease—due to the belief of both parents and physicians that infant cases should be monitored more closely. Therefore, in this analysis the infants' hospitalisation rate was forced to be greater than that of non-infants,i.e. $\eta_{\text{inf}} > \eta_{\text{noninf}}$.

3.2.3.5 *Modelling the Number of Cases*

The number of suspected dengue cases ($\psi_{a,t,h}$) of age group a, at a given time t, and a given hospital h was simulated using the following formula:

$$\psi_{a,t,h} = \eta(a) \times w_{a,h} \times \left(\phi(a) \times \zeta^{(1)} \sum_i \hat{I}^{(1)}_{a,i}(t) + \zeta^{(2)} \sum_i \hat{I}^{(2)}_{a,i}(t) \right)$$

where:
- $\eta(a)$ corresponds to the hospitalisation rate of age group a;[33]
- $w_{a,h}$ is the case proportion that the hospital h contributes to the total number of hospitalisations of age group a (this was calculated directly from the case data);
- $\phi(a)$ is a function of risk ratio that represents the age-dependent effect of maternal immunity on the risk of symptomatic disease during a primary infection;[34]
- $\zeta^{(1)}$ is the probability that a primary infection develops symptoms, without the involvement of maternal immunity;
- $\zeta^{(2)}$ is the probability that a secondary infection becomes symptomatic;
- $\hat{I}^{(1)}_{a,i}(t)$ represents the number of new primary infections of age a, occurring at time t;
- $\hat{I}^{(2)}_{a,i}(t)$ is the number of new secondary infections of age a that are not in the primary protection period, arising at time t.[35]

[33] $\eta(a)$ is defined in §3.2.3.4.

[34] The detailed description of $\phi(a)$ and its formulation are shown in §3.2.3.3.

[35] $\hat{I}^{(1)}_{a,i}(t)$ and $\hat{I}^{(2)}_{a,i}(t)$ were extracted directly from the transmission model that is presented in §3.2.3.1.

The formula above implicitly carries 4 important assumptions:

[1] The hospitalisation rate is the same for all symptomatic infections (as long as they are in the same age group), no matter if they are primary or secondary.

[2] The base symptomatic rates, $\zeta^{(1)}$ and $\zeta^{(2)}$, are allowed to be different between primary and secondary infections, but are not varied by serotype or age.

[3] Infants born with maternal immunity may have their primary symptomatic rate altered (by the function $\phi(a)$).

[4] All secondary infections that occur during the primary protection period are asymptomatic.

Since the case data of this study do not contain information to determine the share of primary and secondary infections in the total number of cases, it was not possible to estimate $\zeta^{(1)}$ and $\zeta^{(2)}$ simultaneously. Therefore, the value of $\zeta^{(2)}$ was fixed at 0.41, based on the result of a pooled analysis of Clapham *et al.* (2017).

3.2.3.6 *Modelling Serotype Switching*

The epidemic size of dengue in Hồ Chí Minh City—which is known to vary across years—may be strongly influenced by the complex interaction between population immunity and alteration of dominant serotypes (Recker *et al.* 2009; Wikramaratna *et al.* 2010). Since detailed data on serotype distribution across the years of study are not available to fully investigate such interaction, each infection in this modelling exercise was assumed to be caused by 1 of 4 hypothetical serotypes, namely serotypes `A`, `B`, `C`, and `D`. Serotype switching was simulated by unevenly distributing the infected individuals across the serotypes at the initial state of the model. The serotype distribution was then intrinsically altered throughout the simulation process due the change in the size of the population susceptible to each serotype over time.

Each simulation of the model was allowed to run freely for 19 years before the model was fitted to the case data. During this initialising process, the size of the population was kept constant by forcing the number of births equal to the number of individuals being removed from the oldest age group (due to ageing). The length of the initialising phase—19 years[36]—was chosen based on the observation that after 10–15 years of free run, the case count

[36] For the equilibrium investigation, the initialising phase of the simulations was let to run up to 100 years. However, in simulations where model fitting was performed, the length of the initialisation was shrunk to 19 years to reduce the runtime of the whole process.

patterns generated by the simulations tended to repeat themselves approximately every 4–10 years (depending on the model configurations). Furthermore, after this time point, the 10-year moving window averages of the simulated annual number of primary infections fluctuated little and were consistently close to the number of new births generated for each year (i.e.around 105,000), indicating the constancy of the susceptible population size, hence the equilibrium of the system.

The proportions of the 4 hypothetical serotypes at the initial state of the simulation—denoted by ω_A, ω_B, ω_C, and ω_D—were estimated relatively to that of serotype A. In other words, the following restriction was applied: $1 = \omega_A > \omega_B > \omega_C > \omega_D$. Additionally, ω_B was empirically fixed at 0.9 to improve the convergence rate of the model fitting.

In this analysis, simpler model configurations—where serotype switching was ignored—were also tested. In these 'base' configurations, infected individuals were uniformly distributed across the serotypes at the initial state (i.e. $\omega_A = \omega_B = \omega_C = \omega_D$). The uniformity of this distribution, and hence the synchronicity of the serotypes were mechanistically preserved throughout simulation since the transmission rates (β) of the serotypes were assumed to be equal,[37] and the models were deterministic.

[37] The modelling of the transmission rates (β) is described in detail in §3.2.3.1 on page 66.

3.2.4 *Model Fitting*

3.2.4.1 *Data Pre-processing*

For this modelling analysis, patients at CH1 and CH2—who were between zero and 14 years of age—were divided into 32 age groups, consisting of 20 infant groups (14 groups with an age-span of 4 weeks and 6 groups with an age-span of 8 weeks) and 12 non-infant groups (each represents 1 year of age). The case counts (from CH1 and CH2) were aggregated into corresponding age groups every fortnight and used for the model fitting. The case counts for each fortnight were assumed to be missing if no case was observed across all of the age groups during that fortnight. Another age group representing all individuals between >14 and 60 years of age was also included in the model to fully simulate the population demographic; however, data for this age group were not available for model fitting.

Although the age-grouping scheme above was empirically chosen so that age-dependent parameters could be effectively estimated, it led to an unwanted side effect. That is, the numbers of observed cases in the infant age groups became low and noisy. Given that the number of infant age groups was more than twice that of non-infants, seasonal signals—that were clearly presented in the non-infant groups—were blurred out by the noisy data of the infant groups during the model fitting. Therefore, to reduce the noise, the time series of each age group were smoothed by a moving average window of 5 fortnights before being fed into the model.

3.2.4.2 *Fitting the Model to Data*

The observed number of cases of age group a admitted to hospital h at time t (denoted by $Y_{a,t,h}$) was assumed to follow a normal distribution with mean of $\psi_{a,t,h}$[38] and SD of σ. Here, a normal distribution was chosen—instead of a (more commonly used) Poisson or a negative binomial distribution—to avoid putting more weight on low-valued data points. Although it is common practice to allow the SD (σ) of this distribution to be estimated via model fitting, such a method cannot be applied for this particular exercise due to the finding (from a preliminary analysis) that all tested samplers had a tendency of lowering the value of σ (since it would lead to an increased likelihood). However, σ was often reduced to the point that the likelihood surface became too rough (with steep peaks surrounded by wide valleys) for the samplers to navigate through. Generally, when σ dropped below 20, the acceptance rate of the samplers started to fall sharply; eventually, the samplers were stuck at a local maximum likelihood as σ decreased further. Therefore, σ was empirically fixed at 20 to avoid this outcome.

[38] $\psi_{a,t,h}$ was simulated by the model, using the formula in §3.2.3.5.

In order to improve the robustness of the inference, the average dengue seroprevalence (denoted by X_a) of each age group (a) in Hồ Chí Minh City between the years 2014–2015 (Table 3.2)—which was estimated by the best fitting continuous model, `HcmCon31`, in CHAPTER 2—was reused as additional data to fit the current model. Here, X_a was assumed to have been sampled from a $\mathcal{B}eta\big(\tau\chi_a\,,\,\tau(1-\chi_a)\big)$ distribution, where χ_a is the corresponding estimated seroprevalence of the current model and τ serves as a hidden parameter that controls the precision of the presented beta distribution (i.e. a higher value of τ leads to a more narrow distribution).

TABLE 3.2 — The median estimate of the dengue seroprevalence in each age group in Hồ Chí Minh City between 2014 and 2015, as calculated by the best fitting continuous model (`HcmCon31`) in CHAPTER 2. Note that the effect of maternal immunity was not considered in these estimations.

Age Group†	Seropre-valence	Age Group†	Seropre-valence	Age Group†	Seropre-valence
(0w, 4w]	0.0046	(44w, 48w]	0.1007	(4y, 5y]	0.4173
(4w, 8w]	0.0138	(48w, 52w]	0.1090	(5y, 6y]	0.4831
(8w, 12w]	0.0228	(52w, 56w]	0.1172	(6y, 7y]	0.5416
(12w, 16w]	0.0318	(56w, 64w]	0.1293	(7y, 8y]	0.5934
(16w, 20w]	0.0407	(64w, 72w]	0.1452	(8y, 9y]	0.6394
(20w, 24w]	0.0495	(72w, 80w]	0.1609	(9y, 10y]	0.6802
(24w, 28w]	0.0582	(80w, 88w]	0.1762	(10y, 11y]	0.7163
(28w, 32w]	0.0669	(88w, 96w]	0.1913	(11y, 12y]	0.7484
(32w, 36w]	0.0755	(96w, 2y]	0.2061	(12y, 13y]	0.7769
(36w, 40w]	0.0840	(2y, 3y]	0.2592	(13y, 14y]	0.8021
(40w, 44w]	0.0924	(3y, 4y]	0.3430	(14y, 60y]	0.9882

† *Round opening brackets and square closing brackets indicate that the intervals are left-open and right-closed; 'w' stands for 'weeks', 'y' stands for 'years'.*

The likelihood of the model was computed as follows:

$$\mathcal{L}\left(\theta \,\middle|\, Y, X\right) = \frac{1}{\sigma\sqrt{2\pi}} \prod_h \prod_t \prod_a \exp\left[-\frac{\left(Y_{a,t,h} - \psi_{a,t,h}\right)^2}{2\sigma^2} \right] \times \prod_a \left[X_a^{\tau\chi_a - 1} \times \left(1 - X_a\right)^{\tau(1-\chi_a)-1} \times \frac{\Gamma\left(\tau\right)}{\Gamma\left(\tau\chi_a\right) \times \Gamma\left(\tau - \tau\chi_a\right)} \right]$$

where: θ denotes the set of all the parameters of the model;

Y represents all the data points in the case count data set;

X denotes the seroprevalence estimates that were extracted from the results of CHAPTER 2 and reused here as data;

Γ is the Gamma function.

In this analysis, 4 model configurations were tested. Two of them—`TypeSwtch-C` and `TypeSwtch-P`—take into account serotype switching[39] while the others—namely `TypeSynch-C` and `TypeSynch-P`—ignore this factor and

[39] The modelling of serotype switching is described in §3.2.3.6 on page 77.

assume that all the 4 serotypes were synchronous.[40] In the models `TypeSwtch-C` and `TypeSynch-C`, the primary protection was assumed to be complete, i.e. fully blocking infection ($\xi = 0$). Meanwhile, the models `TypeSwtch-P` and `TypeSynch-P` assume that the primary protection is partial, i.e. only suppressing symptoms but not altering the risk of infection ($\xi = 1$). The settings of the other parameters and variables were the same in all of these models (Table B.2).

Each model configuration was fitted to the data using the *adaptive Metropolis-within-Gibbs* algorithm (Metropolis *et al.* 1953; Roberts and Rosenthal 2009; Tierney 1994). The parameters were estimated through 4 parallel MCMC chains of 100,000 iterations (with the first 40,000 iterations removed as burn-in). Model convergence was assessed using the diagnostic of Gelman, Rubin, and Brooks (Brooks and Gelman 1998; Gelman and Rubin 1992). The model fitting was implemented in a `Julia` project (Bezanson *et al.* 2017), which incorporated the `DifferentialEquations.jl` package (Rackauckas and Nie 2017) for solving differential equations and the `Mamba.jl` package (Smith 2020) for parameter sampling. The source code of the complete model is accessible at: `https://gitlab.com/lamhm/dengue-sir`.

[40] The models are named after their configurations:
`TypeSwtch` serotype switching is incorporated.
`TypeSynch` serotype synchronicity is imposed.
`-C` with complete primary protection.
`-P` with partial primary protection.

3.3 RESULTS

3.3.1 *Descriptive Data Analysis*

Between 2005 and 2015, the total number of recorded dengue hospitalisations at CH1 and CH2 were 53,396, corresponding to an average of 409 cases per month. Among these patients, 26,126 from CH1 and 14,705 from CH2 were included in the subsequent analyses.[41] In general, the epidemiological patterns of the 2 hospitals were very similar, with all age groups synchronously showing peaks of hospitalisation between August and December of each year (Figure 3.3).

[41] See §3.2.1.2 on page 65 for the inclusion criteria.

Across the years, the age distribution of the patients at both CH1 and CH2 were generally bimodal, with a peak at 6–12 months and another peak at a much later age (Figure 3.4). However, there are a few differences between the 2 hospitals and among the years of the study. Throughout these years, the number of 6–12 months old cases (the first peak in the age distribution)

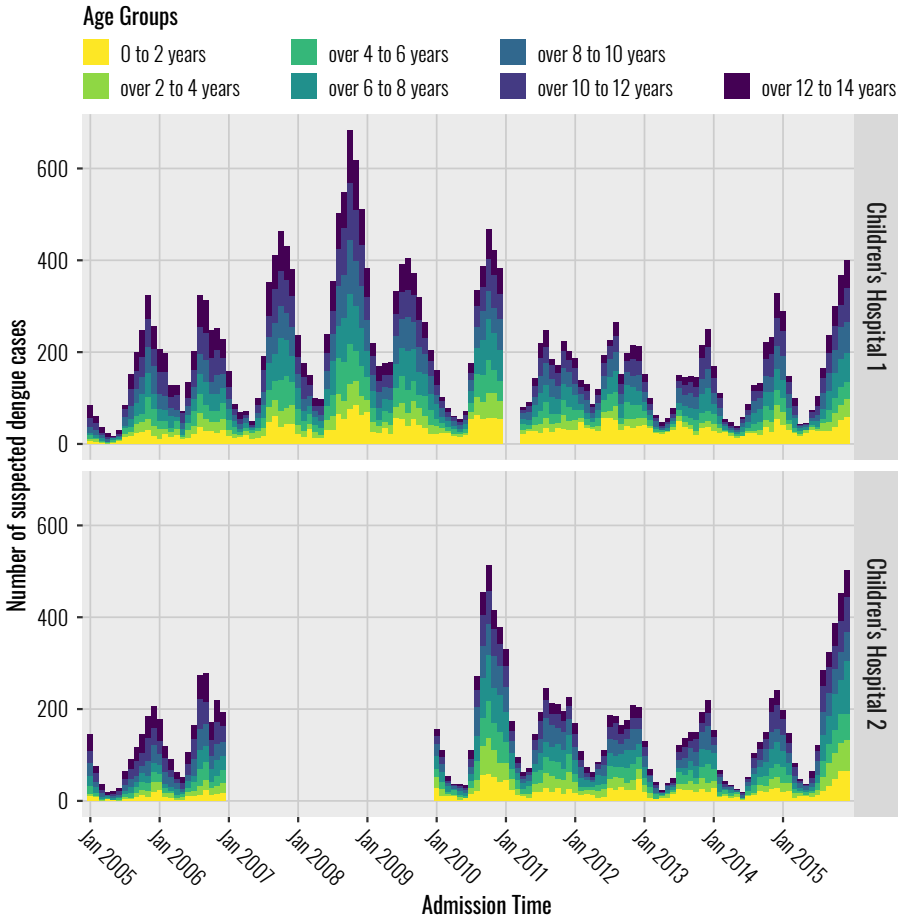


FIGURE 3.3 — Monthly number of cases in each 2-year age group observed at CH1 and CH2 between 2005 and 2015. This graph consists of 26,126 cases from CH1 and 14,705 cases from CH2. The case counts of CH1 between January and March 2011 and those of CH2 during the years 2007–2009 were ignored due to data incompleteness.

observed at CH2 was substantially lower than that of CH1, although the total number of cases at CH2 was only slightly lower than CH1. During 2005–2006, the proportion of infant cases contributing to the total case reports was much lower than that of the later years. In fact, the number of infant cases at CH2 during 2005–2006 was so low that the age distribution of the cases almost became unimodal (with only 1 peak at 12-14 years); however, this number of infant admissions was not lower than that of the 2–4 years age-group. While the data from CH1 showed that the admission rate of non-infants during 2007–2009 was the highest among all the periods of this study, the number of infant cases at CH1 during this period was not higher than those of the later periods. Across the years of study, the median age of non-infant cases at CH1 and CH2 varied to similar degrees (Table 3.3). From 2005 to 2012, the median

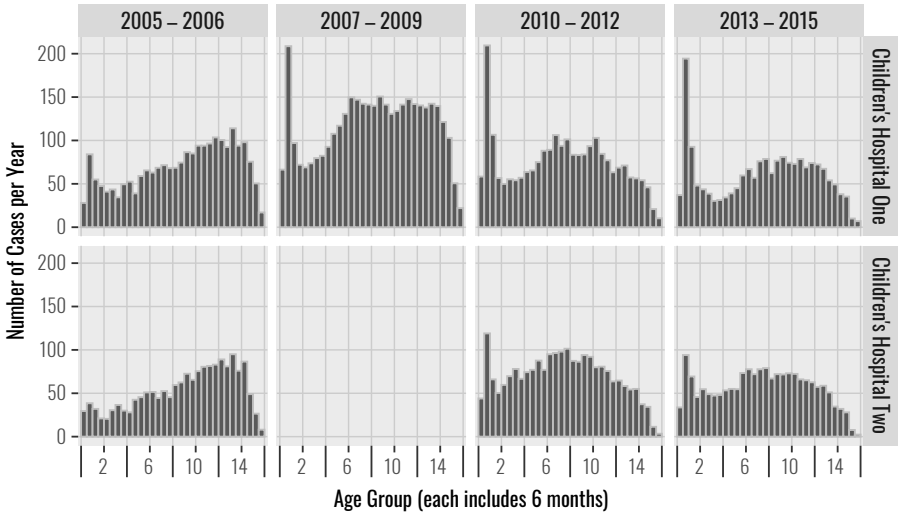


FIGURE 3.4 — The age distribution of the suspected dengue cases observed at CH1 and CH2 between 2005 and 2015. This graph only includes patients whose age information was available. Note that the period of 2005–2006 contains only 2 years while the other periods consist of 3 years. Therefore, for the sake of comparison between the periods, the histograms present the average number of cases observed per year during each period instead of showing the total case reports of the periods. The data from CH2 between 2007 and 2009 were excluded since many of the cases did not have age recorded. The case counts from CH1 in 2011 were scaled up by $1/(1-0.17)$ times to compensate for the missing data from the first 3 months of 2011 (since 0.17 were the average proportion that the first 3 months of each year contributed to the total number of the year).

age gradually decreased from around 9–10 years to 6.5–7.0 years; meanwhile, the years 2013–2015 showed a slightly reversed trend, with the median age of the cases increasing to 7–8 years in 2015.

A majority number of infant hospitalisations at both CH1 and CH2 occurred between 4 and 12 months of age (Figure 3.5). While a correlation test on the calendar month of birth and the calendar month of admission of the non-infant cases does not show evidence for association between these 2 factors (Kendall's rank correlation: 9×10^{-4}; p-value: 0.8283), a similar test on the infant cases indicates a strong correlation between the calendar month of birth and the calendar month of admission of infants (Kendall's rank correlation: −0.03; p-value: 0.0155). Grouping the infant cases by their calendar month of birth and calendar month of admission (Figure 3.6) reveals that the majority of infant cases was contributed by 2 groups: [1] individuals born in the beginning of a year (January–May) and hospitalised during the dengue season (August–December) of the same year, and [2] those who born at the end of a year (August–December) and hospitalised during the dengue season

TABLE 3.3 — The median age (in years) of all cases (0–14 years old) and non-infant cases (2–14 years old), who were admitted to CH1 and CH2 due to suspected dengue between 2005 and 2015. Data from CH2 between 2007 and 2009 were ignored as the age information was missing for many cases.

Admission Year	Children's Hospital One		Children's Hospital Two	
	All Cases	Non-infants	All Cases	Non-infants
2005	8.77	9.28	9.60	10.07
2006	8.67	9.42	9.12	9.50
2007	7.81	8.51	–	–
2008	7.95	8.67	–	–
2009	7.48	8.43	–	–
2010	7.01	7.92	7.05	7.69
2011	6.96	7.96	7.08	7.83
2012	6.42	7.91	7.09	7.99
2013	6.99	8.80	7.28	8.23
2014	7.43	8.74	7.18	8.03
2015	7.79	8.85	7.30	7.97

of the following year. This pattern was observed at both CH1 and CH2 across the years of study, except for 2005–2006 where the data of CH1 was missing and the number of infant cases at CH2 was insufficient for analysis.

3.3.2 *Model Fitting*

Among the 4 tested model configurations, only the `TypeSwtch-C` model (with serotype switching and complete primary protection) and the `TypeSynch-P` model (with serotype synchronicity and partial primary protection) led to converged MCMCs. The `TypeSwtch-P` model (which assumes serotype switching and partial primary protection) did not converge mainly due to the divergence in the estimation of the initial serotype distribution. Meanwhile, the `TypeSynch-C` model (with serotype synchronicity and complete primary protection) suffered from the indeterminability of the duration (δ) of the primary protection. This was caused by the fact that in `TypeSynch-C`, increasing δ and decreasing the transmission rate (β) of the serotypes would lead to a very similar effect, namely reducing the number of observed cases.

Generally, `TypeSwtch-C` and `TypeSynch-P` produce similar estimates for most of the parameters, with the duration of the primary protection

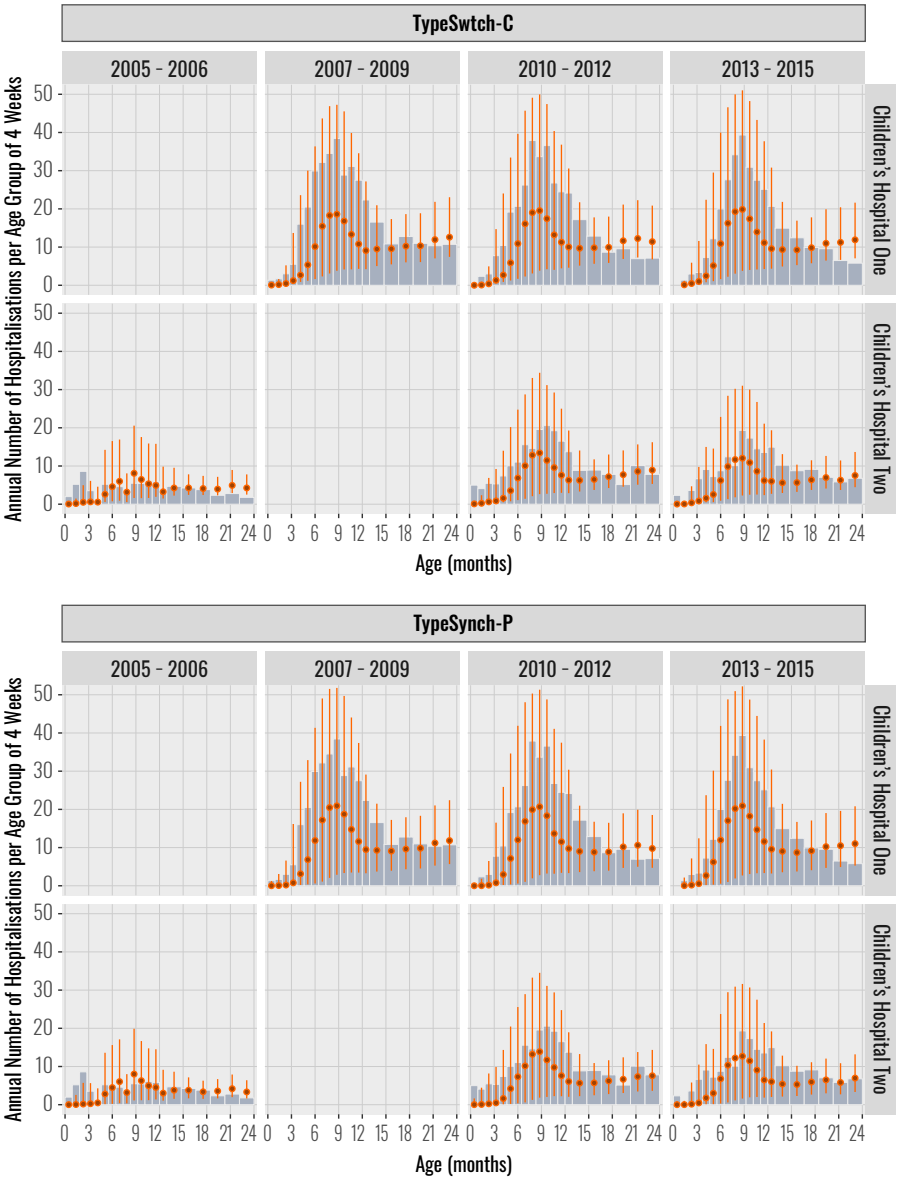


FIGURE 3.5 – The age distribution of the infant cases admitted to CH1 and CH2 across 4 periods: 2005–2006, 2007–2009, 2010–2012, and 2013–2015. The grey columns represent the observed annual numbers of infant hospitalisations (due to suspected dengue) contributed by each age group of 4 weeks.[42] Meanwhile, the orange dots and whiskers, respectively, indicate the median and the 95% CR of the corresponding numbers of simulated cases, generated by the models TypeSwtch-C (the 8 upper panels) and TypeSynch-P (the 8 lower panels). The data of CH1 in 2005–2006 and that of CH2 in 2007-2009 were excluded from this analysis due to heavy data incompleteness.

[42] Although the 6 rightmost columns in each panel represent age groups of 8 weeks (since the patients were categorised this way for the model fitting), the height of the columns were scaled down by half (as if they illustrated the number of cases of 4-week age groups) to give a better visualisation of the case density across ages.

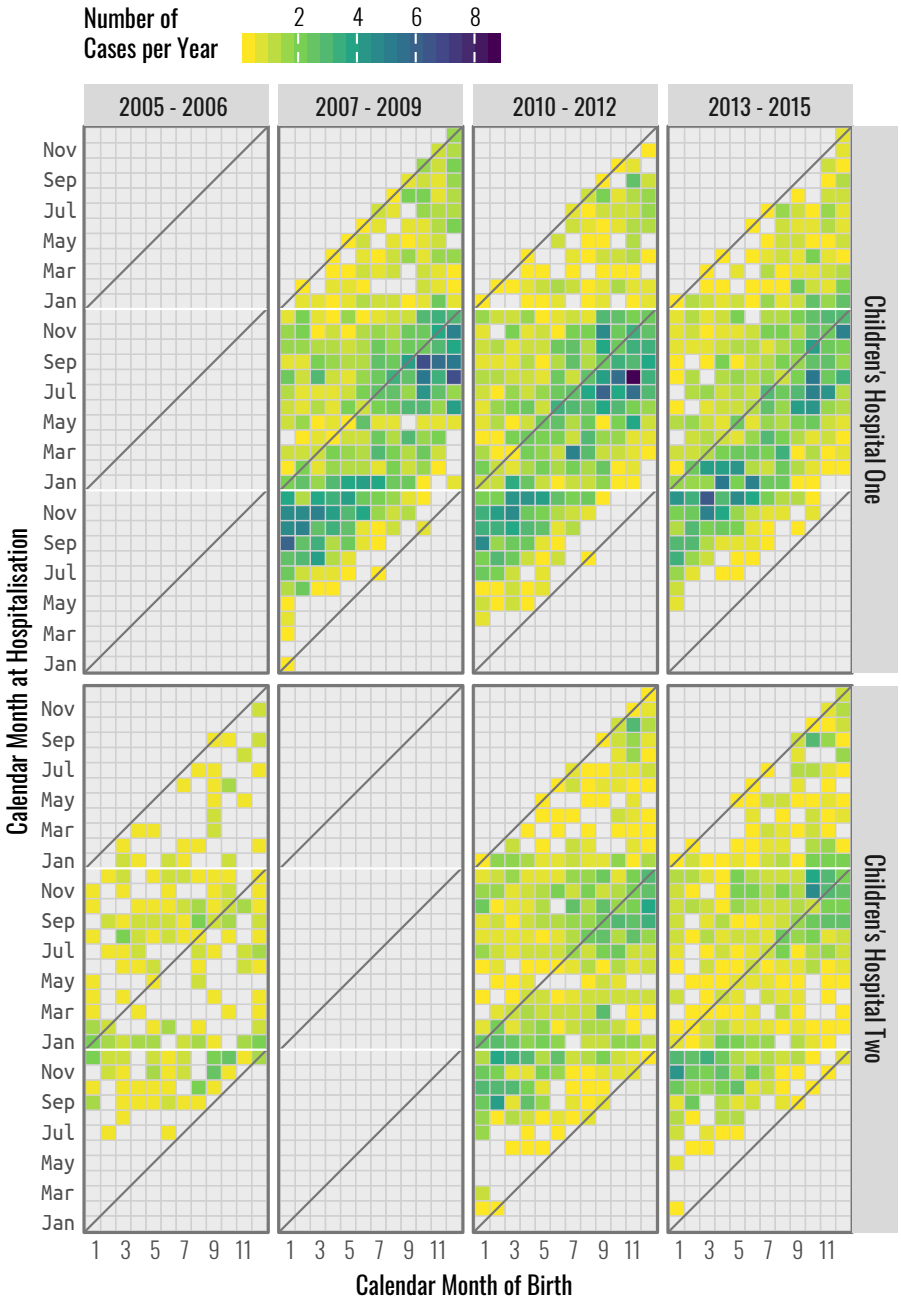


FIGURE 3.6 – The distribution of infant cases by their calendar month of birth and calendar month of admission. The numbers on the x-axis represent the month of birth of the patients. The y-axis indicates the calendar month that the cases were hospitalised. The colour scale represents the average annual number of cases admitted to CH1 (shown in the upper panels) and CH2 (shown in the lower panels) across 4 periods: 2005–2006, 2007–2009, 2010–2012, and 2013–2015. The three black diagonal lines in each panel respectively—from the lowest to the highest—mark the time points when the cases were born, and reached 1 year and 2 years of age.

(δ) being the only exception (Table 3.4). Both of the models consistently estimate that the seasonal transmission rate of dengue in January–April was around 0.39, which was followed by a 20% increase in May–August to peak at 0.47; this number then decreased to 0.44 in September–December. Despite a slight difference in the estimates of the hospitalisation rates (η_{inf} and η_{noninf}), both `TypeSwtch-C` and `TypeSynch-P` suggest that symptomatic infant cases were nearly twice as likely to be hospitalised than symptomatic non-infants. Without the involvement of maternal ADE, the probability that a primary infection becomes symptomatic was estimated to be small (0.2% in `TypeSwtch-C` and 0.5% in `TypeSynch-P`); however, maternal ADE could vastly increase the symptomatic rate of primary infections. Both of the converged models show that 95% of infants affected by maternal ADE were between 4.3 and 12.0 months of age (Figure 3.7). The peak age of maternal ADE was estimated to be around 8 months, at which the risk of symptomatic disease in maternally immune newborns increases by 46.2 times (95% CR: 3.7–309.1 times) as demonstrated by the `TypeSwtch-C` model, or by 15.8 times (95% CR: 1.5–286.2 times) as indicated by the `TypeSynch-P` model. Additionally, the models suggest that maternal immunity may protect infants from symptoms for the first 105–107 days of their life (95% CR: 5.5–267.2 days in `TypeSwtch-C` and 5.4–255.9 days in `TypeSynch-P`).

Since different assumptions were applied for the primary protection in the 2 models, they yield significantly dissimilar estimates for the duration of this protection. In `TypeSwtch-C`, a primary infection would be followed by only 58 days (95% CR: 44–73 days) of complete protection. Meanwhile, in `TypeSynch-P`, recovery from a primary infection would lead to a partial protection of 611 days (95% CR: 171–1683 days).

Although both `TypeSwtch-C` and `TypeSynch-P` successfully reconstruct the general epidemiological pattern of dengue in Hồ Chí Minh City, `TypeSwtch-C` visually fit the data better (Table 3.5, Figure 3.8). Both of the models produce good estimates for the number of infant cases across all the years of study. However, the goodness of fit is less consistent for the non-infant case counts, where the data show more year-to-year variation (as compared to the infant data). While both `TypeSwtch-C` and `TypeSynch-P` adequately capture the numbers of non-infant hospitalisations for most of the years, they slightly overestimate the peaks of 2011–2013 and underestimate those of 2007–2009. The

TABLE 3.4 – Median estimates (with 95% CRs, shown in brackets) of important epidemiological parameters, which were calculated from the TypeSwtch-C and the TypeSynch-P models. In both of these models, the secondary symptomatic probability, $\zeta^{(2)}$, was fixed at 0.41.

Parameter Description	Notation	TypeSwtch–C	TypeSynch–P
Dengue transmission rate in January–April	β	0.3921 (0.3812 ; 0.4094)	0.3993 (0.3875 ; 0.4083)
Dengue transmission rate in May–August	β	0.4690 (0.4582 ; 0.4810)	0.4775 (0.4697 ; 0.4877)
Dengue transmission rate in September–December	β	0.4372 (0.4289 ; 0.4494)	0.4419 (0.4357 ; 0.4482)
Infant hospitalisation rate	η_{inf}	0.2380 (0.1413 ; 0.4433)	0.3419 (0.1731 ; 0.7682)
Non-infant hospitalisation rate	η_{noninf}	0.1379 (0.1266 ; 0.1491)	0.1710 (0.1429 ; 0.2219)
Base primary symptomatic probability	$\zeta^{(1)}$	0.0022 (0.0002 ; 0.0155)	0.0049 (0.0001 ; 0.0378)
Protective duration of maternal immunity (days)	κ_{protect}	106.62 (5.51 ; 267.18)	104.71 (5.42 ; 255.89)
Peak age of maternal ADE (days)	κ_{peak}	244.29 (113.61 ; 374.97)	246.54 (111.80 ; 374.30)
SD of age of infants who experienced maternal ADE (days)	κ_{sd}	58.61 (32.73 ; 92.16)	58.66 (32.88 ; 91.92)
Maximum disease-enhancing effect of maternal ADE	ϕ_{max}	46.16 (3.69 ; 309.11)	15.82 (1.53 ; 286.25)
Duration of the primary protection (days)	δ	58.08 (43.75 ; 73.06)	611.15 (171.08 ; 1682.77)

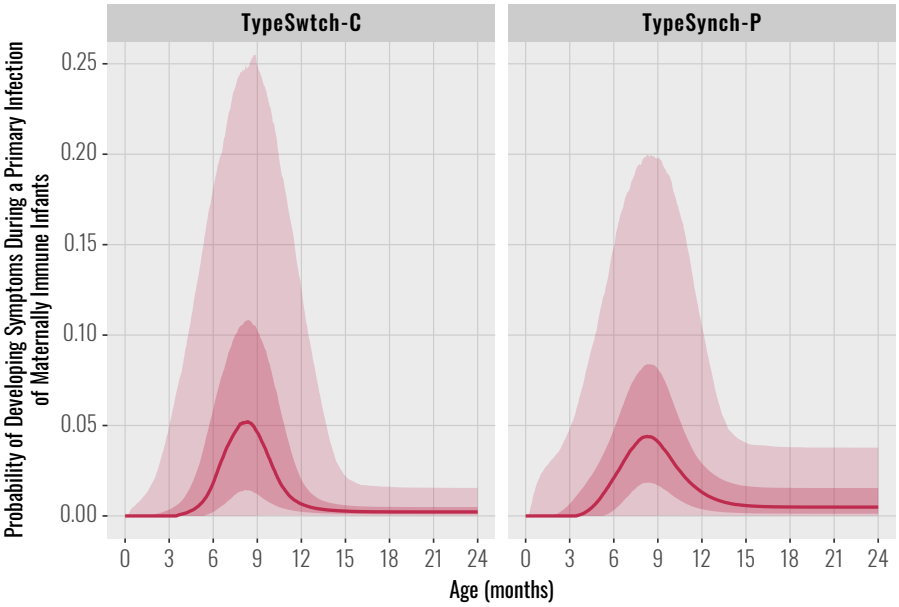


FIGURE 3.7 — Estimated probability of developing symptoms during a primary infection of infants who have maternal immunity to dengue. This probability is the product of the base primary symptomatic probability, $\zeta^{(1)}$, and the age-dependent effect of maternal immunity, $\phi(a)$. The left panel illustrates the result from the TypeSwtch-C model, which enforces serotype switching and complete primary protection. Meanwhile, the right panel shows the results from the TypeSynch-P model, which assumes serotype synchronicity and partial primary protection. The red lines are median estimates. The inner and outer shaded areas represent the 50% and the 95% CRs, respectively.

year 2010 clearly shows the discrepancy between the 2 models, with the simulated non-infant results of TypeSwtch-C closely resembling the data while TypeSynch-P greatly underestimates the case counts.

The results of this modelling analysis consistently suggest that the risk of hospitalisation (due to dengue) of infants in Hồ Chí Minh City strongly correlated with their calendar month of birth (Figure 3.9). In general, children who were born in February were most likely to experience a dengue hospitalisation during their infancy. Meanwhile, the risk was lowest for those born in July–October.

3.4 DISCUSSION

In this analysis, the patterns of suspected dengue cases at 2 paediatric hospitals in Hồ Chí Minh City were examined to cast light on the characteristics of paediatric dengue in Hồ Chí Minh City. This is the first study that attempts

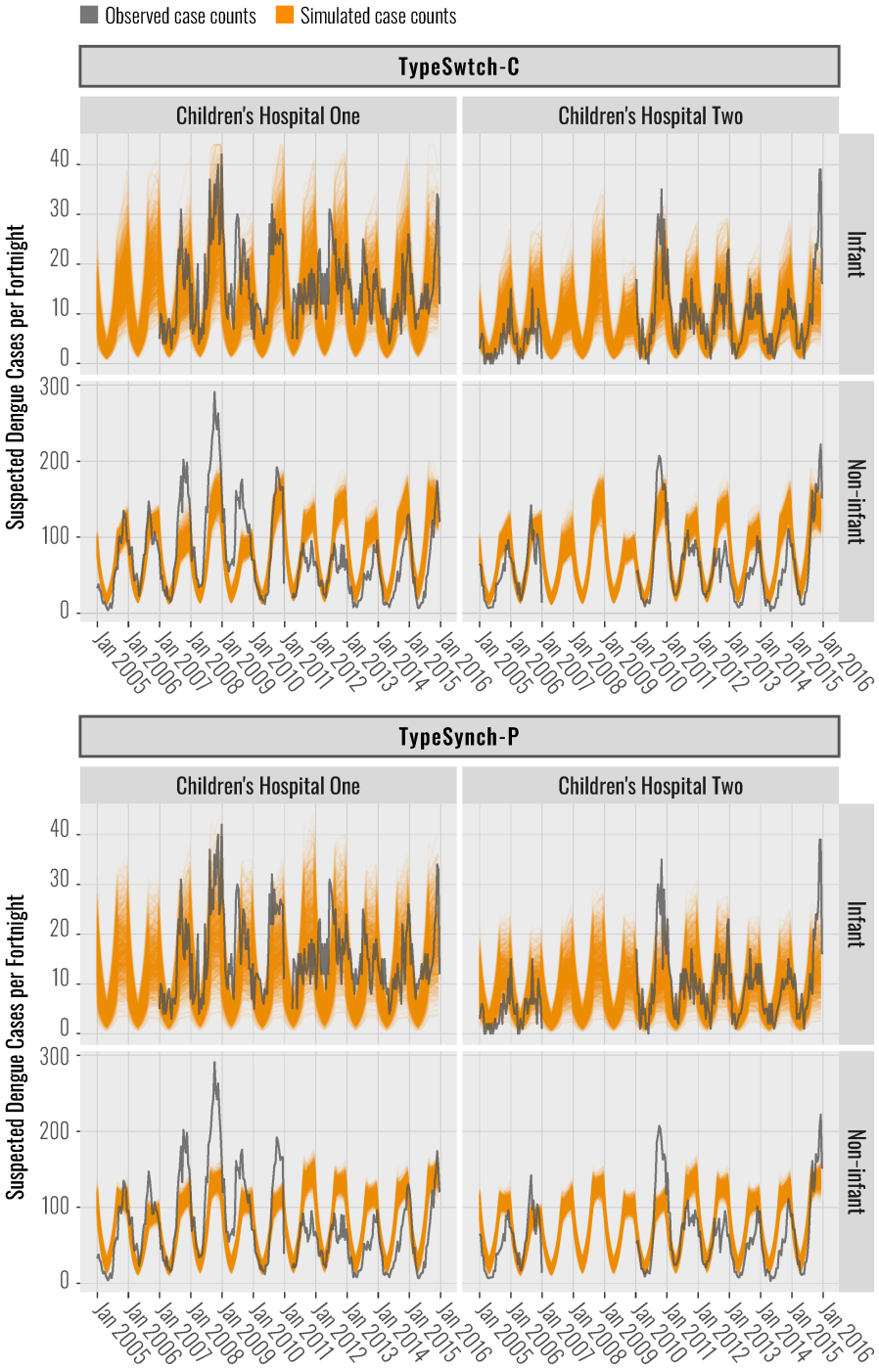


FIGURE 3.8 – The simulated (orange lines) and the observed (black lines) number of suspected dengue cases per fortnight. The simulated numbers of cases were generated from 1,000 posterior samples of the models TypeSwtch-C (the 4 upper panels) and TypeSynch-P (the 4 lower panels).

TABLE 3.5 — Median estimates (with 95% CR, shown in brackets) of the mean squared errors of the TypeSwtch-C and TypeSynch-P models. Each of these errors were calculated by comparing 1,000 simulated time series of fortnightly case counts from the models with the observed data from CH1 and CH2. The number of cases at each hospital were aggregated into 2 age groups: infant (below 2 years of age) and non-infant (between 2 and 14 years old). For the estimation of the mean errors, the case counts of CH1 and CH2 were used together as independent samples of equal weight.

Year	Infant		Non-infant	
	TypeSwtch-C	TypeSynch-P	TypeSwtch-C	TypeSynch-P
2005	14 (6 ; 67)	23 (8 ; 100)	552 (395 ; 830)	1077 (764 ; 1470)
2006	27 (9 ; 115)	12 (8 ; 58)	602 (380 ; 855)	628 (427 ; 884)
2007	48 (43 ; 111)	48 (42 ; 121)	3490 (3321 ; 3929)	3595 (3371 ; 3884)
2008	107 (100 ; 176)	107 (101 ; 171)	6888 (6656 ; 7493)	6777 (6654 ; 7017)
2009	83 (75 ; 112)	84 (75 ; 140)	3641 (3488 ; 3891)	3960 (3623 ; 4460)
2010	57 (34 ; 143)	85 (36 ; 189)	822 (559 ; 1359)	1936 (1456 ; 2439)
2011	30 (22 ; 95)	36 (25 ; 131)	785 (440 ; 1344)	1601 (1019 ; 2392)
2012	67 (50 ; 128)	63 (48 ; 132)	1740 (1092 ; 2872)	1493 (1014 ; 2149)
2013	28 (18 ; 70)	28 (19 ; 76)	1115 (641 ; 1773)	1289 (842 ; 1920)
2014	22 (15 ; 73)	23 (16 ; 71)	1552 (851 ; 2475)	1567 (1070 ; 2186)
2015	68 (48 ; 142)	69 (47 ; 155)	1360 (927 ; 2115)	1121 (820 ; 1520)
All	55 (48 ; 99)	57 (50 ; 108)	2081 (1923 ; 2322)	2292 (2126 ; 2518)

to quantify the epidemiological effect of maternal immunity, which has been the main theorised explanation for the high number of infant dengue cases in Vietnam and other highly endemic countries (Halstead *et al.* 2002). Furthermore, this is also the first time the link between infants' calendar months of birth and their risk of hospitalisation is systematically investigated. Despite a few discrepancies, CH1 and CH2 showed a very similar pattern of dengue hospitalisation with the number of cases plummeted in February–May and peaked in August–December of each year. This epidemiological cycle is consistent with the known seasonality of dengue in Hồ Chí Minh City (H. S. Lee *et al.* 2017), suggesting that the hospitals are robust indicators for dengue epidemics in the city.

Although the number of infant hospitalisations of CH1 was substantially higher than CH2 (which could have been due to the greater popularity of CH1's Infant Department, according to expert opinion), both hospitals

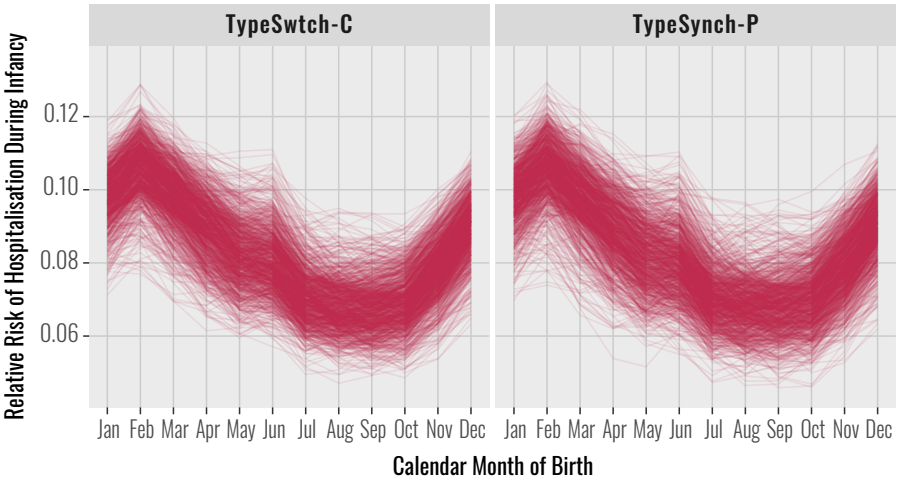


FIGURE 3.9 — Estimated relative risk of hospitalisation during the first 2 years of life of infants in Hồ Chí Minh City, given their calendar months of birth. Each of the 2,000 red lines represents a posterior sample from either the TypeSwtch-C model (left panel) or the TypeSynch-P model (right panel).

clearly showed that children of 2 years old and below contributed a significantly large proportion to the total number of hospitalisations. This high infant case count—which were consistently observed in past data of Vietnam and other highly endemic countries (Halstead *et al.* 2002)—may be a combined results of 2 risk factors, one is behavioural and the other is biological. They are: [1] the speculated preference of clinicians and parents to keep sick newborns at hospitals for better healthcare, and [2] the disease-enhancing effect of dengue maternal immunity. The results of my modelling analysis suggest that between these two, maternal immunity may have played a more important role. The behavioural factor above was estimated to have doubled the hospitalisation likelihood of infants as compared to older children of the same disease severity. Meanwhile, maternal ADE may have increased infants' risk of symptomatic disease—hence the risk of hospitalisation—during their first infection in life by up to 46 times. The finding that maternal immunity may protect newborns for the first 104–106 days and may enhance disease when they are 4–12 months old is strongly consistent with the results of a past *in vitro* study in Hồ Chí Minh City (Chau *et al.* 2009), suggesting that the link between maternal immunity and infants' risk of hospitalisation is likely to exist. The assumption that maternal immunity only protects infants from symptoms rather than from infection may not correctly model the real mechanism of the protection (which is still an unknown). However, the

correctness of this assumption should have little effect on the results of my analysis since the likelihood that infants experience an infection in the first 3–4 months of life is very small even without the involvement of maternal immunity.

The strong correlation between the calendar months of birth and calendar months of hospitalisation of the infant cases in this study suggest that may play an important role in shaping the dynamics of the infant case counts. In this study, I found that children born in February were the most likely to be hospitalised during infancy because they faced the peak of the dengue season (in August–December) while in the high-risk age window of maternal ADE (i.e. 4–12 months of age). Meanwhile, infants born in July–October were the least likely to experience hospitalisation since they were maternally protected during the first dengue season and were mostly out of the age window of maternal ADE in the next dengue season. Interestingly, since the number of births in Hồ Chí Minh City is the highest in July–October and the lowest in February, the number of infant hospitalisations may have been naturally minimised. In summary, infants born in different months may face different degree of risk and be likely to be hospitalised in different periods of the year. This finding may be useful for disease triage as date of birth is very accessible information.

The shifts in the median age of the non-infant cases at both CH1 and CH2 across the years of study may have caused by changes in the age structure of the population and/or alterations in epidemiological characteristics of dengue, as suggested by a previous study in Thailand (Cummings *et al.* 2009). Although it was not possible to thoroughly quantify the effect of demographic changes on the number of non-infant cases due to the lack of precise age-structure information in the census data,[43] it is likely that demographic factors would need a long time to take effect as the age structure of the population altered slowly across the years (Hồ Chí Minh City Statistical Office 2020). In other words, the observed shifts in the median age of the non-infant cases may have been affected more strongly by epidemiological factors of dengue, such as alterations in the overall transmission intensity or changes in the age-specific likelihood of hospitalisation. For instance, when a serotype that is more likely to cause severe primary infection gradually dominate the other serotypes, the proportion of the cases contributed

[43] A complete population census is only carried out every 5 years, and individuals are almost always sorted into age groups of 5 years.

by primary infections would increase, leading to a decrease in the median age of the cases (as primary infections are more likely to occur in younger age groups). Furthermore, serotype switching can also lead to alteration in the FOI since the susceptible population size of each serotype may have been different.

In this analysis, I tested 2 different assumptions for the primary protection. When this protection was assumed to block infection completely, its duration was estimated to be around 2 months. When the primary protection was assumed to only suppress symptoms, the estimated length became 611 days. Both of these estimates, although largely inconsistent with each other, are supported by previous studies. While past epidemiological studies estimated that the duration was around 1–3 years (K. B. Anderson *et al.* 2014; Reich *et al.* 2013), a human challenge study suggested that the protection may last for an average of 2 months (Ooi *et al.* 2014; Sabin 1952). This discrepancy in the estimation may have stemmed from the fact that in all the aforementioned studies, infections were only counted when individuals developed symptoms; therefore, no information was available to distinguish between the effect of a complete protection and that of a partial one.

The finding that my epidemiological model visually fitted to the data better when serotype switching was incorporated is consistent the speculation that serotype interaction may have a strong influence in shaping the dynamics of the transmission (Bosch *et al.* 2016; Lourenço and Recker 2013). However, even in the model `TypeSwtch-C`—where serotype switching was considered—the case counts of the years 2007–2009 were still vastly underestimated, possibly due to 3 reasons. Firstly, the reconstructed serotype dynamics may not be exact as were inferred purely based on the total number of cases, without serotype information. Secondly, because of the design of the model fitting method—in which the likelihood function puts more weight on the infant age groups—the model had a tendency of ignoring the non-infant data if that would improve the fit of the infant groups. Therefore, the increase in the number of cases in 2007–2009 was overlooked since the rise was mainly contributed by non-infants (the number of infant cases was relatively stable compared to the later years). Finally, the models in this analysis did not take into account the possibility that serotypes may have different symptomatic rates on different age groups, the phenomenon that has been

suggested by previous studies (Buchy *et al.* 2005; Clapham *et al.* 2015; Vaughn *et al.* 2000). Hence, underestimation would occur if the years 2007–2009 was dominated by a serotype that substantially increased the likelihood of disease in non-infants but not in infants.

Besides what has been mentioned above, the result of this analysis may have been affected by other minor limitations. Firstly, many of the dengue patients used for this study were only clinically diagnosed, meaning that a fraction of the cases must have been misdiagnosed. However, since most of the parameters of my models were inferred from the temporal trends of the case counts rather than from the absolute numbers of cases, misdiagnosis should not cause major problems as long as the misdiagnosis rate is consistent over time. Secondly, the assumption that primary symptomatic and secondary symptomatic cases have equal chance of hospitalisation if they are in the same age group may not be correct since secondary infections have been found to be more likely to develop severe symptoms (L. Bravo *et al.* 2014; Burke *et al.* 1988; Graham *et al.* 1999; Guzmán and Kourí 2002; Thein *et al.* 1997), and hence more likely to be hospitalised. The 0.2%–0.5% estimate of the probability of symptomatic disease during a primary infection—which is very low compared to the 13% finding of a previous analysis (Clapham *et al.* 2017)—may have been a result of the models trying to compensate for the equal hospitalisation rates. Finally, in my models, an equal transmissibility was assumed for all infected individuals regardless of their number of past infection nor their protection status. While this oversimplification may lead to under- or overestimations of the transmission rate and the FOI, I believe it does not have a major impact on the other parameters.

4

EVALUATING THE EFFECTS OF DENGUE VACCINATION ON THE NUMBER OF DENGUE HOSPITALISATIONS: A SIMULATION STUDY

4.1 INTRODUCTION

According to the suggestion—from recent modelling studies[44]—that routine vaccination may help to reduce the number of dengue hospitalisations in the long term when carried out in a high-transmission setting (Aguiar *et al.* 2016; Coudeville *et al.* 2018; Ferguson *et al.* 2016; Flasche *et al.* 2016), it may be worthwhile to consider vaccinating the population of Hồ Chí Minh City, where the transmission of dengue is high.[45] However, it should be noted that none of the past modelling studies above were specifically designed for Hồ Chí Minh City, hence they may neglect epidemiological factors that could affect the effectiveness of vaccination on this population. Furthermore, despite the prospect that screening is an unavoidable component of dengue vaccination if a *Dengvaxia*®-like vaccine is to be used (Wilder-Smith *et al.* 2018; World Health Organization 2018), and the high likelihood that the effectiveness—both economic and epidemiological—of vaccination programmes depend much on the misclassification rate of screening (España *et al.* 2019; Rodríguez-Barraquer *et al.* 2019), most past modelling studies assumed that screening either is not done (Coudeville *et al.* 2018; Flasche *et al.* 2016) or unrealistically has a sensitivity and a specificity of 100% (Aguiar *et al.* 2016).[46] This oversimplification leaves a great knowledge gap in understanding how a vaccination programme with a practical screening method may impact the population. Therefore, before vaccine deployment can happen in Hồ Chí Minh City, more in-depth risk-benefit analyses that are specifically tailored for this population and based on more realistic assumptions about screening are required.

[44] See §1.7.2 on page 26 for more details.

[45] The inference of CHAPTER 2 indicates that the seroprevalence of 9-year-olds in Hồ Chí Minh City is around 66.8% (see §2.3.2). This is close to the level of a high-transmission setting defined in the modelling studies above (i.e. 70%).

[46] While Ferguson *et al.* (2016) also estimated the effects of vaccination when screening has 90% sensitivity and specificity, this was done solely to demonstrate the potential benefits of pre-vaccination screening; screening performance was not a focus of the analysis.

The primary goal of this chapter is to develop a mathematical simulation to assess the possible impacts of routine dengue vaccination on the population of Hồ Chí Minh City, with averted hospitalisations used as the main proxy to measure the effectiveness of the vaccination programme. Compared to previous studies of the same type, this analysis comprised two major differences. Firstly, as this study was specifically designed for Hồ Chí Minh City, it was implemented to not only take into account demographic properties of the city but also incorporate epidemiological parameter estimates of the population (which were obtained from the inference of CHAPTER 3). Secondly, besides testing the scenarios that vaccination is done without screening and with perfect screening (i.e. 100% sensitivity and 100% specificity), the simulations in this chapter also looked into more realistic scenarios where the performance of screening was assumed to be the same as that of the indirect IgG ELISA used in CHAPTER 2. While the dengue vaccine in this exercise was assumed to have similar immunological characteristics to those of *Dengvaxia*®,[47] vaccine-related parameters were simulated with wide ranges of values to produce results that could be applicable to any *Dengvaxia*®-like vaccines. Both scenarios where the hypothetical vaccine can and cannot block infection were tested.[48] The model structure of this analysis was developed with reusability in mind so that it can be modified easily to simulate dengue vaccination in a different population.

[47] More detailed information on *Dengvaxia*® is provided in §1.5.2.1 on page 20.

[48] See §4.2.1.

4.2 METHODS

4.2.1 *The Simulated Vaccination Programme*

This analysis considered a routine vaccination programme that either includes or does not includes serological screening to assess whether potential vaccine recipients have prior exposures to dengue infection (Figure 4.1). The programme was modelled as a time-continuous process that alters the epidemiological status of the population. In the scenario where screening is incorporated, the following 3 major events—which were simulated instantaneously—would occur to the participants of the programme:

[1] Participants are screened for their dengue serostatus.

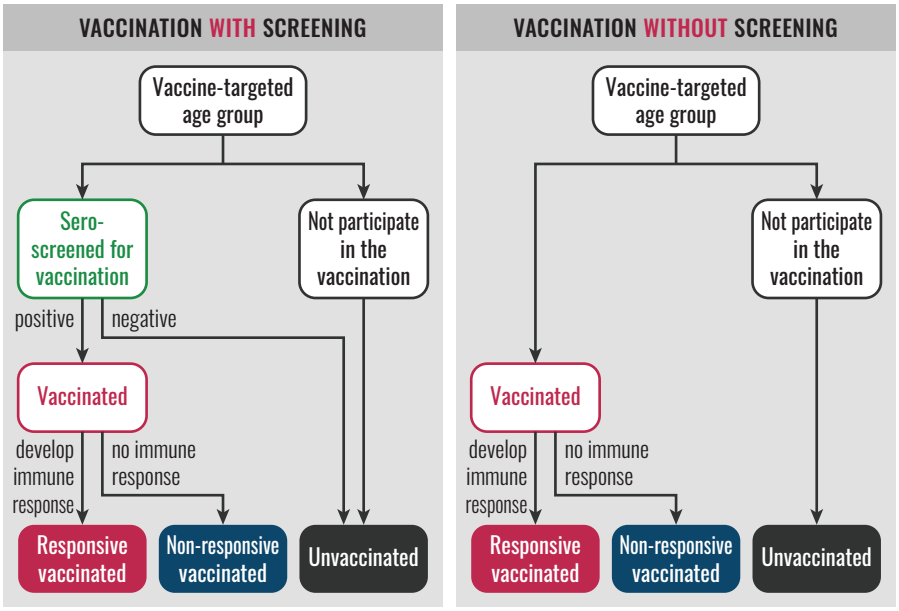


FIGURE 4.1 – The flowchart of the hypothetical vaccination programme, simulated in this analysis.

[2] If the sero-screening leads to positive results (which are indications of a high likelihood that the participants have experienced past dengue infections), the participants would be vaccinated; those with negative screening results would not be vaccinated.[49]

[3] After the vaccination a proportion of vaccinees develop immune response to the vaccine,[50] while the others do not.

This scenario assumes that the probability of being vaccinated of programme participants depends on their number of prior infections as the likelihood of positive screening results generally hinge on the number of past dengue infections that individuals have experienced.[51]

Meanwhile, in scenarios where sero-screening is not part of the vaccination programme, the first event in the list above is jettisoned and all participants would be vaccinated. For convenience, the groups of vaccinees that do and do not develop immune response after the vaccination are hereafter referred to as *responsive* and *non-responsive* individuals, respectively. Non-responsive and unvaccinated individuals were assumed to be immunologically indistinguishable; however they were classified in different groups to avoid re-vaccinating non-responsive vaccinees.

The vaccine in this analysis was assumed to resemble a few immunological characteristics of *Dengvaxia®*. That is, the vaccine-derived immunity

[49] Different assumptions about the accuracy of screening were simulated in this analysis. More details are provided in in §4.2.5 on page 110.

[50] This vaccine-derived immunity may be either protective or harmful.

[51] This argument is clearly supported by the analysis of CHAPTER 2.

would increase the risk of symptomatic disease during a primary infection and would protect vaccinees from developing symptoms during a secondary infection. For the purpose of simplicity, the risk that a responsive vaccinated individual become symptomatic during a primary infection was assumed to increase—instantaneously after the vaccination—to a level identical with that of an unvaccinated one during a secondary infection; the vaccine was assumed not to produce any short-term protection in naive recipients.[52] Additionally, secondary infections of both naive and non-naive responsive recipients were assumed to be completely asymptomatic. In other words, the immune status that naive responsive vaccinees (who are naive to dengue at the time of vaccination) acquire after recovering from a primary infection was assumed to be identical to that of those who are responsive vaccinated after having experience a primary infection. In this simulation, vaccine-derived immunity was assumed to persist for life. This assumption was made to avoid the need of modelling the waning of the vaccine-derived immunity, hence simplifying the models. However, it should be noted that this is not a common choice as most models simulating *Dengvaxia®* assumed a rapid waning rate of vaccine-induced immunity.[53]

To conveniently characterise the vaccination programme in this analysis, the following terminologies are used in the subsequent sections of this chapter:

PROGRAMME COVERAGE (or *coverage* for short, denoted by c_a) is the probability that individuals in a vaccine-targeted age group (a) participate in the vaccination programme. If the programme does not include seroscreening, this number also represents the proportion of the targeted age group that receive the vaccine. However, when screening is incorporated in the programme, the percentage of the targeted age group vaccinated against dengue would be lower than the coverage,[54] as individuals must have positive screening results to be vaccinated.

IMMUNO-EFFECTIVENESS of the vaccine (denoted by $\epsilon_{\mathcal{R}}$) is the probability that recipients are immunologically responsive to the vaccine.[55] This probability was assumed to be the same for all individuals in the population, regardless of their age and their serostatus.

[52] This assumption discriminates vaccination from a natural infection since a recovery from a primary infection was assumed to induce a short-lived heterotypic protection in unvaccinated individuals (see §4.2.2).

[53] See §1.7.1 on page 25 for more details.

[54] Note that this definition of coverage is different from that of a typical vaccination programme where all participants are vaccinated.

[55] In the context of a typical vaccination programme, this quantity is often referred to as '*vaccine efficacy*'. However, since efficacy usually has a semantic association with the protective effects of the vaccine, I chose to use a different term ('immuno-effectiveness') to emphasise the fact that the hypothetical vaccine in this study could also produce harmful effects (besides the protective ones).

TRANSMISSION-BLOCKING EFFECTIVENESS of the vaccine (denoted by $\epsilon_{\mathcal{T}}$) indicates the magnitude of transmission reduction induced by vaccination. In general, vaccines can block transmission in 2 different ways, either by protecting recipients from contracting infection or by suppressing the infectiousness of recipients when they are infected (so they do not transmit the disease). While these 2 mechanisms may lead to different effects at an individual level, their epidemiological impacts are likely to be indistinguishable if infected vaccinees are completely asymptomatic. Therefore, the second case of transmission-blocking (i.e. vaccines only suppress the infectiousness of recipients) was arbitrarily selected for the simulation of this analysis. Here, transmission-blocking effectiveness is defined as the probability that responsive vaccinees are non-infectious during their secondary infection (the vaccine was assumed not to affect the infectivity of recipients during a primary infection).

4.2.2 *Modelling Disease Transmission and Vaccination*

The epidemiological model used in this analysis (Figure 4.2) is an extended version of the model `TypeSynch-P`, introduced in CHAPTER 3.[56] This extended model also groups individuals into compartments based on their age group (denoted by a) and their infection statuses (i.e. susceptible—S, infected—I, and recovered—R). Additionally, these S, I, R compartments were further divided into subclasses of different vaccination categories, namely: unvaccinated ($\mathcal{U}$), responsive vaccinated ($\mathcal{V}r$), and non-responsive vaccinated ($\mathcal{V}n$). The transition of individuals between infection groups were modelled similarly to the original model. This means homotypic infections were only counted once and individuals were assumed to be fully protected from infection after 2 natural infections caused by different dengue serotypes. Furthermore, both unvaccinated and vaccinated individuals were allowed to be infected during their primary cross-protection period with a risk ratio of $\xi = 1$ as compared to a naive unvaccinated individual; however, all infections occurring during the primary protection period were assumed to be asymptomatic. Vaccination was assumed to only take place while individuals are not being infected (i.e. not belonging to an I compartment).[57]

[56] The analysis of CHAPTER 3 yielded to 2 converged models: `TypeSynch-P` (which assumes serotype synchronisation) and `TypeSwtch-C` (which allows serotype switching). However, `TypeSwtch-C` was not used as an initial model structure for this analysis since vaccination may alter inter-serotype interaction and lead to convoluted outcomes.

[57] The accuracy of this assumption should yield little effect on the results of this analysis since vaccination was modelled as a time-continuous process. In reality, individuals may still be vaccinated during an infection if they do not show any clinical symptoms.

The epidemiological model of this chapter is formalised by the following system of ordinary differential equations:

$$
\begin{aligned}
\frac{d}{dt}S_a^{(\mathcal{U},0)} &= -S_a^{(\mathcal{U},0)}\sum_{i=1}^{4}\lambda_i(t) - \frac{\nu(t)}{z_a}\,S_a^{(\mathcal{U},0)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\big(1-c_a\pi_0\big)\times S_{a-1}^{(\mathcal{U},0)} + \mathbb{1}_{a=1}\times B(t) \\
\frac{d}{dt}S_a^{(\mathcal{V}r,0)} &= -S_a^{(\mathcal{V}r,0)}\sum_{i=1}^{4}\lambda_i(t) - \frac{\nu(t)}{z_a}\,S_a^{(\mathcal{V}r,0)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\left[S_{a-1}^{(\mathcal{V}r,0)} + c_a\pi_0\epsilon_{\mathcal{R}}\times S_{a-1}^{(\mathcal{U},0)}\right] \\
\frac{d}{dt}S_a^{(\mathcal{V}n,0)} &= -S_a^{(\mathcal{V}n,0)}\sum_{i=1}^{4}\lambda_i(t) - \frac{\nu(t)}{z_a}\,S_a^{(\mathcal{V}n,0)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\left[S_{a-1}^{(\mathcal{V}n,0)} + c_a\pi_0\big(1-\epsilon_{\mathcal{R}}\big)\times S_{a-1}^{(\mathcal{U},0)}\right] \\
\frac{d}{dt}I_{a,i}^{(\mathcal{U},1)} &= \lambda_i(t)\,S_a^{(\mathcal{U},0)} - \gamma I_{a,i}^{(\mathcal{U},1)} - \frac{\nu(t)}{z_a}\,I_{a,i}^{(\mathcal{U},1)} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\,I_{a-1,i}^{(\mathcal{U},1)} \\
\frac{d}{dt}I_{a,i}^{(\mathcal{V}r,1)} &= \lambda_i(t)\,S_a^{(\mathcal{V}r,0)} - \gamma I_{a,i}^{(\mathcal{V}r,1)} - \frac{\nu(t)}{z_a}\,I_{a,i}^{(\mathcal{V}r,1)} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\,I_{a-1,i}^{(\mathcal{V}r,1)} \\
\frac{d}{dt}I_{a,i}^{(\mathcal{V}n,1)} &= \lambda_i(t)\,S_a^{(\mathcal{V}n,0)} - \gamma I_{a,i}^{(\mathcal{V}n,1)} - \frac{\nu(t)}{z_a}\,I_{a,i}^{(\mathcal{V}n,1)} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\,I_{a-1,i}^{(\mathcal{V}n,1)} \\
\frac{d}{dt}R_{a,i}^{(\mathcal{U},1)} &= \gamma I_{a,i}^{(\mathcal{U},1)} - \left[\frac{1}{\delta}+\xi\sum_{k\neq i}\lambda_k(t)\right]\times R_{a,i}^{(\mathcal{U},1)} - \frac{\nu(t)}{z_a}\,R_{a,i}^{(\mathcal{U},1)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\big(1-c_a\pi_1\big)\times R_{a-1,i}^{(\mathcal{U},1)} \\
\frac{d}{dt}R_{a,i}^{(\mathcal{V}r,1)} &= \gamma I_{a,i}^{(\mathcal{V}r,1)} - \left[\frac{1}{\delta}+\xi\sum_{k\neq i}\lambda_k(t)\right]\times R_{a,i}^{(\mathcal{V}r,1)} - \frac{\nu(t)}{z_a}\,R_{a,i}^{(\mathcal{V}r,1)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\left[R_{a-1,i}^{(\mathcal{V}r,1)} + c_a\pi_1\epsilon_{\mathcal{R}}\times R_{a-1,i}^{(\mathcal{U},1)}\right] \\
\frac{d}{dt}R_{a,i}^{(\mathcal{V}n,1)} &= \gamma I_{a,i}^{(\mathcal{V}n,1)} - \left[\frac{1}{\delta}+\xi\sum_{k\neq i}\lambda_k(t)\right]\times R_{a,i}^{(\mathcal{V}n,1)} - \frac{\nu(t)}{z_a}\,R_{a,i}^{(\mathcal{V}n,1)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\left[R_{a-1,i}^{(\mathcal{V}n,1)} + c_a\pi_1\big(1-\epsilon_{\mathcal{R}}\big)\times R_{a-1,i}^{(\mathcal{U},1)}\right] \\
\frac{d}{dt}I_{a,k}^{(\mathcal{U},\mathcal{P})} &= \xi\lambda_k(t)\sum_{i\neq k}R_{a,i}^{(\mathcal{U},1)} - \gamma I_{a,k}^{(\mathcal{U},\mathcal{P})} - \frac{\nu(t)}{z_a}\,I_{a,k}^{(\mathcal{U},\mathcal{P})} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\,I_{a-1,k}^{(\mathcal{U},\mathcal{P})}
\end{aligned}
$$

$$
\begin{aligned}
\frac{d}{dt} I_{a,k}^{(\mathcal{V}n,\mathcal{P})} &= \xi\lambda_k(t)\sum_{i\neq k} R_{a,i}^{(\mathcal{V}n,1)} - \gamma I_{a,k}^{(\mathcal{V}n,\mathcal{P})} - \frac{\nu(t)}{z_a}\, I_{a,k}^{(\mathcal{V}n,\mathcal{P})} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\, I_{a-1,k}^{(\mathcal{V}n,\mathcal{P})} \\
\frac{d}{dt} S_{a,i}^{(\mathcal{U},1)} &= \frac{1}{\delta}\, R_{a,i}^{(\mathcal{U},1)} - S_{a,i}^{(\mathcal{U},1)}\sum_{k\neq i}\lambda_k(t) - \frac{\nu(t)}{z_a}\, S_{a,i}^{(\mathcal{U},1)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\Big(1-c_a\pi_1\Big)\times S_{a-1,i}^{(\mathcal{U},1)} \\
\frac{d}{dt} S_{a,i}^{(\mathcal{V}r,1)} &= \frac{1}{\delta}\, R_{a,i}^{(\mathcal{V}r,1)} - S_{a,i}^{(\mathcal{V}r,1)}\sum_{k\neq i}\lambda_k(t) - \frac{\nu(t)}{z_a}\, S_{a,i}^{(\mathcal{V}r,1)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\left[S_{a-1,i}^{(\mathcal{V}r,1)} + c_a\pi_1\epsilon_{\mathcal{R}}\times S_{a-1,i}^{(\mathcal{U},1)}\right] \\
\frac{d}{dt} S_{a,i}^{(\mathcal{V}n,1)} &= \frac{1}{\delta}\, R_{a,i}^{(\mathcal{V}n,1)} - S_{a,i}^{(\mathcal{V}n,1)}\sum_{k\neq i}\lambda_k(t) - \frac{\nu(t)}{z_a}\, S_{a,i}^{(\mathcal{V}n,1)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\left[S_{a-1,i}^{(\mathcal{V}n,1)} + c_a\pi_1\Big(1-\epsilon_{\mathcal{R}}\Big)\times S_{a-1,i}^{(\mathcal{U},1)}\right] \\
\frac{d}{dt} I_{a,k}^{(\mathcal{U},2)} &= \lambda_k(t)\sum_{i\neq k} S_{a,i}^{(\mathcal{U},1)} - \gamma I_{a,k}^{(\mathcal{U},2)} - \frac{\nu(t)}{z_a}\, I_{a,k}^{(\mathcal{U},2)} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\, I_{a-1,k}^{(\mathcal{U},2)} \\
\frac{d}{dt} I_{a,k}^{(\mathcal{V}r,2)} &= \lambda_k(t)\sum_{i\neq k}\left[S_{a,i}^{(\mathcal{V}r,1)} + \xi R_{a,i}^{(\mathcal{V}r,1)}\right] - \gamma I_{a,k}^{(\mathcal{V}r,2)} - \frac{\nu(t)}{z_a}\, I_{a,k}^{(\mathcal{V}r,2)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\, I_{a-1,k}^{(\mathcal{V}r,2)} \\
\frac{d}{dt} I_{a,k}^{(\mathcal{V}n,2)} &= \lambda_k(t)\sum_{i\neq k} S_{a,i}^{(\mathcal{V}r,1)} - \gamma I_{a,k}^{(\mathcal{V}n,2)} - \frac{\nu(t)}{z_a}\, I_{a,k}^{(\mathcal{V}n,2)} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\, I_{a-1,k}^{(\mathcal{V}n,2)} \\
\frac{d}{dt} R_a^{(\mathcal{U},2)} &= \gamma\sum_{i=1}^{4}\left[I_{a,i}^{(\mathcal{U},2)} + I_{a,i}^{(\mathcal{U},\mathcal{P})}\right] - \frac{\nu(t)}{z_a}\, R_a^{(\mathcal{U},2)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\Big(1-c_a\pi_2\Big)\times R_{a-1}^{(\mathcal{U},2)} \\
\frac{d}{dt} R_a^{(\mathcal{V},2)} &= \gamma\sum_{i=1}^{4}\left[I_{a,i}^{(\mathcal{V}r,2)} + I_{a,i}^{(\mathcal{V}n,2)} + I_{a,i}^{(\mathcal{V}n,\mathcal{P})}\right] - \frac{\nu(t)}{z_a}\, R_a^{(\mathcal{V},2)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\left[R_{a-1}^{(\mathcal{V},2)} + c_a\pi_2\times R_{a-1}^{(\mathcal{U},2)}\right]
\end{aligned}
$$

where: S, I, and R, respectively, represent the classes of susceptible, infected, and recovered individuals;
the first superscript in the class notations indicates the vaccination status of the group, i.e. $\mathcal{U}$—unvaccinated, $\mathcal{V}r$—responsive vaccinated, $\mathcal{V}n$—non-responsive vaccinated, or $\mathcal{V}$—vaccinated (both responsive and non-responsive);
the second superscript either indicates the number of infections individuals have experienced (i.e. 0, 1, or 2) or shows that individuals are experiencing a secondary infection during the primary protection period (denoted by $\mathcal{P}$);
the first subscript in the class notations represents the age group of individuals;
the second subscript (if exists) indicates the last infecting serotype;

t	denotes time;
a	is the age group index;
i, k	denote dengue serotypes;
$\lambda_i(t)$	is the FOI of serotype i at time t;
γ	is the recovery rate of infected individuals;
c_a	represents the coverage of the age group a;
π_0, π_1, π_2	denote the conditional probability that participants of the vaccination programme are vaccinated, given their number of past dengue infection being zero, 1, or ≥ 2, respectively;[58]
$\nu(t)$	is the seasonal relative birth rate of the population at time t;
z_a	is the age-span of the age group a, measured in the number of days (in this model, $\sum_a z_a = 21{,}900$ days $\approx$ 60 years);
$B(t)$	is the total number of new births at time t;
$\epsilon_{\mathcal{R}}$	is the immuno-effectiveness of the vaccine;
δ	is the average duration of the primary protection period;

[58] When pre-vaccination screening is incorporated in the programme, π_0 can be interpreted as the false-positive rate of screening; meanwhile, π_1 and π_2 are the true-positive rates of screening in individuals with 1 and ≥ 2 prior infections, respectively.

ξ is the relative risk of infection for individuals that are in the primary protection period, as compared to unprotected susceptibles;[59]

$\mathbb{1}_x$ is an indicator function, i.e. $\mathbb{1}_x = \begin{cases} 1 & \text{if } x \text{ is true,} \\ 0 & \text{otherwise} \end{cases}$.

[59] Throughout this simulation analysis, ξ was fixed at 1.0.

In this simulation exercise, vaccination was modelled as if it were part of the ageing process, i.e. individuals are vaccinated when they age into the targeted age group. In a preliminary analysis, I also considered an alternative

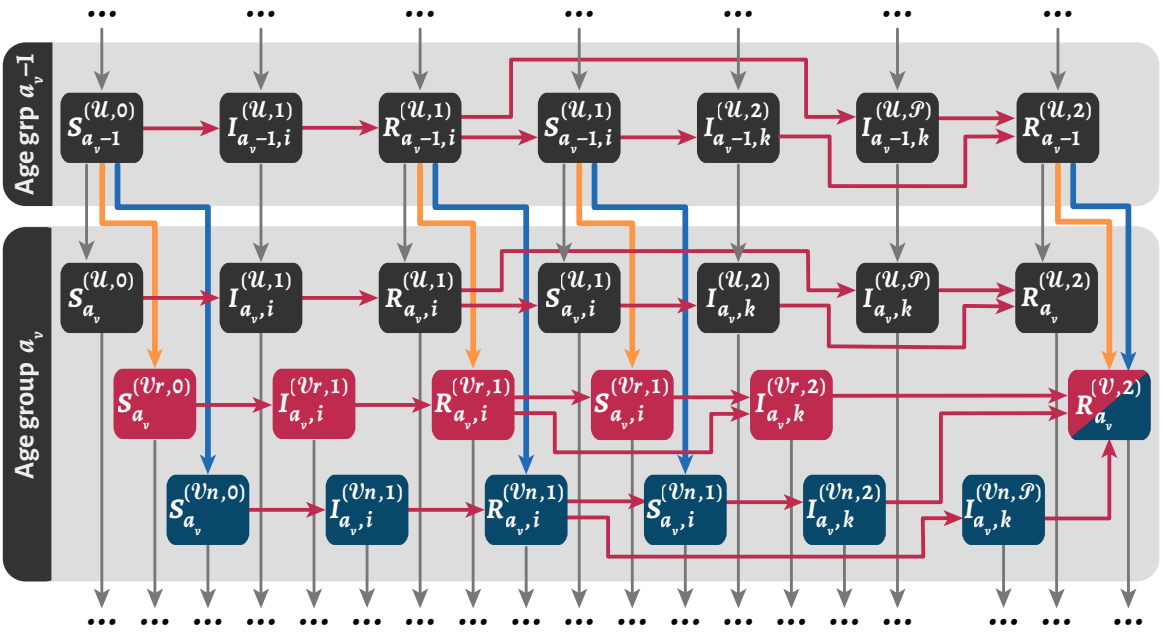


FIGURE 4.2 — The structure of epidemiological model used in this simulation study. The S, I, and R boxes in this diagram, respectively, represent the classes of susceptible, infected, and recovered individuals. The first superscript in the notation of each model compartment indicates the vaccination status of the group, i.e. $\mathcal{U}$—unvaccinated (black boxes), $\mathcal{V}r$—responsive vaccinated (red boxes), $\mathcal{V}n$—non-responsive vaccinated (blue boxes), or $\mathcal{V}$—vaccinated (half-red and half-blue box). The second superscript in the notations either indicates the number of infections individuals have experienced (i.e. 0, 1, or 2) or shows that individuals are experiencing a secondary infection during the primary protection period (denoted by $\mathcal{P}$). The first subscripts of the compartments represent the age group of individuals while the second subscripts (if exist) indicate the last infecting serotype. The red arrows in the graph represent epidemiological transitions caused infection and recovery. The grey arrows represent individuals that are ageing into a higher age group with their vaccination statuses unchanged. Meanwhile, the orange and the blue arrows respectively depict the proportion of individuals being responsive and non-responsive vaccinated when moving into the vaccine-targeted age group (a_v).

model design, in which vaccination was separated from ageing (Figure D.1). However, this alternative model posses a few mathematical disadvantages, and hence produced less reliable results than the one presented here.[60]

[60] See APPENDIX D on page 148 for more detailed explanation.

The FOI, $\lambda_i(t)$, of serotype i at a given time t was calculated based on the assumption that the infectivity is the same for all infected individuals except for responsive vaccinees who are experiencing a secondary infection, i.e. the $I_{a,i}^{(\mathcal{V}r,2)}$ compartment. For these $I_{a,i}^{(\mathcal{V}r,2)}$ individuals, the infectivity was assumed to be reduced by the transmission-blocking effectiveness ($\epsilon_{\mathcal{T}}$) of the vaccine. The calculation of $\lambda_i(t)$ is as follows:

$$\lambda_i(t) = \frac{\beta_i(t)}{N(t)} \times \sum_a \Bigg[I_{a,i}^{(\mathcal{U},1)} + I_{a,i}^{(\mathcal{U},2)} + I_{a,i}^{(\mathcal{U},\mathcal{P})} + I_{a,i}^{(\mathcal{V}n,1)} + I_{a,i}^{(\mathcal{V}n,2)} + I_{a,i}^{(\mathcal{V}n,\mathcal{P})} + I_{a,i}^{(\mathcal{V}r,1)} + \left(1 - \epsilon_{\mathcal{T}}\right) I_{a,i}^{(\mathcal{V}r,2)} \Bigg]$$

where: $\beta_i(t)$ represents the transmission rate of serotype i at time t;

$N(t)$ is the total population size at the time;

$\epsilon_{\mathcal{T}}$ is the transmission-blocking effectiveness of the vaccine;

the large sum after the fraction $\frac{\beta_i(t)}{N(t)}$ corresponds to the total number of infectious individuals of serotype i at the time.

To be consistent with the TypeSynch-P model, the following 3 assumptions about dengue infections were enforced on the extended model:

[1] All 4 dengue serotypes share an equal transmission rate ($\beta_1 = \beta_2 = \beta_3 = \beta_4 = \beta$) and an equal FOI ($\lambda_1 = \lambda_2 = \lambda_3 = \lambda_4$) at all time t.

[2] The transmission rate (β) has a seasonal pattern that is strictly preserved across the years (i.e. year-to-year variation is not allowed).

[3] All infections have an equal recovery rate (γ) of $1/7$.

For this analysis, the transmission rate of dengue (β) and the duration of the primary protection period (δ) were sampled from the corresponding posterior of the TypeSynch-P model (Table 4.1).

TABLE 4.1 — List of parameters in the simulation model. The values of the parameters were either fixed or sampled from a distribution, as indicated below.

	Parameter Description	Notation	Sampling Distribution or Fixed Value†
All Screening Scenarios	Dengue transmission rate in January–April	β	0.3993 (0.3875 ; 0.4083)
	Dengue transmission rate in May–August	β	0.4775 (0.4697 ; 0.4877)
	Dengue transmission rate in September–December	β	0.4419 (0.4357 ; 0.4482)
	Duration of the primary protection (days)	δ	611.15 (171.08 ; 1682.77)
	Primary symptomatic probability	$\zeta^{(1)}$	0.0049 (0.0001 ; 0.0378)
	Secondary symptomatic probability	$\zeta^{(2)}$	0.41
	Recovery rate	γ	$1/7$
	Coverage of the 9-year age group	c_9	50% or 70% or 90%
	Immuno-effectiveness of the vaccine	$\epsilon_{\mathcal{R}}$	40% or 60% or 80%
	Transmission-blocking effectiveness of the vaccine	$\epsilon_{\mathcal{T}}$	0 or 1
No Screening	Probability that naive participants are vaccinated	π_0	1
	Probability that participants with 1 prior infection are vaccinated	π_1	1
	Probability that participants with >1 prior infection are vaccinated	π_2	1
Perfect Screening	Probability that naive participants are vaccinated	π_0	0
	Probability that participants with 1 prior infection are vaccinated	π_1	1
	Probability that participants with >1 prior infection are vaccinated	π_2	1
Realistic Screening	Probability that naive participants are vaccinated	π_0	0.0005 (0.0001 ; 0.0042)
	Probability that participants with 1 prior infection are vaccinated	π_1	0.6031 (0.4872 ; 0.7162)
	Probability that participants with >1 prior infection are vaccinated	π_2	1.0 (1.0 ; 1.0)

† *A single number or a list of numbers separated by 'or' indicates that the parameter was fixed at the given value(s). Meanwhile, if the parameter was sampled from a distribution, the distribution is shown by its median value and its 2.5th–97.5th percentile range (presented in brackets, underneath the median). The values of β, δ, and $\zeta^{(1)}$ were sampled from the corresponding posterior distributions of the* TypeSynch-P *model in* CHAPTER 3. *For the realistic-screening scenario, the sampling distributions of π_0, π_1, and π_2 were calculated based on the results of the* HcmCon31 *model in* CHAPTER 2.

4.2.3 *Modelling Demographic Changes*

Compared to the original model of CHAPTER 3, the model in this analysis shares similar assumptions about the demographic dynamics of the population, namely: [1] disease-related deaths are ignored, [2] the ageing rate of each age group, a, is $\frac{\nu(t)}{z_a}$, and [3] individuals are removed from the system as soon as they reach 60 years of age. The only major difference is that the current model does not incorporate immigration (while the original does) since adding immigration into a vaccine simulation would require multiple assumptions on the vaccination status of immigrants, hence unnecessarily complicating the analysis. For the current model, the number of new births, $B(t)$, that are added into the population at a given time t was modelled as a sum of 2 products:

$$B(t) = \frac{\nu(t)}{z_A} \times \left\{ \sum_{q \in \{\mathcal{U},\, \mathcal{V}_r,\, \mathcal{V}_n\}} \left[S_A^{(q,0)} + \sum_i \left(I_{A,i}^{(q,1)} + R_{A,i}^{(q,1)} + S_{A,i}^{(q,1)} + I_{A,i}^{(q,2)} \right) \right] + \sum_i \left(I_{A,i}^{(\mathcal{U},\mathcal{P})} + I_{A,i}^{(\mathcal{V}_n,\mathcal{P})} \right) + R_A^{(\mathcal{U},2)} + R_A^{(\mathcal{V},2)} \right\} + N(t) \times g(t)$$

where:
- $\nu(t)$ represents the seasonal relative birth rate of the population at time t;
- z_A is the age-span (measured in the number of days) of the oldest age group (denoted by A) in the model;
- q denotes individuals' vaccination status;
- i denotes serotype;
- $N(t)$ is the total population size at time the time;
- $g(t)$ is the *daily* natural increase rate of the population;[61]

The first product in the sum above, i.e. $\frac{\nu(t)}{z_A} \times \{\cdots\}$, is equal to the number of individuals that reach the maximum allowed age in the model (i.e. 60 years) and are removed from the system. Meanwhile, the second product in the sum, $N(t) \times g(t)$, is the number of additional births that are needed to match the natural growth rate of the population.

[61] This daily growth rate was also converted from the annual rate (reported in the population census), using the same method presented in CHAPTER 3. See §3.2.3.2 on page 71 for more details.

Since Hồ Chí Minh City was the focal population in this analysis, all the demographic parameters above were fixed based on the census data of the city. More specifically, the size of the population at the beginning of the vaccination was set at $N(0) = 9{,}038{,}600$, based on the data of 2019[62] (Hồ Chí Minh City Statistical Office 2020). The annual natural increase rate of the population was fixed at 1.04%, which is approximately the average annual natural increase rate of Hồ Chí Minh City between the years 2010 and 2019 (Hồ Chí Minh City Statistical Office 2020). Meanwhile, the same monthly relative birth rates that were calculated in §3.2.3.2[63] were reused for this analysis. These parametrisations were chosen to mimic the current demographic conditions of Hồ Chí Minh City, so that the result from the simulation could be consider as a prediction for the impacts of vaccination on the population if the vaccine deployment were just started very recently (in 2019).

[62] The most recent population census of Hồ Chí Minh City was conducted in 2019.

[63] See page 71.

4.2.4 *Calculating the Percentage of Hospitalisations Averted by Vaccination*

Since it is not known how dengue vaccination would affect infants' risk of symptomatic disease and hospitalisation (indirectly via maternally transferred immunity), the number of averted hospitalisations was only calculated for the older age groups (who are above 2 years of age). For the purpose of simplicity, this exercise assumes that the ratio between the number of hospitalisations and the number of symptomatic cases in the population is a constant that is not influenced by vaccination. Therefore, the percentage of hospitalisations that is prevented by vaccination (Δ_ψ) is approximately the percentage of symptomatic cases averted by the vaccine:

$$\Delta_\psi = \frac{\psi_\mathcal{N} - \psi_\mathcal{V}}{\psi_\mathcal{N}} \times 100$$

with $\psi_\mathcal{V}$ and $\psi_\mathcal{N}$ being the simulated numbers of symptomatic cases in the population where vaccines are and are not deployed, respectively.

The total number of symptomatic cases of a simulation scenario (with or without vaccination) was estimated by the following formula:

$$\psi = \sum_a \sum_i \left[\zeta^{(1)} \times \left(I_{a,i}^{(\mathcal{U},1)} + I_{a,i}^{(\mathcal{V}n,1)} \right) + \zeta^{(2)} \times \left(I_{a,i}^{(\mathcal{U},2)} + I_{a,i}^{(\mathcal{V}n,2)} + I_{a,i}^{(\mathcal{V}r,1)} \right) \right]$$

where: a denotes age;

i denotes serotype;

$\zeta^{(1)}$ is the probability that unvaccinated individuals become symptomatic during a primary infection;

$\zeta^{(2)}$ is the probability that unvaccinated individuals become symptomatic during a secondary infection;

$I_{a,i}^{(\mathcal{U},1)}$, $I_{a,i}^{(\mathcal{V}n,1)}$, $I_{a,i}^{(\mathcal{V}r,1)}$ are, respectively, the numbers of primary infections by serotype i occurring in unvaccinated, non-responsive vaccinated, and responsive vaccinated individuals of age group a;

$I_{a,i}^{(\mathcal{U},2)}$, $I_{a,i}^{(\mathcal{V}n,2)}$ are the numbers of secondary infections by serotype i occurring in unvaccinated and non-responsive vaccinated individuals of age group a, respectively.

In the equation above, the quantities $I_{a,i}^{(\mathcal{U},1)}$, $I_{a,i}^{(\mathcal{V}n,1)}$, $I_{a,i}^{(\mathcal{V}r,1)}$, $I_{a,i}^{(\mathcal{U},2)}$, and $I_{a,i}^{(\mathcal{V}n,2)}$ were generated by the epidemiological model. The primary symptomatic rate, $\zeta^{(1)}$, was sampled from the corresponding posterior of the model `TypeSynch-P` in CHAPTER 3 (Table 4.1). Meanwhile, the secondary symptomatic rate, $\zeta^{(2)}$ was fixed at 0.41, as it was also fixed at the same value in `TypeSynch-P`, based on the finding of Clapham *et al.* (2017).

In this analysis, averted hospitalisation was summarised in 2 different ways to thoroughly assess both the instantaneous and the long-term risks and benefits of vaccination:

[1] The *annual* averted hospitalisation is the percentage of hospitalisations prevented by vaccination *within a given year*.

[2] Meanwhile, the *cumulative* averted hospitalisation is the percentage of hospitalisations prevented *since the beginning of the vaccination programme up to a given year*.

4.2.5 *Simulation Scenarios*

This simulation study tested 54 vaccination scenarios, which are combinations of 2 different values of the transmission-blocking effectiveness ($\epsilon_{\mathcal{T}}$) with 3 qualities of the immuno-effectiveness ($\epsilon_{\mathcal{R}}$), 3 options of coverage (c_a), and 3 pre-vaccination screening strategies. Since it is unknown whether dengue vaccines (including *Dengvaxia®*) can reduce transmission, the transmission-blocking effectiveness of the hypothetical vaccine was simulated as either *complete* (i.e. all responsive vaccinees are not infectious during their secondary infection, $\epsilon_{\mathcal{T}} = 1$) or *non-existent* (i.e. the vaccine does not alter individuals' infectivity at all, $\epsilon_{\mathcal{T}} = 0$).[64] Meanwhile, the immuno-effectiveness of the vaccine ($\epsilon_{\mathcal{R}}$) was varied among 3 values—i.e. 40%, 60%, and 80%—which were chosen to roughly cover the efficacy range of *Dengvaxia®*.

As the serological inference of CHAPTER 2 suggests that 9-year-olds in Hồ Chí Minh City may be suitable targets for dengue vaccination, the simulations in this chapter also assume that only the age group of 9 years would be vaccinated. This was implemented by setting a coverage value of 50%, 70%, or 90% for the 9-year-olds (c_9) while fixing the coverage of the other age groups at zero. For convenience, in the subsequent text, the term 'coverage' is used to implicitly refer to the coverage of the 9-year age group only.

The 3 simulated screening scenarios were: [1] vaccination *without screening* (no screening, the worst-case scenario), [2] vaccination *with perfect screening* (the best-case scenario), and [3] vaccination *with realistic screening* (a realistic scenario). These scenarios were modelled by altering the parameters π_0, π_1, and π_2[65]—which denote the conditional probability that programme participants are vaccinated, given their number of prior infections being zero, 1, or ≥ 2, respectively. Firstly, for the no-screening scenario, all the probabilities π_0, π_1, and π_2 were fixed at 1, resulting in vaccinating all programme participants regardless of their number of past dengue infections. Secondly, the perfect-screening scenario assumes that the accuracy of the pre-vaccination screening is 100% (i.e. both the false-positive and the false-negative rates of the screening method are zero), hence participants would be vaccinated

[64] Although moderate values of transmission-blocking effectiveness ($0 < \epsilon_{\mathcal{T}} < 1$) can also be tested, they was excluded to reduce the complexity of the analysis.

[65] π_0, π_1, and π_2 are defined in §4.2.2 on page 100.

if and only if they have experienced prior dengue infections. This perfect-screening scenario was modelled by fixing $\pi_0 = 0$ and $\pi_1 = \pi_2 = 1$. Finally, the realistic-screening scenario assumes that the IgG ELISA used in CHAPTER 2 of this thesis is employed for the pre-vaccination screening, and that the probability of receiving the vaccine of participants is identical to the probability of their ELISA results being above 10 Panbio® unit.[66] The scenario was simulated by sampling the probabilities π_0, π_1, and π_2 from the seronegative, the primary-seropositive, and the secondary-seropositive IgG-level distributions of Hồ Chí Minh City, which were inferred by the model `HcmCon31` in CHAPTER 2. It is worth to note that this implementation led to low values (near zero) of π_0 and very high values (close to 1) of π_2 (Table 4.1); therefore, the major different between the realistic-screening and the perfect-screening strategies was π_1, namely the chance that participants with 1 prior infection get vaccinated (while perfect screening assumed $\pi_1 = 1$, realistic screening assumed that π_1 ranges between 0.49 and 0.72).

[66] 10 Panbio® unit was the cut-off used in the analysis of CHAPTER 2. See §2.2.3.1 on page 38 for how the value was chosen.

To generate a baseline for the assessment of the vaccination scenarios, simulations of dengue epidemics without vaccine deployment were also carried out. These no-vaccine simulations were implemented by fixing the programme coverage at zero while maintaining the other parameters at identical values to those in the vaccination scenarios. Each of the epidemiological scenarios above (both with and without vaccination) was evaluated based on the 20-year results of 1,000 simulations, with the initial conditions of each simulation roughly mimicking the estimated epidemiological and demographic state of Hồ Chí Minh City at the beginning of the year 2019. In the subsequent sections, simulation results are reported with their median values followed by the 95% uncertainty intervals (UIs, which are the range between the 2.5th and the 97.5th percentiles of the simulation estimates).

This analysis was implemented as a `Julia` project (Bezanson *et al.* 2017), which employed the `DifferentialEquations.jl` package (Rackauckas and Nie 2017) for solving differential equations. The complete source code of the analysis is accessible at: `https://gitlab.com/lamhm/DengueVacSim`.

4.3 RESULTS

According to my simulations, without vaccine interventions, dengue epidemics in Hồ Chí Minh City would lead to around 51,888–96,602 symptomatic cases per year (Figure C.1). This annual number was estimated to have a general upward trend across the year due to population growth. A routine vaccination programme that includes screening and targets 9-year-olds was estimated to reduce this number substantially in the long run, regardless of the transmission-blocking effectiveness of the vaccine.

4.3.1 *Without Transmission-blocking Effectiveness* ($\epsilon_{\mathcal{T}} = 0$)

In scenarios where the vaccine does not induce any transmission-blocking effect ($\epsilon_{\mathcal{T}} = 0$), the simulations show that the number of hospitalisations in Hồ Chí Minh City would still be reduced if vaccination is carried out with screening; however, administering the vaccine without prior screening was estimated to yield little or no benefit in lowering the number of hospitalisations (Figure 4.3 and Figure 4.4). In the scenarios of realistic screening and perfect screening, both the cumulative and the annual averted hospitalisations were found to increase over time (with slight fluctuations) and be positively correlated with the immuno-effectiveness ($\epsilon_{\mathcal{R}}$) of the vaccine and the coverage of the programme. With a low immuno-effectiveness of 40% and a minimal coverage of only 50%, the cumulative averted hospitalisations still reached 3.46% (95% UI: 2.83%–4.13%) in the scenario of realistic screening, or 5.67% (95% UI: 5.30%–5.92%) in the scenario of perfect screening by the 20^{th} year of the vaccination. When the immuno-effectiveness is 80% and the coverage is 90%, 20 years of vaccination with realistic screening prevented 12.09% (95% UI: 9.78%–14.46%) of the hospitalisations; meanwhile, vaccination with perfect screening led to 20.00% (95% UI: 18.70%–20.91%) of hospitalisations averted.

When screening was not part of the vaccination programme, a slight reduction in the annual number of hospitalisations was still observed at the second year of the programme (Figure 4.4) since the number of symptomatic secondary cases reduced by vaccination was higher than the increase of symptomatic primary infections caused by vaccinating naive individuals.

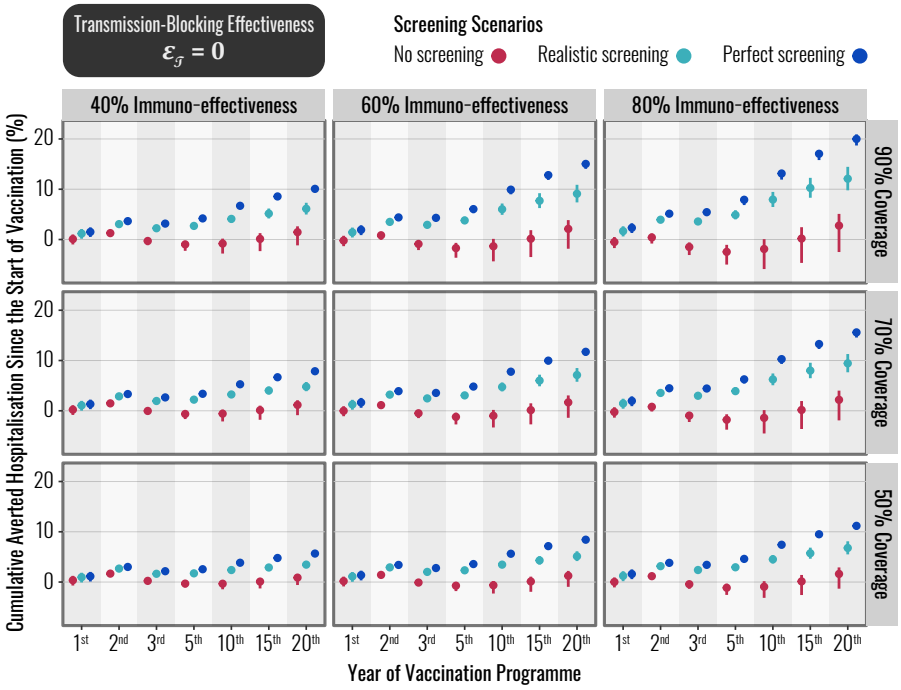


FIGURE 4.3 — The *cumulative* percentage of hospitalisations (since the start of the vaccination programme) that would be prevented if the vaccine does not block transmission ($\epsilon_{\mathcal{J}} = 0$). Negative percentages indicate increases in the number of hospitalisations. The dots and the whiskers, respectively, represent the median value and the 95% UI of the corresponding estimates, each of which was generated from 1,000 simulations.

However, negative numbers of averted hospitalisations were estimated for the later years—especially between the third and the 15$^{\text{th}}$ year of the programme—since the number of naive recipients experiencing a symptomatic primary infection as well as the percentage of hospitalisations contributed by naive vaccinees consistently increased over time (Figure C.8). While the magnitude of this negative effect was small when the coverage and the immuno-effectiveness of the vaccine were low, the annual number of hospitalisations increased by as much as 5.61% in the scenario where the immuno-effectiveness was 40% and the coverage was 50%. Meanwhile, with 80% immuno-effectiveness and 90% coverage, vaccination without screening raised the annual number of hospitalisations by up to 10.28%. After the 15$^{\text{th}}$ year, this negative effect slowly diminished since the number of averted symptomatic secondary infections kept increasing while the increased contribution of infected naive vaccinees to hospitalisations started to saturate (at 36.0% [95% UI: 33.6%–39.6%] in the scenario of 80% immuno-effectiveness

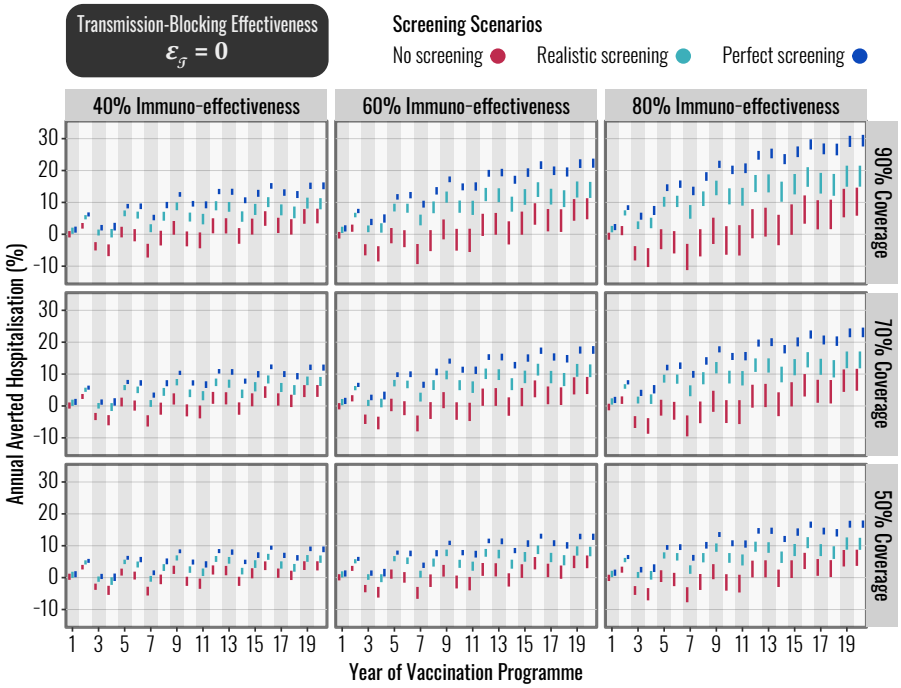


FIGURE 4.4 – The *annual* percentage of hospitalisations that would be prevented if the vaccine does not block transmission ($\epsilon_{\mathcal{J}} = 0$). Negative percentages indicate increases in the number of hospitalisations. Each colour bar in the plots represents the 95% UI of the corresponding estimate, which was generated from 1,000 simulations.

and 90% coverage, or at 9.5% [95% UI: 8.7%–10.9%] for the scenario of 40% immuno-effectiveness and 50% coverage, as calculated for the 15th year).

The number of vaccines given to the population each year—while not influenced by the immuno-effectiveness of the vaccine—was clearly driven by the programme coverage and the screening strategy (Table 4.2 and Figure C.3). As expected, higher coverages led to more individuals being vaccinated. For the same coverage, realistic screening resulted in the fewest vaccinees while no screening yields the highest number. For a population of size of Hồ Chí Minh City, the number of vaccines required to achieve a coverage of 90% in the first year of the vaccination was estimated to be 227,058 (95% UI: 227,010–227,107) for the no-screening scenario, 135,576 (95% UI: 134,008–136,855) for perfect screening, and 101,610 (95% UI: 91,408–111,466) for realistic screening. In all the tested scenarios, the annual numbers of vaccines given to the population gradually rose across the years—due to population growth—with similar rates that added up to a total increase of between 17.5% and 19.5% in 20 years. The proportion of vaccines given to each group of serostatus (i.e. naive to dengue, having 1 prior infection, and

TABLE 4.2 – The median estimates and the 95% UIs (in brackets) of the number of vaccines required each year (calculated for the first and the 20th year of the vaccination programme) to meet a specified level of coverage (Cov.), given the screening scenario and the immuno-effectiveness (IE) of the vaccine. The vaccine was assumed to have no transmission-blocking effect in this analysis ($\epsilon_{\mathcal{T}} = 0$).

	Cov. (%)	Number of Vaccines (×1,000) Deployed in the 1st Year			Number of Vaccines (×1,000) Deployed in the 20th Year		
		40% IE	60% IE	80% IE	40% IE	60% IE	80% IE
No Screening	50	126.1 (126.1; 126.1)	126.1 (126.1; 126.1)	126.1 (126.1; 126.1)	150.7 (150.6; 150.8)	150.7 (150.6; 150.8)	150.7 (150.6; 150.8)
	70	176.6 (176.6; 176.6)	176.6 (176.6; 176.6)	176.6 (176.6; 176.6)	211.1 (211.0; 211.2)	211.1 (211.0; 211.2)	211.1 (211.0; 211.2)
	90	227.1 (227.0; 227.1)	227.1 (227.0; 227.1)	227.1 (227.0; 227.1)	271.4 (271.3; 271.5)	271.4 (271.3; 271.5)	271.4 (271.3; 271.5)
Realistic Screening	50	56.5 (50.8; 61.9)	56.5 (50.8; 61.9)	56.5 (50.8; 61.9)	66.6 (60.2; 73.0)	66.6 (60.2; 73.0)	66.6 (60.2; 73.0)
	70	79.0 (71.1; 86.7)	79.0 (71.1; 86.7)	79.0 (71.1; 86.7)	93.2 (84.2; 102.1)	93.2 (84.2; 102.1)	93.2 (84.2; 102.1)
	90	101.6 (91.4; 111.5)	101.6 (91.4; 111.5)	101.6 (91.4; 111.5)	119.8 (108.3; 131.3)	119.8 (108.3; 131.3)	119.8 (108.3; 131.3)
Perfect Screening	50	75.3 (74.5; 76.0)	75.3 (74.5; 76.0)	75.3 (74.5; 76.0)	89.0 (88.7; 89.3)	89.0 (88.7; 89.3)	89.0 (88.7; 89.3)
	70	105.5 (104.2; 106.4)	105.5 (104.2; 106.4)	105.5 (104.2; 106.4)	124.6 (124.2; 125.1)	124.6 (124.2; 125.1)	124.6 (124.2; 125.1)
	90	135.6 (134.0; 136.9)	135.6 (134.0; 136.9)	135.6 (134.0; 136.9)	160.3 (159.7; 160.8)	160.3 (159.7; 160.8)	160.3 (159.7; 160.8)

having $\geq$2 prior infections) was constant over the years of vaccination and was neither affected by the coverage nor the immuno-effectiveness of the vaccine (Figure C.4). When screening was included in the vaccination process, none or very few of naive individuals were vaccinated (as π_0 was fixed at zero in perfect screening, or sampled from a close-to-zero distribution in realistic screening). However, this group of individuals were estimated to take up 40.85% (95% UI: 39.73%–41.56%) of the vaccines if screening is not done. The proportion of vaccinees having 1 prior infection was highest with perfect screening (median estimate: 63.99%; 95% UI: 63.17%–64.50%) and lowest without screening (median estimate: 37.85%; 95% UI: 37.67%–38.07%). Meanwhile, realistic screening was estimated to result in 51.74% (95% UI: 46.33%–56.14%) of the vaccines being used on individuals with 1 past infection. The proportion of vaccines spent on recipients with $\geq$2 prior

infections were estimated to be 21.75% (95% UI: 21.22%–22.20%) for the no-screening scenario, 36.43% (95% UI: 35.95%–36.83%) for perfect screening, and 48.64% (95% UI: 44.34%–53.65%) for realistic screening. Despite these different percentages, the absolute number of vaccines given to individuals with ≥ 2 infections was estimated to be identical regardless of screening scenarios (since everyone in this group of recipients was vaccinated in all the scenarios).

4.3.2 *With Complete Transmission-blocking Effectiveness ($\epsilon_{\mathcal{T}} = 1$)*

Compared to the scenario of no transmission-blocking effectiveness (i.e. $\epsilon_{\mathcal{T}} = 0$), vaccination was found to prevent more hospitalisations when the transmission-blocking effectiveness of the vaccine was complete (i.e. $\epsilon_{\mathcal{T}} = 1$). In this scenario, vaccination still led to positive numbers of cumulative averted hospitalisations even if screening was not included (Figure 4.5). The proportion of hospitalisations averted by vaccination was positively correlated with the coverage and the immuno-effectiveness of the vaccine. With a 40% immuno-effectiveness and a 50% coverage, by the 20$^{\text{th}}$ year, the proportion of cumulative hospitalisation prevented by vaccination was 8.78% (95% CI: 7.90%–9.07%) with perfect screening, 5.41% (95% UI: 4.42%–6.33%) with realistic screening, and 6.23% (95% UI: 3.75%–7.35%) without screening. Meanwhile, with an immuno-effectiveness of 80% and a coverage of 90%, the estimate was 31.08% (95% UI: 28.37%–31.58%) for perfect screening, 18.26% (95% UI: 14.72%–21.64%) for realistic screening, and 20.58% (95% UI: 12.57%–23.96%) for no screening.

Differently from the scenario of zero transmission-blocking effectiveness, when complete transmission suppression was assumed (i.e. $\epsilon_{\mathcal{T}} = 1$), the annual averted hospitalisations did not demonstrate a general upward trend but rather widely fluctuated, with negative values in some years even for perfect screening (Figure 4.6). In these simulations, the number of averted hospitalisations quickly increased in the first 2 years of the programme since the vaccine considerably lowered the risk of infection in the population by suppressing the infectiousness of responsive recipients during their secondary infection. However, as the FOI decreased, the number of susceptible

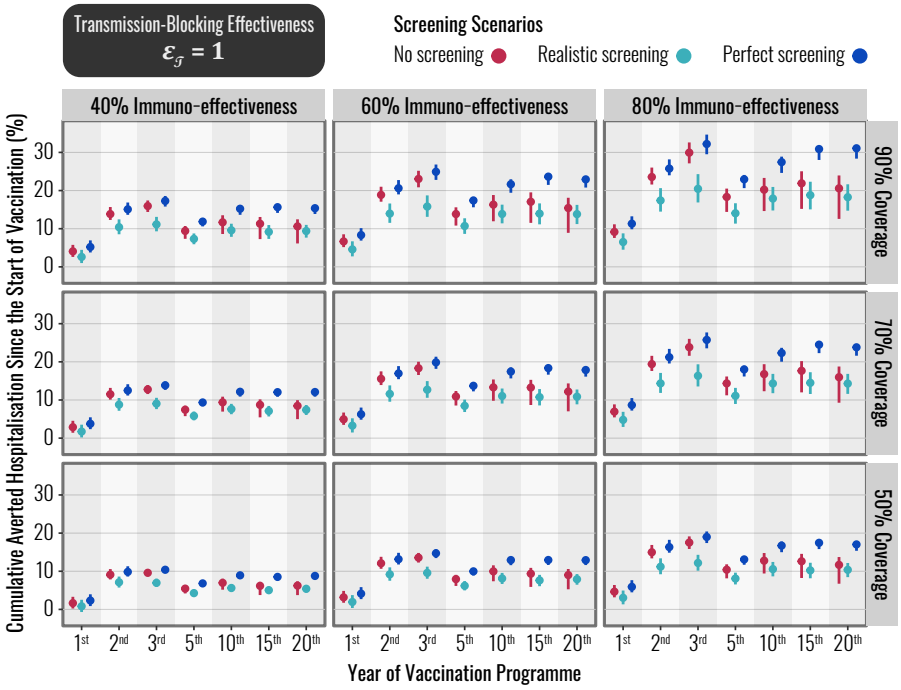


FIGURE 4.5 — The *cumulative* percentage of hospitalisations (since the start of the vaccination programme) that would be prevented if the vaccine completely blocks transmission during the secondary infection of vaccinees ($\epsilon_{\mathcal{J}} = 1$). Negative percentages indicate increases in the number of hospitalisations. The dots and the whiskers, respectively, represent the median value and the 95% UI of the corresponding estimates, each of which was generated from 1,000 simulations.

individuals gradually stacked up over the years. By the third year of the programme, the susceptible population became so large that the small risk of infection could still generate a substantial number of new infections, hence the FOI rebounded, so did the number of hospitalisations. This downturn in the averted hospitalisations was maintained only for 2 years until the high FOI depleted the susceptible population. Then, the FOI was brought down again, and the 4-year cycle of ups and downs in the number of averted hospitalisations repeated. Across the 20 years of the simulations, the number of annual averted hospitalisations climaxed at the years 2, 6, 10, 14, 18 and dropped to a nadir at the years 4, 8, 12, 16, 20. The difference between these peaks and troughs was generally wider when the vaccine immuno-effectiveness and the coverage were higher.

When transmission suppression was assumed ($\epsilon_{\mathcal{J}} = 1$), vaccination without screening also led to a continuous surge in the proportion of hospitalisations accounted by naive vaccinees (Figure C.8). Comparing between the 2 scenarios of transmission-blocking effectiveness ($\epsilon_{\mathcal{J}} = 0$ and $\epsilon_{\mathcal{J}} = 1$),

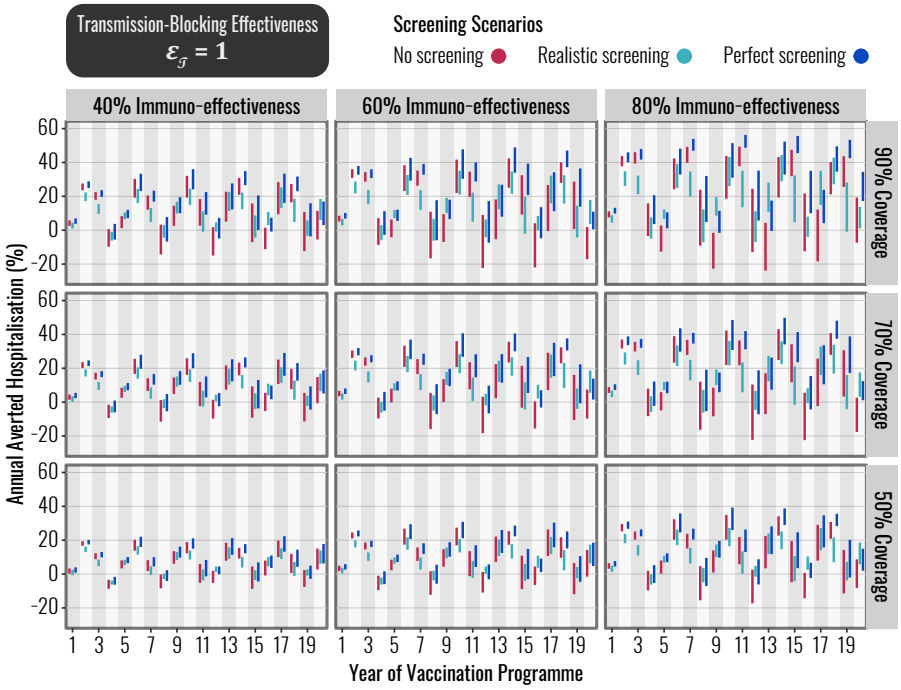


FIGURE 4.6 – The *annual* percentage of hospitalisations that would be prevented if the vaccine completely blocks transmission during the secondary infection of vaccinees ($\epsilon_{\mathcal{J}} = 1$). Negative percentages indicate increases in the number of hospitalisations. Each colour bar in the plots represents the 95% UI of the corresponding estimate, which was generated from 1,000 simulations.

the increase in the absolute number of naive vaccinees experiencing a symptomatic primary infection was generally similar, although more year-to-year variation was estimated when $\epsilon_{\mathcal{J}} = 1$. However, the proportion of annual hospitalisations accounted by naive vaccinees grew faster with $\epsilon_{\mathcal{J}} = 1$, especially when the vaccine immuno-effectiveness and the coverage were high. By the 20$^{\text{th}}$ year of an unscreened vaccination programme, with an immuno-effectiveness of 40% and a coverage of 50%, naive vaccinees were estimated to account for 11.03% (95% UI: 10.09%–12.94%) of the annual hospitalisations. Meanwhile, the proportion was estimated to be 48.62% (95% UI: 46.38%–52.09%) when the immuno-effectiveness was 80% and the coverage was 90%.

Surprisingly, vaccination without screening—which led the worst results when $\epsilon_{\mathcal{J}} = 0$—was found to cumulatively produce more averted hospitalisations than vaccination with realistic screening when $\epsilon_{\mathcal{J}} = 1$ (Figure 4.5). However, in terms of annual results, vaccination without screening was still

the only strategy that could yield substantially negative averted hospitalisations in some years. This negative effect was amplified when the coverage and the immuno-effectiveness of the vaccine were higher. For instance, when the vaccine immuno-effectiveness was 40% and the coverage was 50%, the lowest estimated level of annual averted hospitalisation was −8.68%. Meanwhile, with an immuno-effectiveness of 80% and a coverage of 90%, annual averted hospitalisation could reach as low as −23.88%.

The total number of vaccines required each year to meet a given coverage was clearly associated with the chosen screening strategy (Table 4.3 and Figure C.6). More specifically, vaccination without screening demanded the highest number of vaccines while vaccination with perfect screening required the lowest number. In all the tested scenarios, the annual number of vaccines needed to be deployed increased over the years. When vaccination was carried out without screening, the annual number of vaccines was estimated to rise by 19.5% in 20 years. Although the vaccine immuno-effectiveness and the coverage did not affect this growth rate in the scenario of no screening, they did alter the number when screening was part of the vaccination process. In other words, a higher immuno-effectiveness and a greater coverage resulted in a smaller rise in the annual number of vaccines. Additionally, the increase was estimated to be slighter for perfect screening (between 4.6% and 15.6% in 20 years) than for realistic screening (between 9.6% and 15.9% in 20 years).

The proportion of vaccine shots given to each sero-group of vaccinees was strongly affected by the screening strategies (Figure C.7). By design, almost no naive individual received the vaccine when realistic screening or perfect screening was simulated. However, conducting vaccination without screening resulted in 40.3% (95% UI: 39.7%–41.0%) of the vaccines in the first year being given to naive recipients (regardless of the immuno-effectiveness and the coverage). This number gradually increased over time with a rate that was positively correlated with the vaccine immuno-effectiveness and the coverage. By the 20^{th} year of the vaccination (without screening), naive individuals accounted for between 43.6% (with 40% immuno-effectiveness and 50% coverage; 95% UI: 43.4%–44.0%) and 54.1% (with 80% immuno-effectiveness and 90% coverage; 95% UI: 53.6%–54.4%) of the total annual number of vaccine shots. When screening was included in the vaccination, more than

TABLE 4.3 — The median estimates and the 95% UIs (in brackets) of the number of vaccines required each year (calculated for the first and the 20th year of the vaccination programme) to meet a specified level of coverage (Cov.), given the screening scenario and the immuno-effectiveness (IE) of the vaccine. In this analysis, the vaccine was assumed to completely block the transmission during the secondary infection of effectively vaccinated individuals ($\epsilon_{\mathcal{T}} = 1$).

	Cov. (%)	Number of Vaccines (×1,000) Deployed in the 1st Year			Number of Vaccines (×1,000) Deployed in the 20th Year		
		40% IE	60% IE	80% IE	40% IE	60% IE	80% IE
No Screening	50	126.1 (126.1; 126.1)	126.1 (126.1; 126.1)	126.1 (126.1; 126.1)	150.8 (150.7; 150.9)	150.8 (150.7; 150.9)	150.8 (150.7; 150.9)
	70	176.6 (176.6; 176.6)	176.6 (176.6; 176.6)	176.6 (176.6; 176.6)	211.0 (211.0; 211.2)	211.0 (211.0; 211.2)	211.0 (211.0; 211.2)
	90	227.1 (227.0; 227.1)	227.1 (227.0; 227.1)	227.1 (227.0; 227.1)	271.4 (271.3; 271.5)	271.4 (271.3; 271.5)	271.4 (271.3; 271.5)
Realistic Screening	50	56.5 (51.1; 61.9)	56.5 (51.1; 61.9)	56.5 (51.1; 61.9)	65.5 (59.3; 71.6)	65.0 (58.9; 71.0)	64.4 (58.4; 70.3)
	70	79.1 (71.5; 86.7)	79.1 (71.5; 86.7)	79.1 (71.5; 86.7)	91.1 (82.5; 99.5)	90.0 (81.6; 98.2)	88.6 (80.6; 96.3)
	90	101.7 (91.9; 111.3)	101.7 (91.9; 111.3)	101.7 (91.9; 111.3)	116.3 (105.5; 127.1)	114.2 (103.9; 124.3)	111.5 (102.0; 120.6)
Perfect Screening	50	75.4 (74.5; 76.1)	75.4 (74.5; 76.1)	75.4 (74.5; 76.1)	87.2 (86.7; 87.6)	86.1 (85.4; 86.7)	84.7 (83.8; 85.5)
	70	105.5 (104.3; 106.5)	105.5 (104.3; 106.5)	105.5 (104.3; 106.5)	120.9 (119.9; 121.6)	118.1 (116.9; 119.3)	114.5 (113.3; 115.8)
	90	135.5 (134.0; 136.8)	135.5 (134.0; 136.8)	135.5 (134.0; 136.8)	153.6 (152.0; 154.8)	147.9 (146.4; 149.6)	141.8 (141.0; 142.9)

half of the vaccinees (46.3%–59.8% for realistic screening, 63.2%–69.6% for perfect screening) were individuals with 1 prior infection. Meanwhile, this sero-group only accounted for 34.1%–37.8% of the vaccinees when screening was not done. Across the years, the proportion of vaccines given to recipients with 1 prior infection slightly increased for the realistic screening and the perfect screening scenarios, but marginally decreased for the no-screening scenario. At the first year of the programme, the proportion of vaccinees that have experienced ≥ 2 prior infections were estimated to be 21.78% (95% UI: 21.28%–22.23%) for no screening, 48.70% (95% UI: 44.43%–53.72%) for realistic screening, and 36.46% (95% UI: 36.00%–36.87%) for perfect screening. Over the years of the vaccination, this proportion decreased with a rate that was positively correlated with the immuno-effectiveness of the vaccine and

the coverage of the programme. For example, at the 20th year, with a 40% immuno-effectiveness and a 50% coverage, the proportion fell to 18.93% (95% UI: 18.66%–19.15%) in the no-screening scenario, to 47.32% (95% UI: 42.99%–52.53%) in realistic screening, and to 34.77% (95% UI: 34.50%–34.99%) in perfect screening. Meanwhile, a 60% immuno-effectiveness in combination with a 90% coverage was found to drop the percentage to 11.99% (95% UI: 11.79%–12.26%) when screening was not done, to 45.04% (95% UI: 40.05%–50.75%) with realistic screening, and to 30.61% (95% UI: 30.37%–30.93%) with perfect screening, by the 20th year of the programme.

4.4 DISCUSSION

This simulation study is a proof of concept of how serological and case data from a specific population can be combined to parametrise a dengue vaccine simulation, hence more realistic and detailed estimates on the impact of vaccination could be generated. The serological data was used to reconstruct the chance of misclassifying individuals when pre-vaccination screening is employed. Parameters estimated from fitting the model to the case data were used to build a base structure for the disease transmission simulation.

Consistently with the recommendation of the WHO (Wilder-Smith *et al.* 2018; World Health Organization 2018), the results from this study suggest that routine dengue vaccination in Hồ Chí Minh City should not be conducted without screening if a *Dengvaxia*®-like vaccine is to be used for the programme. If the vaccine cannot block transmission ($\epsilon_{\mathcal{T}} = 0$), this vaccination strategy was estimated to yield either no benefit or a slight increase in the number of hospitalisations (Figure 4.3). This is a conservative estimate when compared to the predictions from past simulation studies, which estimated that routinely vaccinating 80% of the 9-year-olds (regardless of their serostatus) in a population where 70% individuals are seropositive by the age of 9 years would lead to a 10%–30% reduction in hospitalisations within 10 years (Ferguson *et al.* 2016; Flasche *et al.* 2016). Nevertheless, Flasche *et al.* (2016) have pointed out that after the 'honeymoon period' of around 10 years, their models predicted a rebound in the number of hospitalisations

due to more naive vaccinees being infected and hospitalised, hence the cumulative proportion of hospitalisations averted in 30 years was estimated to be much smaller[67] than that of the first 10 years. In my simulations, the so-called 'honeymoon period' was not observed possibly due to the stringent assumption that the vaccine instantaneously increase the risk of symptomatic disease in naive recipients (while past studies often assumed that the disease-enhancing effect of the vaccine in naive recipients is preceded by a transient heterologous protection that can last 0.5–1.5 years). In other words, my model may have underestimated the benefits and overestimated the risks of vaccination in the medium term (the first 10 years of the programme). However, the analysis was intentionally designed this way since I believe that excessive caution is better than over-optimism when deliberating vaccinating a whole population with a vaccine that could yield harmful effects.

[67] Some of the models of Flasche *et al.* (2016) even predicted that the 30-year cumulative number of averted hospitalisations would be close to zero.

In scenarios where the vaccine can reduce transmission via suppressing the infectiousness of responsive recipients during their secondary infections ($\epsilon_{\mathcal{T}} = 1$), vaccination without screening was estimated to substantially decrease the cumulative number of hospitalisations in the first few years of the programme (Figure 4.5). This reduction, if happens in real-life vaccination programmes, could dangerously lead to a false impression of a highly beneficial vaccine. In fact, the big drop in the number of hospitalisations is expected to last for only a few years, while the number of hospitalisations prevented—due to a lower transmission intensity and due to secondary infections becoming asymptomatic in responsive vaccinees—is still larger than the number of additional hospitalisations caused by vaccinating naive individuals. In the later years of the programme, when the proportion of naive vaccinees in the population becomes greater and the ratio of naive individuals to the total size of the vaccine-targeted age group increases, this vaccination strategy is anticipated to yield much worse results, with the possibility that vaccination may escalate hospitalisations in some years. The finding that the no-screening strategy could yield more averted hospitalisations than realistic screening (when $\epsilon_{\mathcal{T}} = 1$) suggests that at a population level, the added averted hospitalisations caused by additionally vaccinating 40% of the primary-seropositive participants[68] could surpass the extra number of hospitalised cases resulting from vaccinating all naive participants. However, a vaccination programme without screening is definitely not a viable option

[68] In the realistic screening scenario, approximately 40% of the primary-seropositive participants were not vaccinated.

for Hồ Chí Minh City since such programme would—in the long term—lead to a large number of naive vaccinees confronted by a high risk of hospitalisation. Let alone the ethical issues that this vaccination strategy would bring, its cost-effectiveness is questionable since 40%–54% of the vaccines—if used in Hồ Chí Minh City—is expected to be wasted on mis-vaccinating naive recipients, and only 34%–38% of the vaccines would be correctly given to individuals with 1 prior infection (i.e. the primary targets of the vaccination).

My simulation results indicate that when screening is included, routinely vaccinating 9-year-olds in Hồ Chí Minh City would reduce the number of dengue hospitalisations significantly in the long term. This beneficial impact is expected even if the vaccine cannot block transmission (i.e. $\epsilon_{\mathcal{T}} = 0$, hence not producing indirect protection), although a larger benefit would be achieved if the vaccine can suppress transmission (i.e. $\epsilon_{\mathcal{T}} = 1$). With this screen-then-vaccinate strategy, the higher the vaccine's immuno-effectiveness and the programme coverage are, the more averted hospitalisations are anticipated. Vaccination with realistic screening—while found to prevent fewer hospitalisations than with perfect screening (as expected)—was estimated to perform very well on the population of Hồ Chí Minh City. This promising outcome may be partly rooted from the optimistic assumption about realistic screening (based on the results of CHAPTER 2) that its accuracy is near perfect in naive and in secondary-seropositive individuals (i.e. the main difference between realistic screening and perfect screening was the assumed sensitivity in primary-seropositive individuals).[69] At first glance, the assumed accuracy above seems to be higher than the 93.4% specificity (95% CI: 89.6%–97.2%) and the 95.2% sensitivity (95% CI: 94.2%–96.2%) that were recently estimated by Lopez *et al.* (2020) based on serological data of children in the Philippines.[70] However, it should be noted that the slightly lower specificity in the study of Lopez *et al.* (2020) was estimated based on an ELISA cut-off that corresponds to 9 Panbio® unit, lower than the 10-unit cut-off assumed for this simulation analysis. Furthermore, the reported 95.2%sensitivity above was calculated from all seropositive samples combined; when compensating for the poor sensitivity of only 77.1%—measured in the same study—among children with immunity to just 1 dengue serotype, the sensitivity of the ELISA is likely to be significantly higher than 95.2% in

[69] See the values of π_0, π_1 and π_2 in Table 4.1 on page 106.

[70] The study of Lopez *et al.* (2020) employed the same commercial ELISA as the one used in CHAPTER 2 of this thesis.

Philippine children with prior multitypic exposures. In other words, the assumed performance of the ELISA—for the realistic-screening scenario in the analysis herein—is not dissimilar from the cohort-based estimates of Lopez *et al.* (2020). Therefore, the promising vaccination outcomes simulated from my model are likely to be reasonable predictions.

When the vaccine can fully block transmission ($\epsilon_{\mathcal{T}} = 1$) the annual number of averted hospitalisations was estimated to strongly fluctuate across the years due to the oscillation of the FOI between being lowered by the vaccination and being intensified by the abundance of susceptibles.[71] This repeating up and down of the FOI (and thus of the hospitalisations) after the vaccine deployment is likely to be a dengue-specific problem. Unlike in non-dengue vaccination programmes (where both the number of infectious and the number of susceptible individuals are constantly reduced by the vaccination), dengue vaccination requires leaving out most naive individuals (since a *Dengvaxia*®-like vaccine is used). Consequently, a big susceptible population is preserved for the transmission to not only sustain but also bounce back when the proportion of naive individuals in the population becomes larger (due to the lowered FOI). My simulations predicted that this rebound of the transmission could lead to a substantial wave of increased infections and hospitalisations every 4 years. This cycle length of 4 years, however, was unlikely a result of the vaccination but rather was carried from the inference of the TypeSynch-P model in CHAPTER 3. In other words, the 4-year cycle length was implicitly assumed for the vaccine-free transmission of dengue in Hồ Chí Minh City;[72] and the cycle only became more pronounced with vaccination variability of the transmission intensity was enhanced by the vaccine intervention. In reality, the dynamics of dengue transmission and its interaction with vaccination is likely to be more complex than this estimated 4-year cycle. Despite this fact, a strong fluctuations in the FOI is still very probable if a routine dengue vaccination programme—utilising a vaccine that can suppress infection—only protects seropositive individuals. Therefore, the effectiveness of such programme in reducing the number of hospitalisations is expected to temporally vary greatly.

[71] This observed simulated phenomenon is described in more details in §4.3.2.

[72] See Figure C.1 on page 140.

Since the model used in this simulation analysis was parametrised and calibrated based on the TypeSynch-P model of CHAPTER 3, it also inherits the limitations of the TypeSynch-P model. Firstly, this model assumed that

dengue serotypes always circulate with identical FOI and the effectiveness of the vaccine is the same for all the serotypes. However, in real life, dengue circulation in Hồ Chí Minh City has been and may always be predominated by 1 or 2 serotypes with serotype switching occurring frequently (Ha *et al.* 2000; Hang *et al.* 2010; Recker *et al.* 2009; Wikramaratna *et al.* 2010). When combining this phenomenon with the prospect that dengue vaccines (including *Dengvaxia*®) will have different efficacy on different serotypes, the future results of dengue vaccination become much more difficult to predict. Although my mathematical model could be extended to cover these complicated serotype-serotype and serotype-vaccine interactions, such overly complex model does not necessarily lead to more accurate predictions due to the lack of serotype-specific data to calibrate the model. Secondly, by reusing the posteriors of the `TypeSynch-P` model, this analysis implicitly assumed that the hospitalised paediatric cases—to which the `TypeSynch-P` model was fit—are representative for the whole population. Nonetheless, this is unlikely to be a major issue unless the dynamics of dengue infection an transmission change significantly after adolescence.

Another limitation of this study is that it assumed the misclassification rate of the realistic screening is constant over time. This assumption—while plausible in the short term—may not hold when vaccination is continuously conducted over decades, especially if the vaccine can alter the transmission rate of the disease (as in the scenario of $\epsilon_{\mathcal{T}} = 1$). If the chance of infection of the population is reduced (by the vaccine or by a different epidemiological factor), the waiting time between infections of individuals would increase, hence potentially lowering the IgG levels of seropositive individuals (even if their numbers of dengue exposures are unchanged). In this case, the chance that seropositives are misclassified as seronegatives would rise if the screening method remains the same.

Despite that this simulation analysis was based on a few oversimplified assumptions about dengue transmission and dengue vaccination, its results are still valuable for understanding the effect of dengue vaccination on the population of Hồ Chí Minh City. In summary, the study suggest that if pre-vaccination screening is included, routinely vaccinating 9-years-olds with a *Dengvaxia*®-like vaccine would decrease the number of hospitalisations in the

long term. This reduction is expect to be larger when the programme coverage and the immuno-effectiveness of the vaccine are higher. If the vaccine can block transmission (i.e. having a non-zero transmission-blocking effectiveness), the beneficial impact of the vaccination would be greater (compared to when the vaccine does not block transmission); however, the transmission-blocking effect of the vaccine is also expected to enhance the variability of the transmission intensity and lead to fluctuation in the effectiveness of the vaccination programme over years. Although this study was specifically designed for Hồ Chí Minh City, its modelling framework can be modified and reuse for a different population.

5

GENERAL DISCUSSION

This thesis addresses several questions regarding the links between the epidemiology of dengue, the population immune responses, and the potential of dengue vaccination. Firstly, CHAPTER 2 introduces a new quantitative approach of using IgG levels (measured from population sera by indirect ELISAs) to not only estimate the seroprevalence of dengue in the population but also probabilistically identify primary-seropositive individuals, i.e. the group that would benefit the most from dengue vaccination. Secondly, CHAPTER 3 presents a novel method to quantify the protective and the disease-enhancing effects of maternally derived immunity in infants, using dengue epidemiological data. Finally, CHAPTER 4 is the first vaccine simulation study for dengue where the accuracy of pre-vaccination screening was realistically simulated (by reusing the estimates from CHAPTER 2). While the focal study context of this thesis is Vietnam, particularly Hồ Chí Minh City, all the modelling approaches introduced herein can be fruitfully applied elsewhere. The methodology developed throughout the chapters provides a modelling framework for assessing the future epidemiological impacts of dengue vaccination on a vaccine-free population, if a *Dengvaxia*®-like vaccine is to be used. In summary, this assessment methodology consists of 3 major steps:

[1] Estimating the performance accuracy of the pre-vaccination screening method via population serum samples (CHAPTER 2).

[2] Reconstructing the vaccine-free transmission dynamic of dengue in the population (CHAPTER 3).

[3] Reusing the inferred results of step 1 and 2 to build a vaccine simulation to evaluate the epidemiological effects of vaccination on the population (CHAPTER 4).

5.1 THE LINK BETWEEN DENGUE EPIDEMIOLOGY AND POPULATION IMMUNE RESPONSE

One of the key results of this thesis is the statistical correlation between the level of anti-DENV IgG of individuals and their number of past infections.[73] This study also found that given a same number of past heterotypic exposures, the IgG levels of individuals tend to be higher in populations with higher transmission intensity, implying that the distribution of IgG levels from population samples could be employed to monitor the transmission intensity of dengue in vaccine-free settings. Similarly to the observed results of Kucharski *et al.* (2018), this work suggests that a recent large outbreak of dengue could be revealed as a shift in the IgG-level distribution of the population.

[73] See CHAPTER 2.

Additionally, the inferred association between the transmission intensity and the distribution of IgG levels may be particularly important when put into the context of maternal immunity against dengue since past research has shown that the antibody titres of mothers may affect not only the quantity of maternally transferred antibody but also the opportunistic time window for ADE to occur in newborns (Castanha *et al.* 2016; Kliks *et al.* 1988; Libraty *et al.* 2009; Perret *et al.* 2005). In other words, the epidemiological characteristics of maternal ADE—hence infants' risk of hospitalisation—may strongly depend on the transmission dynamics of dengue, at least in vaccine-free populations. This dependency—while not investigated in my analyses herein due to data insufficiency—is worthy of further research since it may provide key information to reduce the number of infant cases. A potential future study is to extend the modelling method of CHAPTER 3 to directly estimate the number of infants born to mothers with 1 and ≥ 2 past infections (based on the FOI) and to assume that the effect size and the time frame of maternal ADE depends on the number past infections of the mothers.[74] By fitting this complex model simultaneously to case data of multiple populations of different transmission intensity, one may be able to quantify the effect of the transmission on the dynamics of maternal immunity.

[74] CHAPTER 3 assumes that all children in Hồ Chí Minh City were born with maternal immunity and the effect of the maternal immunity was identical in all infants.

Although newborns are unlikely to be included in future dengue vaccination programmes, they may be indirectly affected by vaccination in many

different ways due to the possible interactions among transmission intensity, mothers' levels of antibody, and maternal ADE. On the one hand, vaccination may reduce the transmission and result in fewer children born to naturally immune mothers. On the other hand, vaccination may lead to more infants born with vaccine-derived maternal immunity. Additionally, since vaccines may also boost the levels of antibody in already immune mothers, the level of maternally transferred antibody in infants may be altered. Furthermore, vaccine-derived maternal immunity may have different dynamics (e.g. a dissimilar waning rate, or a different time window of ADE) compared to one inherited from a naturally protected mother. In short, all of these possible side effects of vaccination on the infant age group need to be addressed in future research as they may yield negative epidemiological impacts in the long term.

5.2 PROSPECTS FOR DENGUE VACCINATION

Collectively, the findings from this work suggest a few potential approaches to improve not only the epidemiological impact but also the cost-effectiveness of dengue vaccination programmes. Firstly, the results of CHAPTER 2 indicate that when a *Dengvaxia*®-like vaccine is to be used and pre-vaccination screening is based on a quantitative measurement of antibodies, the optimal measurement cut-off—for separating seronegative individuals from seropositive ones—is likely to vary across different populations and different age groups (in the same population). Thus, the performance of screening—on which the cost-effectiveness of vaccination depends (España *et al.* 2019)—may be improved by recalibrating the cut-off specifically for the targeted age group in the targeted population. Secondly, CHAPTER 2 also implies the possibility of using the IgG level as a predictor to identify and *not* to vaccinate secondary-seropositive individuals. By excluding individuals with ≥ 2 prior infections from vaccination programmes, this strategy may vastly improve the cost-effectiveness of vaccination since secondary-seropositive individuals—if not excluded—would take up a large number of vaccines,[75] but this sero-group are unlikely to receive direct benefit from the vaccination (as they are probably naturally protected from disease). This

[75] Results from the realistic-screening scenarios of CHAPTER 4 indicate that more than 40% of the vaccines would be given to individuals with ≥ 2 prior infections.

vaccination scheme (namely, excluding secondary-seropositive individuals) may be beneficial even when the employed vaccine does not yield negative effects on seronegative individuals (in which case seronegative individuals would also be vaccinated along with primary-seropositive ones). However, the feasibility of this vaccination strategy as well as of the suggested classification method (for identifying individuals with ≥ 2 prior infections) still needs to be assessed thoroughly with more research. Finally, the simulation results of CHAPTER 4 indicate that routine vaccination alone—using a *Dengvaxia*®-like vaccine—is unlikely to lead to disease elimination since the transmission would always be sustained by the abundance of susceptible naive individuals (who are excluded from the vaccination). Therefore, even if a high efficacy vaccine is available, vaccination should still be combined with vector control methods to yield more suppressive effects on the transmission of dengue (Scott and Morrison 2010).

The combined findings from the analyses of this thesis consistently predict that routinely vaccinating 9-year-olds in Hồ Chí Minh City with a *Dengvaxia*®-like vaccine would be likely to yield epidemiological benefits. However, it should be noted that this optimistic prospect may have primarily stemmed from the currently high endemicity of dengue and from the inferred high specificity of the assumed serological screening assay since both of these factors would significantly reduce the chance of mis-vaccinating naive individuals. In practice, by the time of vaccine deployment, the transmission intensity in Hồ Chí Minh City may have already changed substantially or a different screening assay may be used, thus the results presented herein may no longer applicable. Even if this is the case, the modelling framework developed throughout this thesis could still be reused on the updated data to produce a complete re-evaluation of the vaccination programme.

APPENDICES

A

SUPPLEMENTARY FIGURES FOR CHAPTER 2

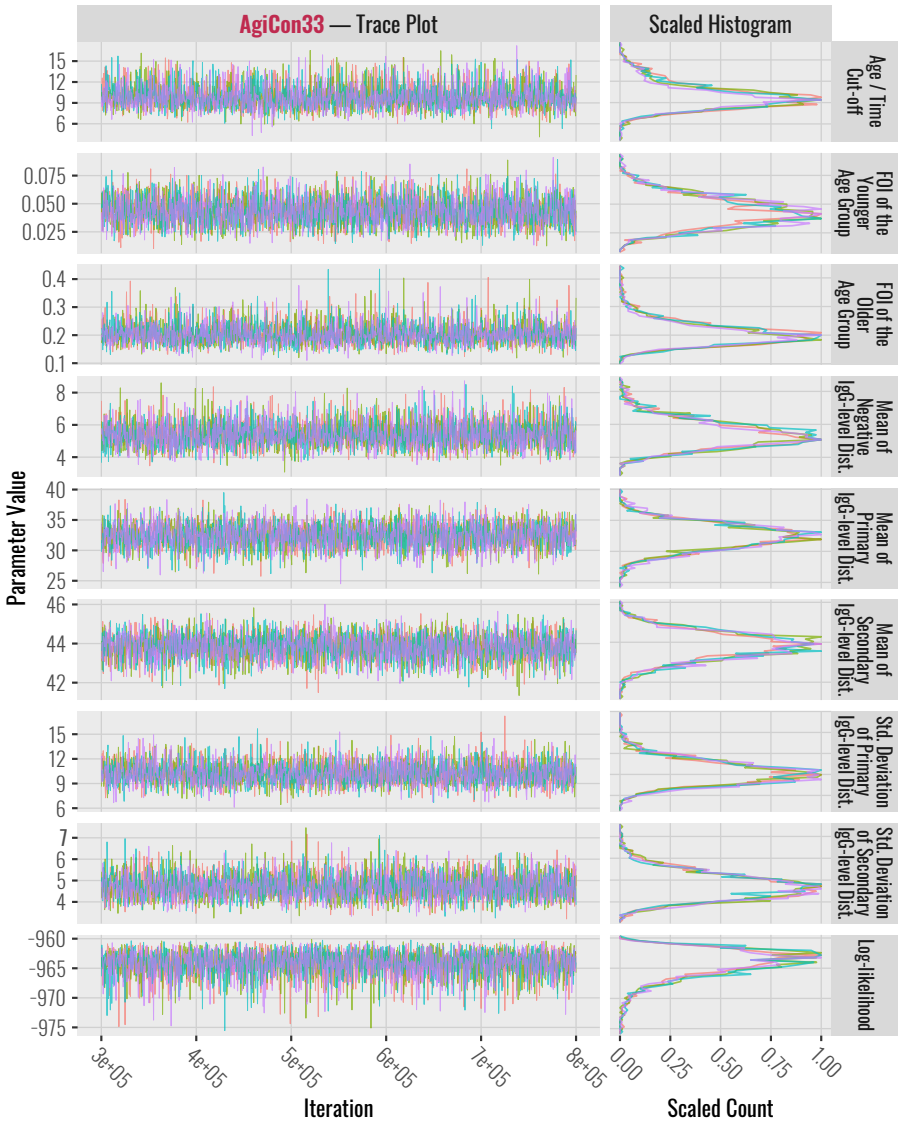


FIGURE A.1 – Trace plots (left panels) and histograms (right panels) of the posterior samples (the first 8 figure rows) and the log-likelihood (the bottom figure row) of the `AgiCon33` model (one of the best-fitting models for An Giang). Each plot comprises the results of 4 independent MCMCs, presented in 4 different colours. Each of the MCMCs consists of 800,000 iterations, with the first 300,000 iterations removed as burn-in. The histograms were scaled down to make the count of the most frequently occurred bin in each graph equal to 1.

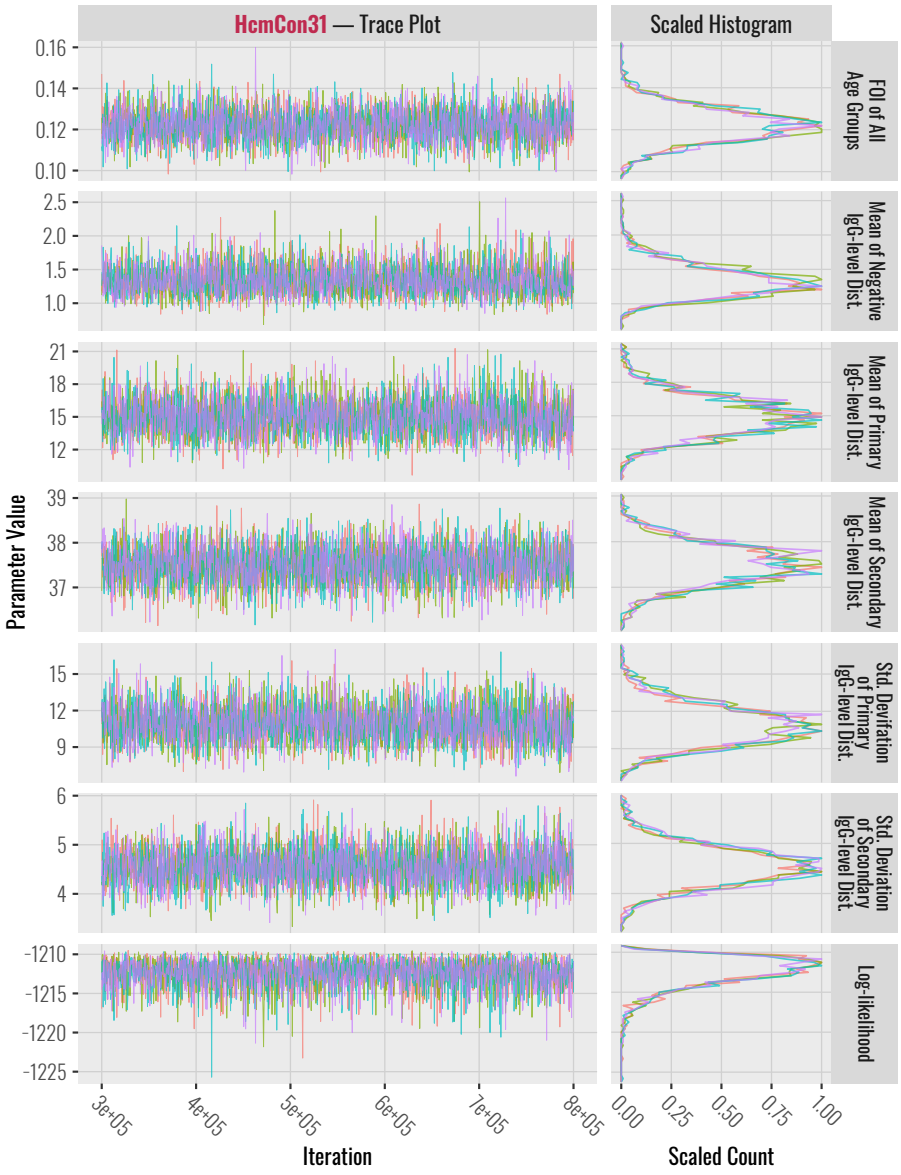


FIGURE A.2 — Trace plots (left panels) and histograms (right panels) of the posterior samples (the first 6 figure rows) and the log-likelihood (the bottom figure row) of the HcmCon31 model (one of the best-fitting models for Hồ Chí Minh City). Each plot comprises the results of 4 independent MCMCs, presented in 4 different colours. Each of the MCMCs consists of 800,000 iterations, with the first 300,000 iterations removed as burn-in. The histograms were scaled down to make the count of the most frequently occurred bin in each graph equal to 1.

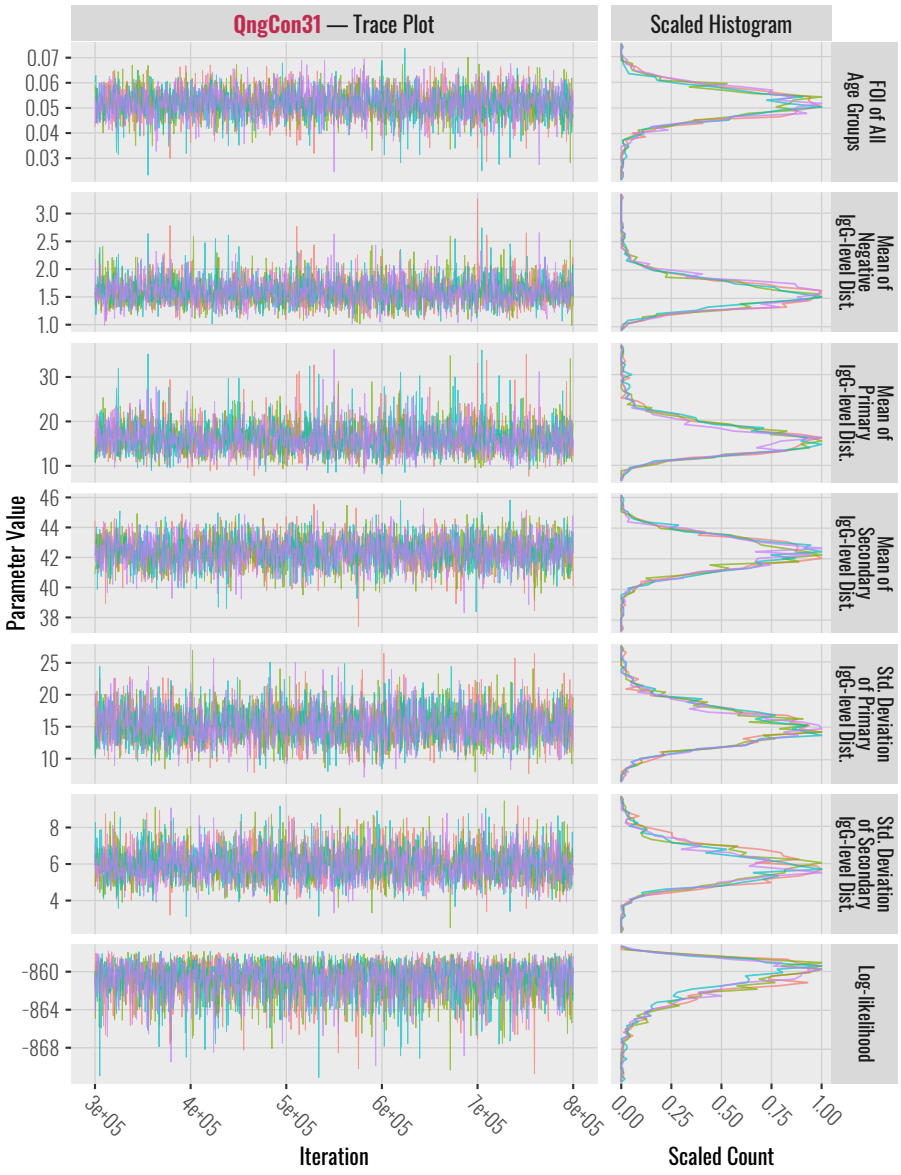


FIGURE A.3 — Trace plots (left panels) and histograms (right panels) of the posterior samples (the first 6 figure rows) and the log-likelihood (the bottom figure row) of the QngCon31 model (one of the best-fitting models for Quảng Ngãi). Each plot comprises the results of 4 independent MCMCs, presented in 4 different colours. Each of the MCMCs consists of 800,000 iterations, with the first 300,000 iterations removed as burn-in. The histograms were scaled down to make the count of the most frequently occurred bin in each graph equal to 1.

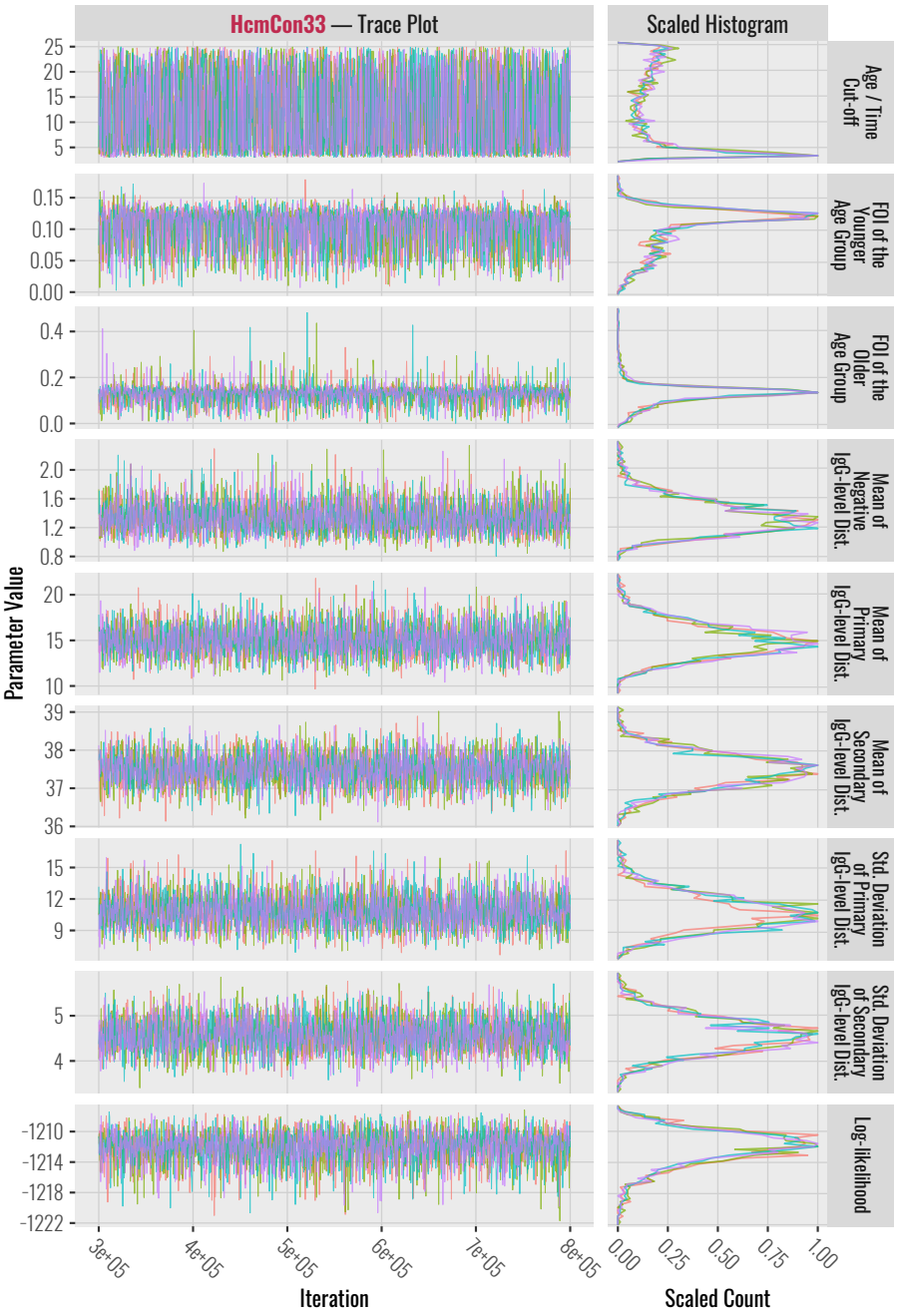


FIGURE A.4 – Trace plots (left panels) and histograms (right panels) of the posterior samples (the first 8 figure rows) and the log-likelihood (the bottom figure row) of the HcmCon33 model. Note that this model was rejected since the estimation of the age/time cut-off (shown in the first figure row) did not converge. Each panel in the plot above comprises the results of 4 independent MCMCs, presented in 4 different colours. Each of the MCMCs consists of 800,000 iterations, with the first 300,000 iterations removed as burn-in. The histograms were scaled down to make the count of the most frequently occurred bin in each graph equal to 1.

B

SUPPLEMENTARY INFORMATION FOR CHAPTER 3

TABLE B.1 – List of variables in the epidemiological model of CHAPTER 3.

NOTATION	PARAMETER DESCRIPTION
a	The age group index.
A	The index of the oldest age group in the model.
z_a	The age-span of the age group a, measured in the number of days. The total age-span of all the age groups in this model is: $\sum_a z_a = 21,900$ days ≈ 60 years.
t	The time at which the system state is estimated.
$N(t)$	The total size of the population at time t.
$v(t)$	The relative birth rate at time t. $v(t)$ has an annual mean of 1.0.
$g(t)$	The daily natural population growth rate of the population at time t. $g(t)$ is estimated from the population census data.
$m(t)$	The daily net migration rate at time t. $m(t)$ is estimated from the population census data.

TABLE B.2 – List of important parameters and functions in the epidemiological model of CHAPTER 3.

Notation	Parameter Description	Prior Distribution / Fixed Value†
$\beta_i(t)$	Transmission rate of serotype i at time t	$\mathit{Uniform}\big(0.2,\ 1.0\big)$
$\lambda_i(t)$	FOI of serotype i at time t; $\lambda_i(t)$ is a composite function of $\beta_i(t)$ and the number of infectious individuals of serotype i at time t	–
γ	Recovery rate of infected individuals	$1/7$
ξ	Relative risk of infection for individuals that are in the primary protection period, as compared to unprotected susceptibles	0 or 1‡
η_{inf}	Infant hospitalisation rate	$\mathit{Uniform}\big(0.0,\ 1.0\big)$
η_{noninf}	Non-infant hospitalisation rate.	$\mathit{Uniform}\big(0.0,\ 0.5\big)$
$\zeta^{(1)}$	Probability that a primary infection develops symptoms, without the involvement of maternal immunity	$\mathit{Uniform}\big(0.0,\ 1.0\big)$
$\zeta^{(2)}$	Probability that a secondary infection becomes symptomatic	0.41
κ_{protect}	Protective duration of maternal immunity (days)	$\mathit{Uniform}\big(0.0,\ \kappa_{\text{peak}}\big)$
κ_{peak}	Peak age of maternal ADE (days)	$\mathit{Gamma}\big(5.03,\ \text{scale: } 46.97\big)$ $\mathbb{T}[30.0,\ 730.0]$
κ_{sd}	SD of age of infants who experienced maternal ADE (days)	$\mathit{Normal}\big(30.0,\ 38.7\big)$ $\mathbb{T}[30.0,\ 150.0]$
ϕ_{max}	Maximum disease-enhancing effect of maternal ADE	$\mathit{Normal}\big(1.0,\ 215.0\big)$ $\mathbb{T}[1.0,\ 1000.0]$
δ	Duration of the primary protection (days)	$\mathit{Uniform}\big(30,\ 2000\big)$

† $\mathbb{T}[a, b]$ *denotes truncation of the given distribution at* a *and* b.

‡ $\xi = 0$ *in* `TypeSwtch-C` *and* `TypeSynch-C`;
$\xi = 1$ *in* `TypeSwtch-P` *and* `TypeSynch-P`.

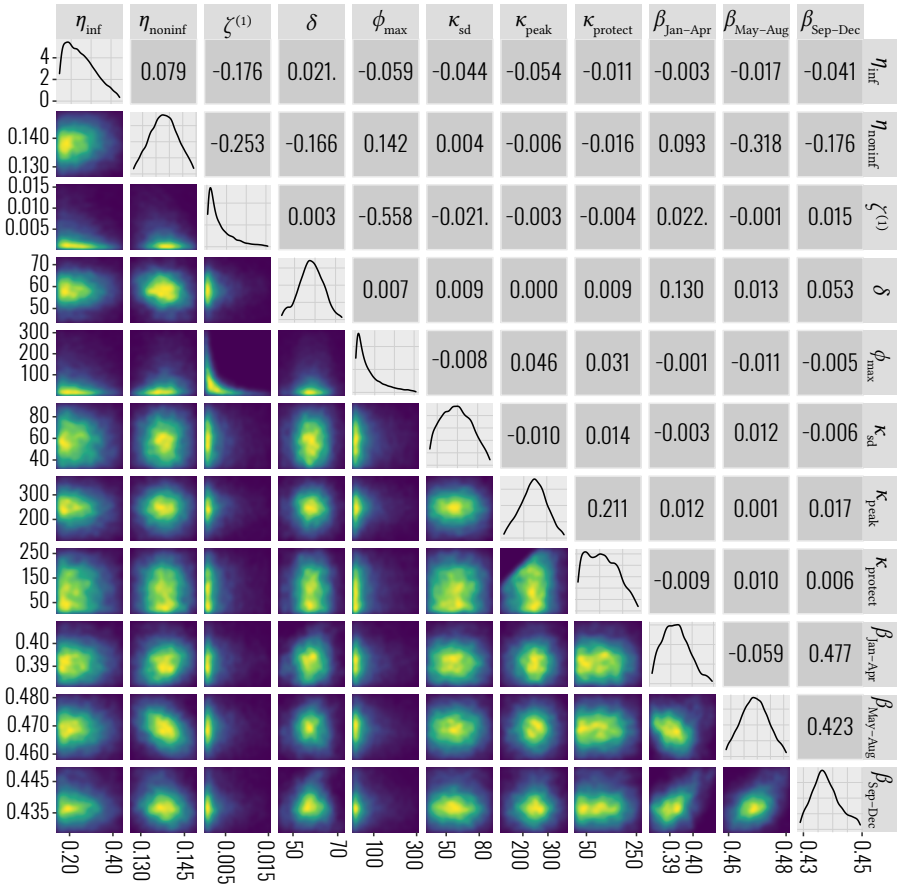


FIGURE B.1 – The marginal (shown in the diagonal panels) and the pairwise joint posteriors (shown in the lower diagonal panels) of the parameters estimated from the TypeSwtch-C model. The numbers in the upper diagonal panels represent the Pearson's correlation coefficient of each pair of the parameters. The parameters are: η_{inf}—infant hospitalisation rate, η_{noninf}—non-infant hospitalisation rate, $\zeta^{(1)}$—base primary symptomatic probability, δ—duration of the primary protection, ϕ_{max}—maximum disease-enhancing effect of maternal ADE, κ_{sd}—SD of age of infants who experienced maternal ADE, κ_{peak}—peak age of maternal ADE, $\kappa_{protect}$—protective duration of maternal immunity, $\beta_{Jan-Apr}$—transmission rate of each serotype between January and April, $\beta_{May-Aug}$—transmission rate of each serotype between May and August, $\beta_{Sep-Dec}$—transmission rate of each serotype between September and December.

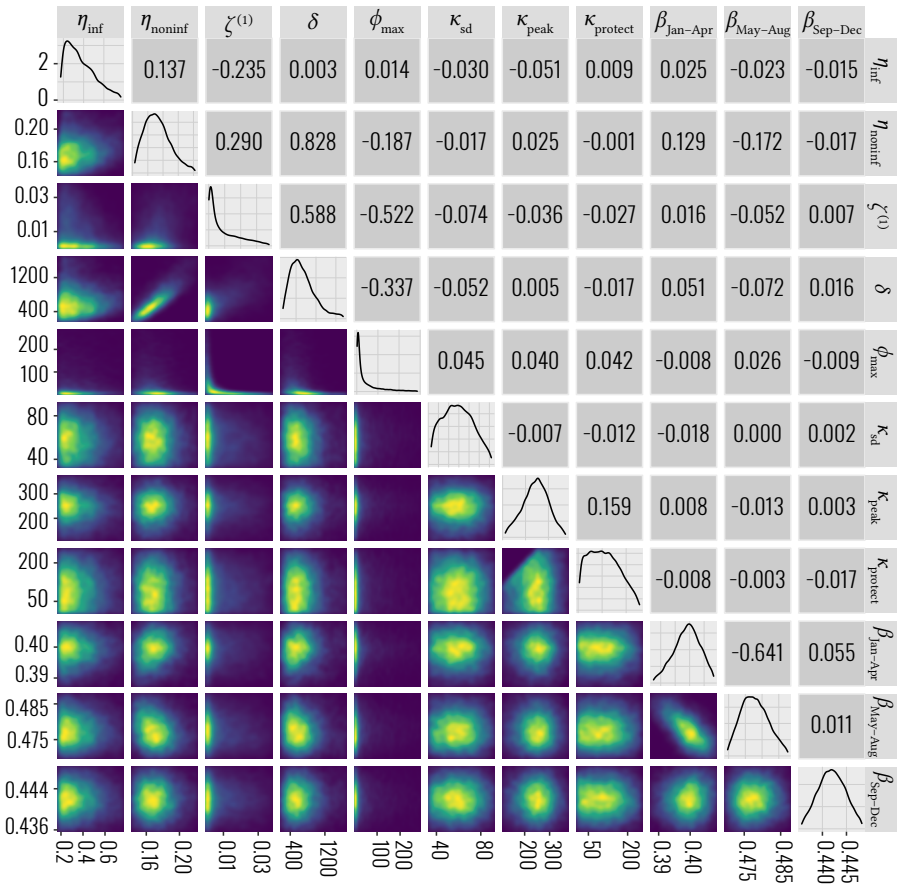


FIGURE B.2 – The marginal (shown in the diagonal panels) and the pairwise joint posteriors (shown in the lower diagonal panels) of the parameters estimated from the Type-Synch-P model. The numbers in the upper diagonal panels represent the Pearson's correlation coefficient of each pair of the parameters. The parameters are: η_{inf}—infant hospitalisation rate, η_{noninf}—non-infant hospitalisation rate, $\zeta^{(1)}$—base primary symptomatic probability, δ—duration of the primary protection, ϕ_{max}—maximum disease-enhancing effect of maternal ADE, κ_{sd}—SD of age of infants who experienced maternal ADE, κ_{peak}—peak age of maternal ADE, $\kappa_{protect}$—protective duration of maternal immunity, $\beta_{Jan-Apr}$—transmission rate of each serotype between January and April, $\beta_{May-Aug}$—transmission rate of each serotype between May and August, $\beta_{Sep-Dec}$—transmission rate of each serotype between September and December.

C

SUPPLEMENTARY FIGURES FOR CHAPTER 4

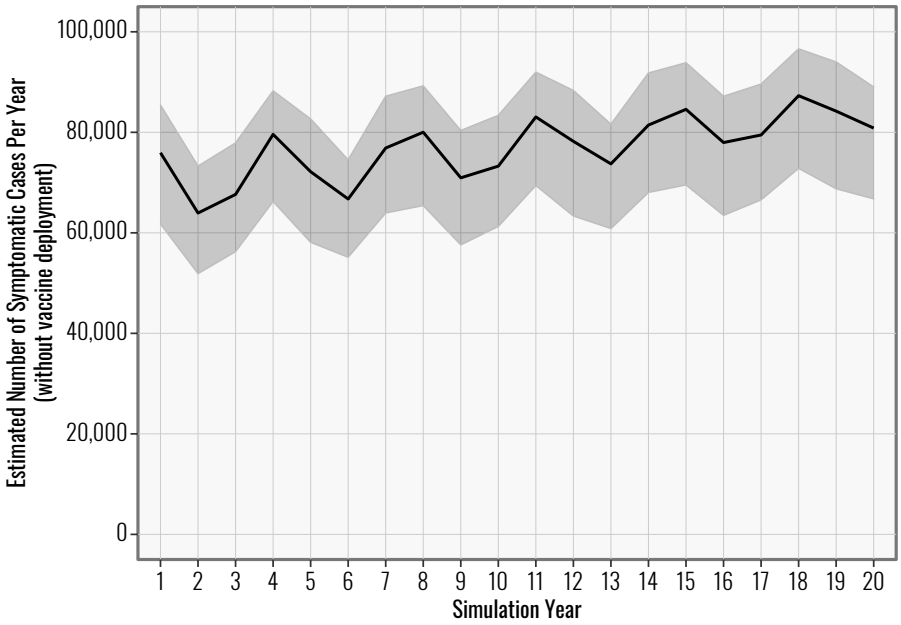


FIGURE C.1 — The annual number of symptomatic cases in Hồ Chí Minh City across the years, estimated from 1,000 simulations for the scenario that dengue vaccines are not deployed in the population. The black line and the grey area, respectively, represent the median value and the 95% UI of the annual estimates.

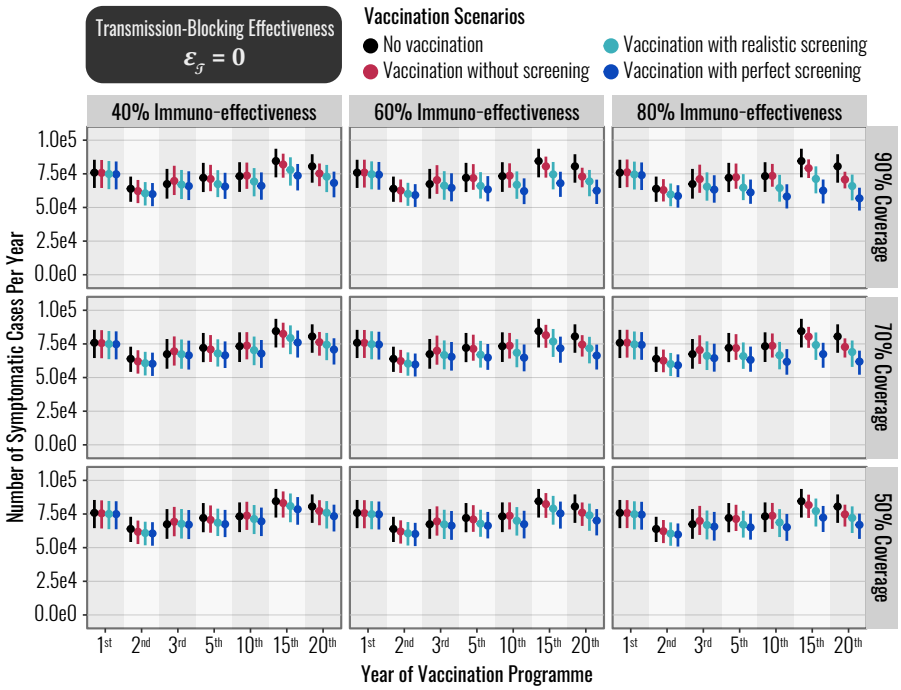


FIGURE C.2 — The annual number of symptomatic cases across the years of the vaccination programme, estimated for the scenario that the vaccine does not block transmission ($\epsilon_{\mathcal{T}} = 0$). Four vaccination scenarios were tested in this analysis: [1] no vaccination (black), [2] vaccination without screening (red), [3] vaccination with realistic screening (teal), and [4] vaccination with perfect screening (blue). Panels on the same column assume the same immune-effectiveness for the vaccine (i.e. 40%, 60%, or 80%). Meanwhile, panels on the same row assume the same coverage for the 9-year-olds (i.e. 50%, 70%, or 90%). The dots and the whiskers in the plots, respectively, represent the median value and the 95% UI of the estimates, each of which was generated from 1,000 simulations.

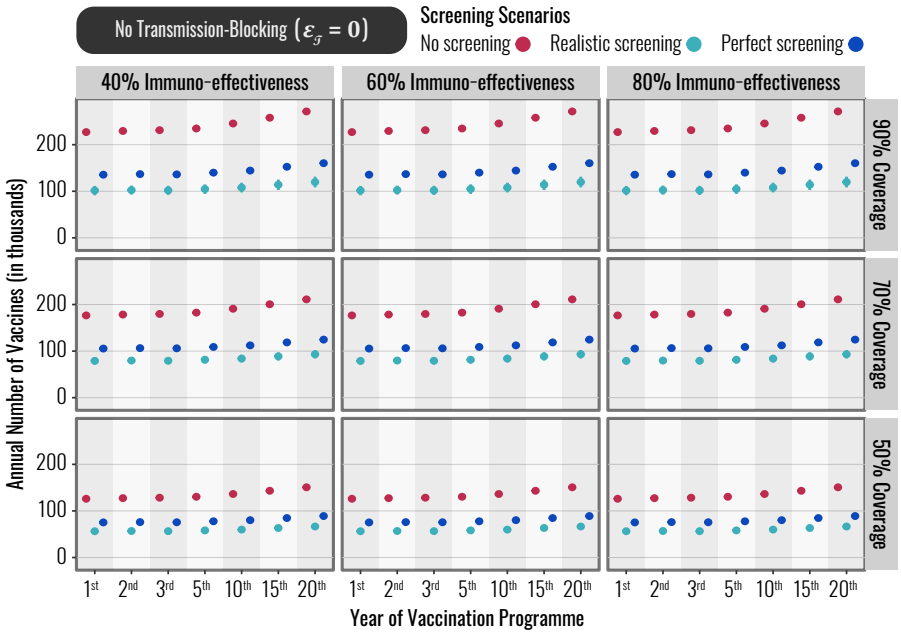


FIGURE C.3 – The median estimates[76] of the number of vaccines required each year, calculated for the scenarios where the vaccine does not block transmission ($\epsilon_{\mathcal{J}} = 0$). The colours indicate the tested screening scenarios: no screening (red), realistic screening (teal), and perfect screening (blue). Each of the estimates was generated from 1,000 simulations.

[76] It is not possible to show the 95% UIs of the estimates on these graphs since the intervals are too small compared to the scale of the y-axes.

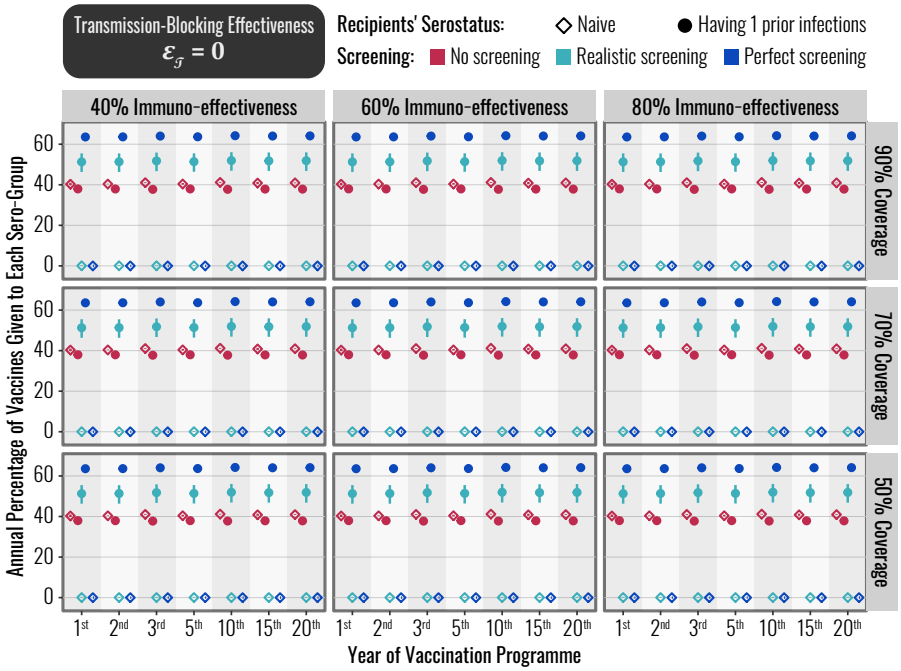


FIGURE C.4 – The annual percentage of vaccinees being naive (diamonds) or having 1 prior infection (round dots) in the scenarios where the vaccine does not block transmission ($\epsilon_{\mathcal{J}} = 0$). The colours indicate the tested screening scenarios, i.e. no screening (red), realistic screening (teal), and perfect screening (blue). The diamonds/dots and the whiskers, respectively, represent the median values and the 95% UIs[77] of the corresponding estimates, each of which was calculated from 1,000 simulations.

[77] Some of the UIs are not visible on the graph due to their small widths.

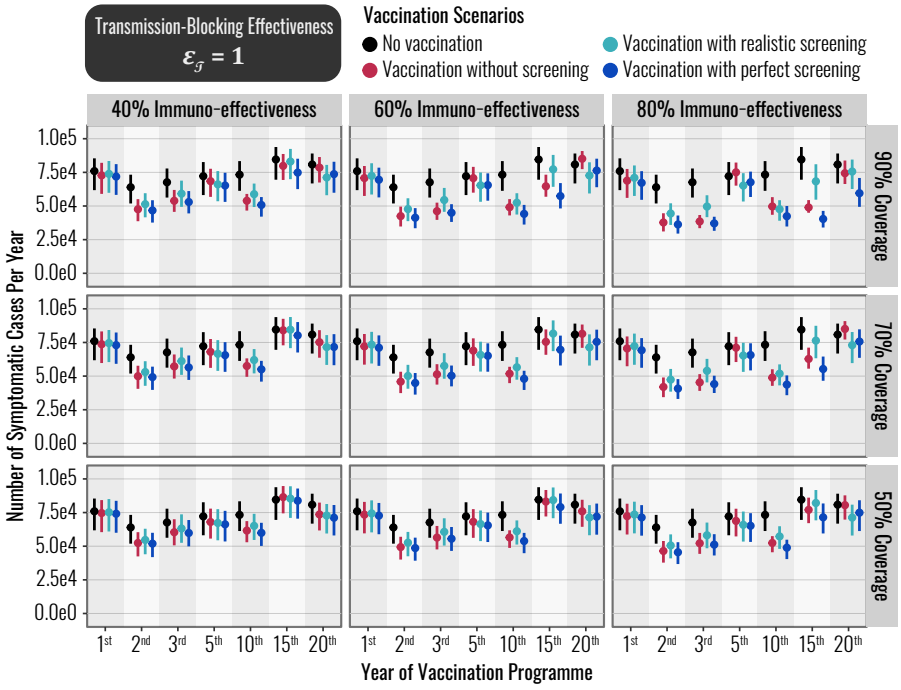


FIGURE C.5 – The annual number of symptomatic cases across the years of the vaccination programme, estimated for the scenario that the vaccine completely blocks transmission during the secondary infection of vaccinees ($\epsilon_{\mathcal{T}} = 1$). Four vaccination scenarios were tested in this analysis: [1] no vaccination[78] (black), [2] vaccination without screening (red), [3] vaccination with realistic screening (teal), and [4] vaccination with perfect screening (blue). Panels on the same column assume the same immune-effectiveness for the vaccine (i.e. 40%, 60%, or 80%). Meanwhile, panels on the same row assume the same coverage for the 9-year-olds (i.e. 50%, 70%, or 90%). The dots and the whiskers in the plots, respectively, represent the median value and the 95% UI of the estimates, each of which was generated from 1,000 simulations.

[78] Note that the results for the no-vaccination scenario in this figure are identical to those in Figure C.2 since the only different assumption between the 2 analyses is the transmission-blocking effectiveness of the vaccine ($\epsilon_{\mathcal{T}}$), which does not affect the no-vaccination scenario.

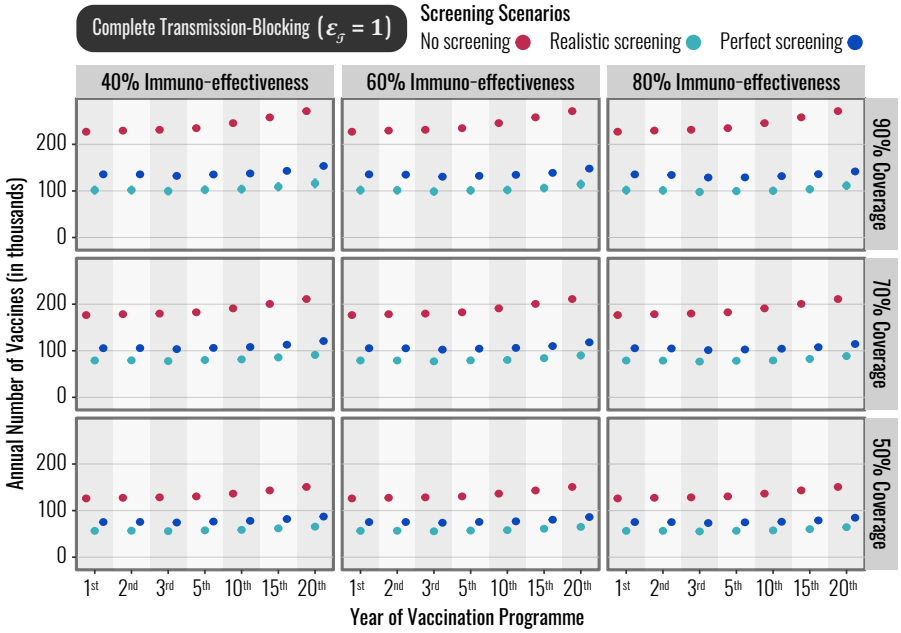


FIGURE C.6 – The median estimates[79] of the number of vaccines required each year, calculated for the scenarios where the vaccine completely blocks transmission during the secondary infection of vaccinees ($\epsilon_{\mathcal{J}} = 1$). The colours indicate the tested screening scenarios: no screening (red), realistic screening (teal), and perfect screening (blue). Each of the estimates was generated from 1,000 simulations.

[79] It is not possible to show the 95% UIs of the estimates on these graphs since the intervals are too small compared to the scale of the y-axes.

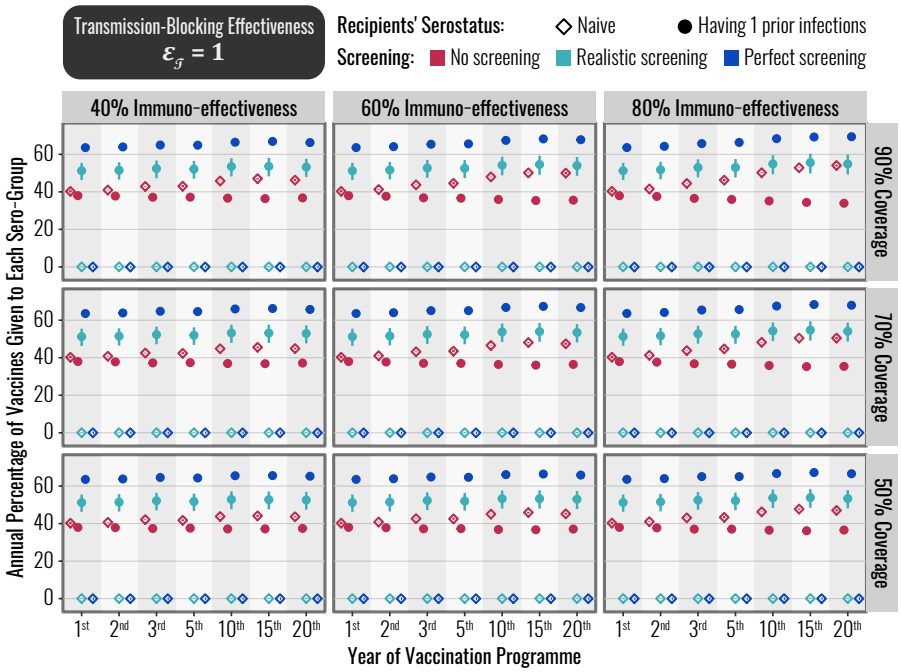


FIGURE C.7 – The annual percentage of vaccinees being naive (diamonds) or having 1 prior infection (round dots) in the scenarios where the vaccine completely blocks transmission during the secondary infection of vaccinees ($\epsilon_{\mathcal{T}} = 1$). The colours indicate the tested screening scenarios: no screening (red), realistic screening (teal), and perfect screening (blue). The diamonds/dots and the whiskers, respectively, represent the median values and the 95% UIs[80] of the corresponding estimates, each of which was calculated from 1,000 simulations.

[80] Some of the UIs are not visible on the graph due to their small widths.

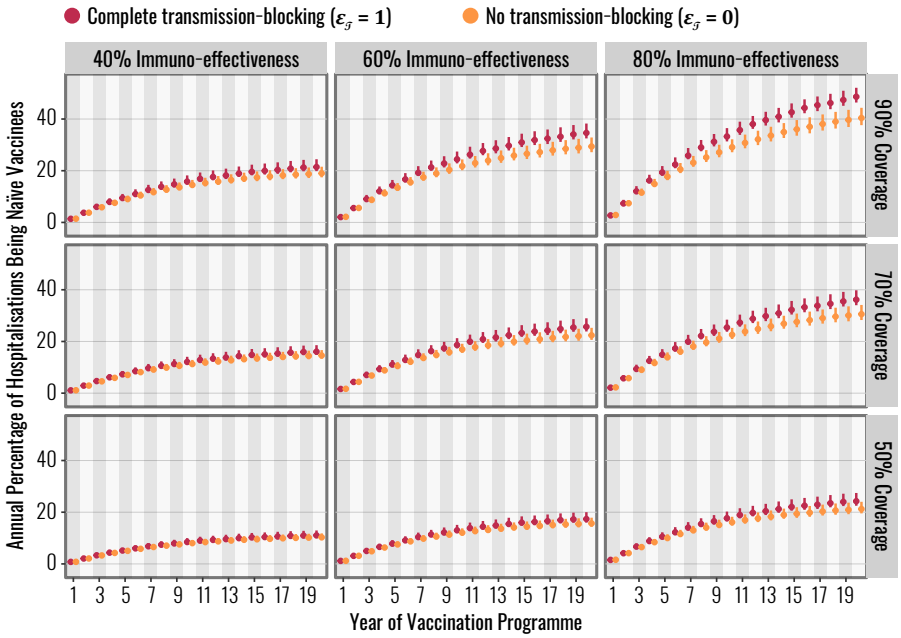


FIGURE C.8 — The estimated annual percentage of hospitalisations being naive vaccinees. The red and the orange dots represent the median estimates (generated from 1,000 simulations) for 2 scenarios of transmission-blocking effectiveness, namely $\epsilon_{\mathcal{J}} = 1$ and $\epsilon_{\mathcal{J}} = 0$, respectively. The whiskers around the dots indicate the 95% UIs of the corresponding calculations.

D

AN ALTERNATIVE MODEL FOR SIMULATING DENGUE VACCINATION

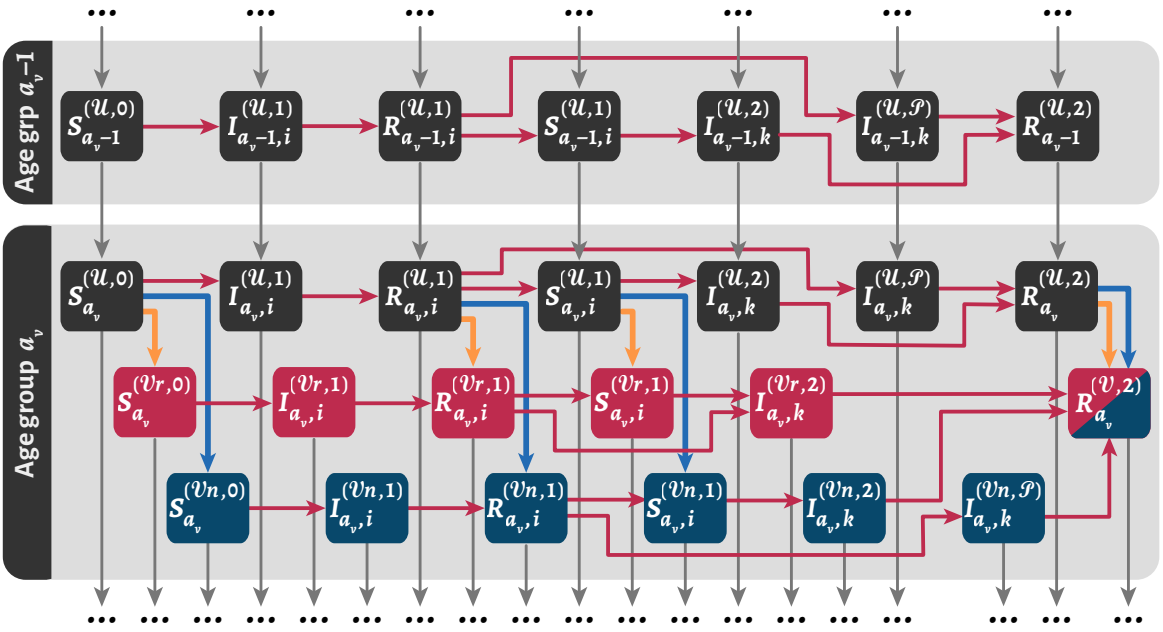


FIGURE D.1 – An alternative epidemiological model for the vaccine simulation of CHAPTER 4. The S, I, and R boxes in this diagram, respectively, represent the classes of susceptible, infected, and recovered individuals. The first superscript in the notation of each model compartment indicates the vaccination status of the group, i.e. $\mathcal{U}$—unvaccinated (black boxes), $\mathcal{V}r$—responsive vaccinated (red boxes), $\mathcal{V}n$—non-responsive vaccinated (blue boxes), or $\mathcal{V}$—vaccinated (half-red and half-blue box). The second superscript in the notations either indicates the number of infections individuals have experienced (i.e. 0, 1, or 2) or shows that individuals are experiencing a secondary infection during the primary protection period (denoted by $\mathcal{P}$). The first subscripts of the compartments represent the age group of individuals while the second subscripts (if exist) indicate the last infecting serotype. The red arrows in the graph represent epidemiological transitions caused infection and recovery. The grey arrows represent individuals that are ageing into a higher age group with their vaccination statuses unchanged. Meanwhile, the orange and the blue arrows respectively depict the proportion of individuals becoming responsive and non-responsive vaccinated while in the vaccine-targeted age group (a_v).

This section presents an alternative mathematical model (Figure D.1) that was also considered for the simulation analysis of CHAPTER 4. In this alternative model, the process of individuals moving across infection compartments due to infection and recovery was formulated the same way as presented in CHAPTER 4.[81] However, vaccination was modelled differently in the alternative model. Here, all individuals that reached the targeted age group of the vaccination, a_v, were moved into unvaccinated compartments of a_v before being considered for vaccination. In other words, vaccination happened while individuals were in the a_v age group rather than occurring right at the moment they moved from a_{v-1} to a_v (as formulated in the main model).

[81] See §4.2.2 on page 100 for the detailed formulation of the epidemiological model of CHAPTER 4.

The alternative model is formalised by the following system of ordinary differential equations:[82]

$$
\begin{aligned}
\frac{d}{dt}S_a^{(\mathcal{U},0)} &= -S_a^{(\mathcal{U},0)}\sum_{i=1}^{4}\lambda_i(t) - \frac{\nu(t)}{z_a}S_a^{(\mathcal{U},0)} - c'_a\pi_0 \times S_a^{(\mathcal{U},0)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}S_{a-1}^{(\mathcal{U},0)} + \mathbb{1}_{a=1}\times B(t) \\
\frac{d}{dt}S_a^{(\mathcal{V}r,0)} &= -S_a^{(\mathcal{V}r,0)}\sum_{i=1}^{4}\lambda_i(t) - \frac{\nu(t)}{z_a}S_a^{(\mathcal{V}r,0)} + c'_a\pi_0\epsilon_{\mathcal{R}} \times S_a^{(\mathcal{U},0)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}S_{a-1}^{(\mathcal{V}r,0)} \\
\frac{d}{dt}S_a^{(\mathcal{V}n,0)} &= -S_a^{(\mathcal{V}n,0)}\sum_{i=1}^{4}\lambda_i(t) - \frac{\nu(t)}{z_a}S_a^{(\mathcal{V}n,0)} + c'_a\pi_0\left(1-\epsilon_{\mathcal{R}}\right) \times S_a^{(\mathcal{U},0)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}S_{a-1}^{(\mathcal{V}n,0)} \\
\frac{d}{dt}I_{a,i}^{(\mathcal{U},1)} &= \lambda_i(t)\,S_a^{(\mathcal{U},0)} - \gamma I_{a,i}^{(\mathcal{U},1)} - \frac{\nu(t)}{z_a}I_{a,i}^{(\mathcal{U},1)} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}I_{a-1,i}^{(\mathcal{U},1)} \\
\frac{d}{dt}I_{a,i}^{(\mathcal{V}r,1)} &= \lambda_i(t)\,S_a^{(\mathcal{V}r,0)} - \gamma I_{a,i}^{(\mathcal{V}r,1)} - \frac{\nu(t)}{z_a}I_{a,i}^{(\mathcal{V}r,1)} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}I_{a-1,i}^{(\mathcal{V}r,1)} \\
\frac{d}{dt}I_{a,i}^{(\mathcal{V}n,1)} &= \lambda_i(t)\,S_a^{(\mathcal{V}n,0)} - \gamma I_{a,i}^{(\mathcal{V}n,1)} - \frac{\nu(t)}{z_a}I_{a,i}^{(\mathcal{V}n,1)} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}I_{a-1,i}^{(\mathcal{V}n,1)} \\
\frac{d}{dt}R_{a,i}^{(\mathcal{U},1)} &= \gamma I_{a,i}^{(\mathcal{U},1)} - \left[\frac{1}{\delta} + \xi\sum_{k\neq i}\lambda_k(t)\right]\times R_{a,i}^{(\mathcal{U},1)} - \frac{\nu(t)}{z_a}R_{a,i}^{(\mathcal{U},1)} \\
&\quad - c'_a\pi_1 \times R_{a,i}^{(\mathcal{U},1)} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}R_{a-1,i}^{(\mathcal{U},1)}
\end{aligned}
$$

[82] Except for c'_a (which is newly introduced here), all the notations are identical to those presented in §4.2.2. Compared to the equations of the main model, the infectious compartment (I's) are unchanged, but the calculation of the other classes are different.

$$\begin{aligned}
\frac{d}{dt}R_{a,i}^{(\mathcal{V}r,1)} &= \gamma I_{a,i}^{(\mathcal{V}r,1)} - \left[\frac{1}{\delta} + \xi\sum_{k\neq i}\lambda_k(t)\right]\times R_{a,i}^{(\mathcal{V}r,1)} - \frac{\nu(t)}{z_a}\,R_{a,i}^{(\mathcal{V}r,1)} \\
&\quad + c_a'\pi_1\epsilon_{\mathcal{R}}\times R_{a,i}^{(\mathcal{U},1)} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\,R_{a-1,i}^{(\mathcal{V}r,1)} \\
\frac{d}{dt}R_{a,i}^{(\mathcal{V}n,1)} &= \gamma I_{a,i}^{(\mathcal{V}n,1)} - \left[\frac{1}{\delta} + \xi\sum_{k\neq i}\lambda_k(t)\right]\times R_{a,i}^{(\mathcal{V}n,1)} - \frac{\nu(t)}{z_a}\,R_{a,i}^{(\mathcal{V}n,1)} \\
&\quad + c_a'\pi_1\Big(1-\epsilon_{\mathcal{R}}\Big)\times R_{a,i}^{(\mathcal{U},1)} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\,R_{a-1,i}^{(\mathcal{V}n,1)} \\
\frac{d}{dt}I_{a,k}^{(\mathcal{U},\mathcal{P})} &= \xi\lambda_k(t)\sum_{i\neq k}R_{a,i}^{(\mathcal{U},1)} - \gamma I_{a,k}^{(\mathcal{U},\mathcal{P})} - \frac{\nu(t)}{z_a}\,I_{a,k}^{(\mathcal{U},\mathcal{P})} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\,I_{a-1,k}^{(\mathcal{U},\mathcal{P})} \\
\frac{d}{dt}I_{a,k}^{(\mathcal{V}n,\mathcal{P})} &= \xi\lambda_k(t)\sum_{i\neq k}R_{a,i}^{(\mathcal{V}n,1)} - \gamma I_{a,k}^{(\mathcal{V}n,\mathcal{P})} - \frac{\nu(t)}{z_a}\,I_{a,k}^{(\mathcal{V}n,\mathcal{P})} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\,I_{a-1,k}^{(\mathcal{V}n,\mathcal{P})} \\
\frac{d}{dt}S_{a,i}^{(\mathcal{U},1)} &= \frac{1}{\delta}\,R_{a,i}^{(\mathcal{U},1)} - S_{a,i}^{(\mathcal{U},1)}\sum_{k\neq i}\lambda_k(t) - \frac{\nu(t)}{z_a}\,S_{a,i}^{(\mathcal{U},1)} - c_a'\pi_1\times S_{a,i}^{(\mathcal{U},1)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\,S_{a-1,i}^{(\mathcal{U},1)} \\
\frac{d}{dt}S_{a,i}^{(\mathcal{V}r,1)} &= \frac{1}{\delta}\,R_{a,i}^{(\mathcal{V}r,1)} - S_{a,i}^{(\mathcal{V}r,1)}\sum_{k\neq i}\lambda_k(t) - \frac{\nu(t)}{z_a}\,S_{a,i}^{(\mathcal{V}r,1)} + c_a'\pi_1\epsilon_{\mathcal{R}}\times S_{a,i}^{(\mathcal{U},1)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\,S_{a-1,i}^{(\mathcal{V}r,1)} \\
\frac{d}{dt}S_{a,i}^{(\mathcal{V}n,1)} &= \frac{1}{\delta}\,R_{a,i}^{(\mathcal{V}n,1)} - S_{a,i}^{(\mathcal{V}n,1)}\sum_{k\neq i}\lambda_k(t) - \frac{\nu(t)}{z_a}\,S_{a,i}^{(\mathcal{V}n,1)} \\
&\quad + c_a'\pi_1\Big(1-\epsilon_{\mathcal{R}}\Big)\times S_{a,i}^{(\mathcal{U},1)} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\,S_{a-1,i}^{(\mathcal{V}n,1)} \\
\frac{d}{dt}I_{a,k}^{(\mathcal{U},2)} &= \lambda_k(t)\sum_{i\neq k}S_{a,i}^{(\mathcal{U},1)} - \gamma I_{a,k}^{(\mathcal{U},2)} - \frac{\nu(t)}{z_a}\,I_{a,k}^{(\mathcal{U},2)} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\,I_{a-1,k}^{(\mathcal{U},2)} \\
\frac{d}{dt}I_{a,k}^{(\mathcal{V}r,2)} &= \lambda_k(t)\sum_{i\neq k}\left[S_{a,i}^{(\mathcal{V}r,1)} + \xi R_{a,i}^{(\mathcal{V}r,1)}\right] - \gamma I_{a,k}^{(\mathcal{V}r,2)} - \frac{\nu(t)}{z_a}\,I_{a,k}^{(\mathcal{V}r,2)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\,I_{a-1,k}^{(\mathcal{V}r,2)} \\
\frac{d}{dt}I_{a,k}^{(\mathcal{V}n,2)} &= \lambda_k(t)\sum_{i\neq k}S_{a,i}^{(\mathcal{V}r,1)} - \gamma I_{a,k}^{(\mathcal{V}n,2)} - \frac{\nu(t)}{z_a}\,I_{a,k}^{(\mathcal{V}n,2)} + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\,I_{a-1,k}^{(\mathcal{V}n,2)} \\
\frac{d}{dt}R_{a}^{(\mathcal{U},2)} &= \gamma\sum_{i=1}^{4}\left[I_{a,i}^{(\mathcal{U},2)} + I_{a,i}^{(\mathcal{U},\mathcal{P})}\right] - \frac{\nu(t)}{z_a}\,R_{a}^{(\mathcal{U},2)} - c_a'\pi_2\times R_{a}^{(\mathcal{U},2)} \\
&\quad + \mathbb{1}_{a>1}\times\frac{\nu(t)}{z_{a-1}}\,R_{a-1}^{(\mathcal{U},2)}
\end{aligned}$$

$$\frac{d}{dt}R_a^{(\mathcal{V},2)} = \gamma \sum_{i=1}^{4}\left[I_{a,i}^{(\mathcal{V}r,2)} + I_{a,i}^{(\mathcal{V}n,2)} + I_{a,i}^{(\mathcal{V}n,\mathcal{P})}\right] - \frac{\nu(t)}{z_a} R_a^{(\mathcal{V},2)} + c_a'\pi_2 \times R_a^{(\mathcal{U},2)} + \mathbb{1}_{a>1} \times \frac{\nu(t)}{z_{a-1}} R_{a-1}^{(\mathcal{V},2)}$$

where: S, I, and R, respectively, represent the classes of susceptible, infected, and recovered individuals;
the first superscript in the class notations indicates the vaccination status of the group, i.e. $\mathcal{U}$—unvaccinated, $\mathcal{V}r$—responsive vaccinated, $\mathcal{V}n$—non-responsive vaccinated, or $\mathcal{V}$—vaccinated (both responsive and non-responsive);
the second superscript either indicates the number of infections individuals have experienced (i.e. 0, 1, or 2) or shows that individuals are experiencing a secondary infection during the primary protection period (denoted by $\mathcal{P}$);
the first subscript in the class notations represents the age group of individuals;
the second subscript (if exists) indicates the last infecting serotype;

- t denotes time;
- a is the age group index;
- i, k denote dengue serotypes;
- $\lambda_i(t)$ is the FOI of serotype i at time t;
- γ is the recovery rate of infected individuals;
- c_a' is the proportion of unvaccinated individuals in the age group a participating in the vaccination programme each day;
- π_0, π_1, π_2 denote the conditional probability that participants of the vaccination programme are vaccinated, given their number of past dengue infection being zero, 1, or more than 1, respectively;
- $\epsilon_{\mathcal{R}}$ is the immuno-effectiveness of the vaccine;
- δ is the average duration of the primary protection period;

ξ is the relative risk of infection for individuals that are in the primary protection period, as compared to unprotected susceptibles;[83]

$v(t)$ is the seasonal relative birth rate of the population at time t;

z_a is the age-span of the age group a, measured in the number days (in this model, $\sum_a z_a = 21,900$ days ≈ 60 years);

$B(t)$ is the total number of new births at time t;

$\mathbb{1}_x$ is an indicator function, i.e. $\mathbb{1}_x = \begin{cases} 1 & \text{if } x \text{ is true,} \\ 0 & \text{otherwise} \end{cases}$.

[83] Throughout this simulation analysis, ξ was fixed at 1.0.

The proportion of unvaccinated individuals in the age group a participating in the vaccination programme each day, c'_a,[84] was calculate from the original (yearly) coverage, c_a, using the following approximation:

$$c'_a = \frac{-\ln(1 - c_a)}{365} \qquad \big(\text{where: } 0 < c_a < 1\big)$$

[84] c'_a can be interpreted as the daily coverage of the vaccination program.

In the model above, the *numbers of newly vaccinated individuals* in a targeted age group at a time are represented by the quantities:

$$c'_a \pi_0 \, S_a^{(\mathcal{U},0)}, \quad c'_a \pi_1 \, R_{a,i}^{(\mathcal{U},1)}, \quad c'_a \pi_1 \, S_{a,i}^{(\mathcal{U},1)}, \quad \text{and } c'_a \pi_2 \, R_a^{(\mathcal{U},2)}.$$

These quantities are *proportional to the number of unvaccinated individuals in the same age group* since the values of c'_a, π_0, π_1, and π_2 are fixed. Therefore, as vaccination alters the number of unvaccinated individuals in the targeted age group, the number of new vaccinations at each time point is also changed (even when the total number of individuals in the targeted age group is constant). In other words, the number of newly vaccinated individuals was over-estimated at the beginning of the vaccination (since a large proportion of the targeted age group was unvaccinated) and under-estimated in the later years of the vaccination (when the size of unvaccinated groups became smaller).[85] In the main model (presented in CHAPTER 4), this particular problem does not exist since the number of newly vaccinated individuals in the vaccine-targeted age group, a_v, is proportional to the size of the a_{v-1} age group (which is stable as it is not altered by vaccination). Despite that the alternative model

[85] Note the difference between the estimates of the first year and those of the later years in Figure D.2.

suffered from this reliability issue in calculating the number of vaccines for each time point, its estimates for the impact of vaccination on hospitalisation (Figure D.3) are generally consistent with those from the main model.

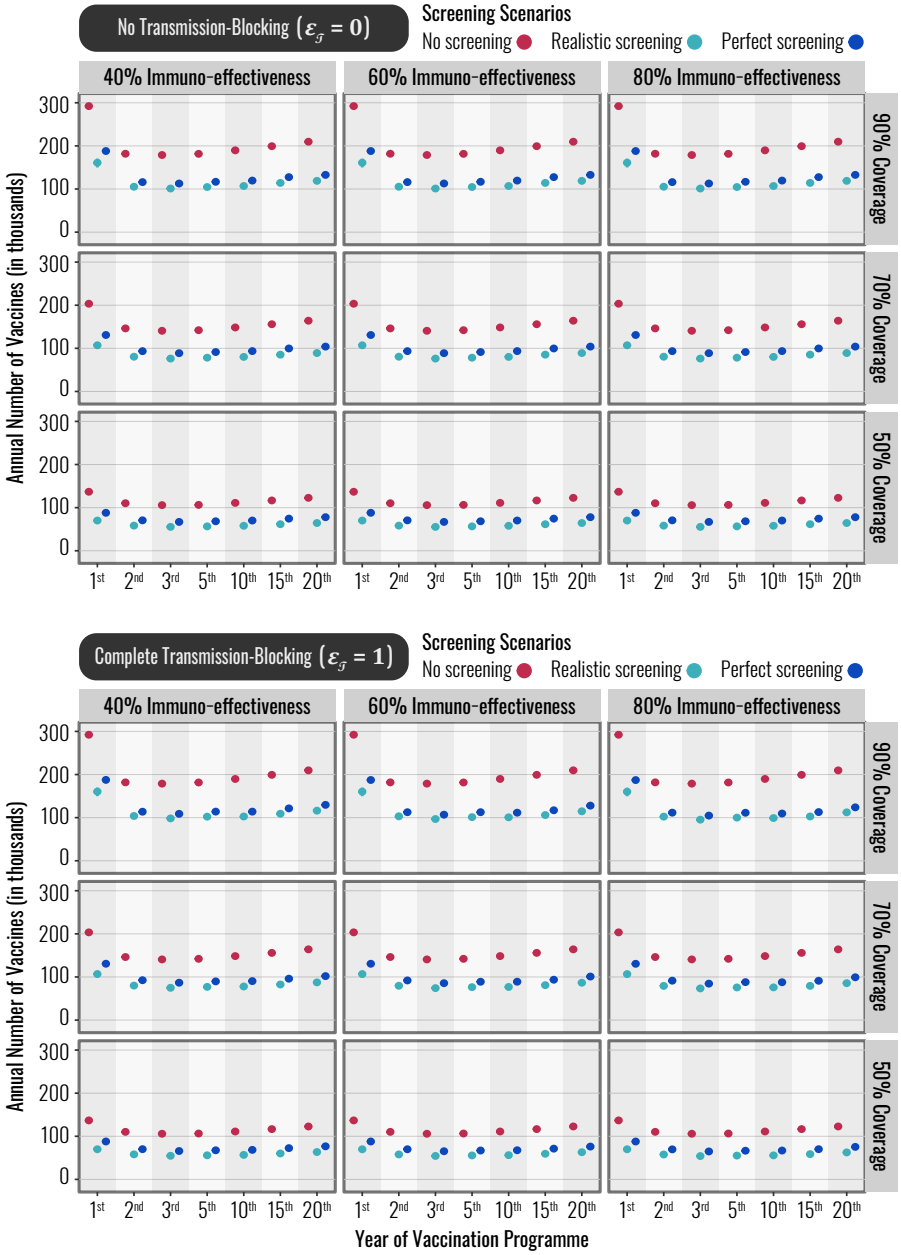


FIGURE D.2 – The median estimates[86] of the number of vaccines required each year, calculated from the alternative model (1,000 simulations were run for each vaccination scenario). The 9 upper panels illustrate the estimates for the scenarios where the vaccine does not block transmission ($\epsilon_{\mathcal{J}} = 0$). Meanwhile, the 9 lower panels show the results for when the vaccine completely blocks transmission during the secondary infection of vaccinees ($\epsilon_{\mathcal{J}} = 1$). The colours indicate the tested screening scenarios: no screening (red), realistic screening (teal), and perfect screening (blue).

[86] It is not possible to show the 95% UIs of the estimates on these graphs since the intervals are too small compared to the scale of the y-axes.

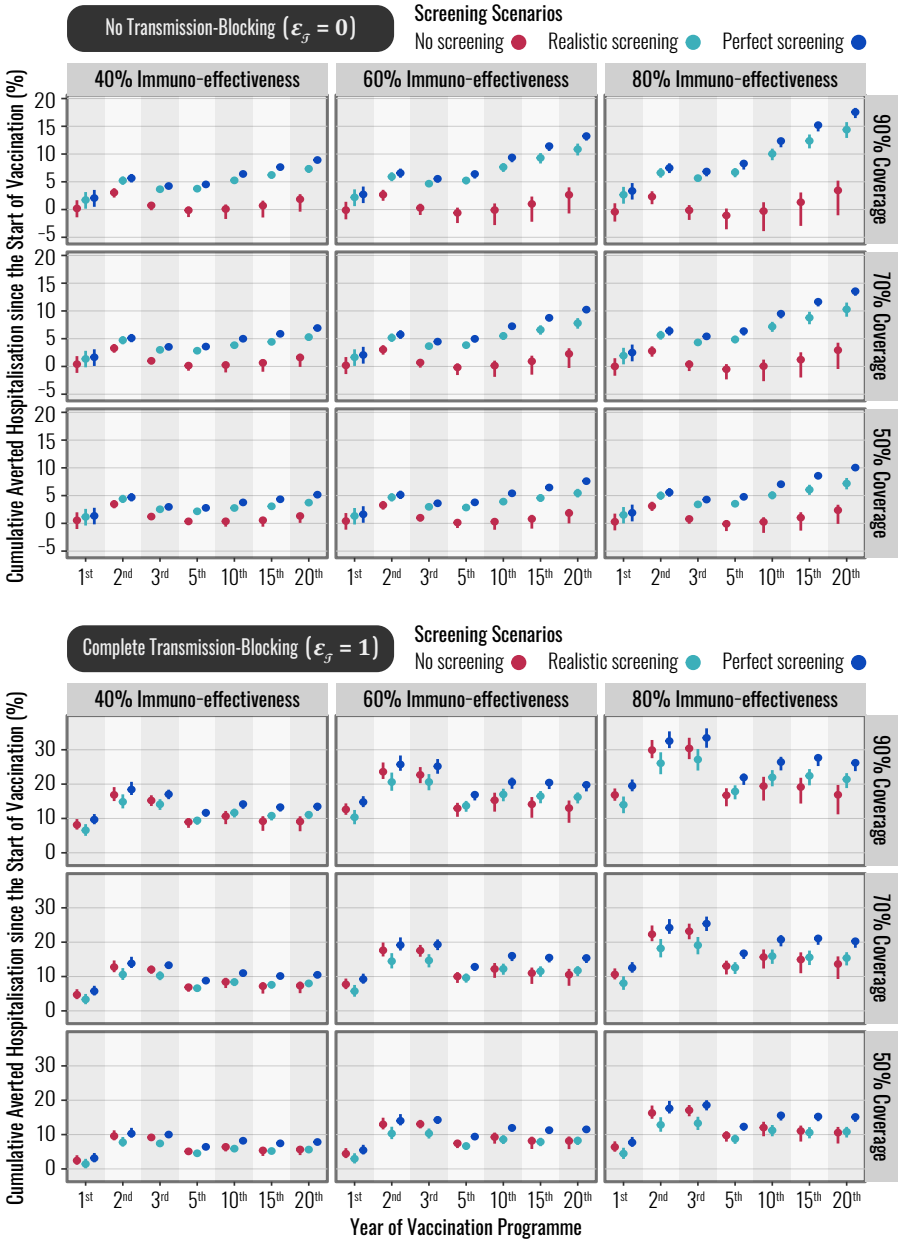


FIGURE D.3 — The *cumulative* percentage of hospitalisations (since the start of the vaccination programme) that would be prevented, estimated by the alternative model. The 9 upper panels illustrate the estimates for the scenario that the vaccine does not block transmission ($\epsilon_{\mathcal{J}} = 0$). Meanwhile, the 9 lower panels show the results for when the vaccine completely blocks transmission during the secondary infection of vaccinees ($\epsilon_{\mathcal{J}} = 1$). Negative percentages indicate increases in the number of hospitalisations. The dots and the whiskers, respectively, represent the median value and the 95% UI of the corresponding estimates, each of which was generated from 1,000 simulations. Note that the scale of the y-axes is different between the upper and the lower panels.



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The end of a melody is not its goal; but nonetheless,
had the melody not reached its end, it would not have reached its goal either.

— Friedrich Nietzsche

COLOPHON

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