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Modeling the Effects of Vaccination on Dengue Pathogenesis Evolution

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Modeling the Effects of Vaccination on Dengue Pathogenesis Evolution

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B.A., Smith College, 2017

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Abstract

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By Ellie Mainou

Theory on evolution of viral replication posits that parasites face trade-offs between transmission and the duration of infection, as both cannot simultaneously be optimized. A higher transmission rate requires higher parasite replication, whereas longer durations of infection (e.g., via a lower clearance rate) requires lower parasite production. Under some circumstances, this trade-off can lead to the evolution of an intermediate level of parasite production, which maximizes the parasite's reproduction rate. Such fitness trade-offs have been empirically demonstrated in dengue. Further, a quantitative analysis of these trade-offs indicate that viral transmission potential depends on dengue's epidemiological context. Specifically, in the case of dengue, peak viral load is highly associated with the manifestation of dengue hemorrhagic fever or dengue shock syndrom (DHF/DSS). Here, we examine how a licensed dengue vaccine (Dengvaxia) may impact the evolution of dengue strains that cause DHF/DSS. Dengvaxia is a recombinant live-attenuated, imperfect vaccine that is thought to act like a silent infection. As such, vaccination with Dengvaxia would alter the epidemiological context in which dengue transmits, which in turn should impact virulence-associated selection pressures on the virus. To examine the potential effect of Dengvaxia vaccination on dengue evolution, we develop a nested, multi-scale model of viral replication and transmission. The model includes deterministic within-host dynamics, which differ by host infection status and the replication phenotype of a viral strain. The model also includes epidemiological dynamics simulated through an individual-based model in a dengue-endemic context. By introducing vaccination into the population, we examine whether Dengvaxia would select for strains that cause more or less DHF/DSS cases. We place our findings in the context of the imperfect vaccine-driven virulence evolution literature.

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Chapter 1

Introduction

1.1 Dengue Background

Dengue is a RNA virus belonging to the Flaviviridae family and is transmitted between humans by *Aedes aegypti* mosquitos (92). It is endemic in tropical and subtropical climates and can be found in over 100 countries. Dengue has geographically expanded, and remarkably increased in incidence with an estimated 390 million cases a year (9). Dengue infection can be subclinical or present a range of symptoms from febrile illness (DF) to dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), which can be life-threatening (1). DF consists of fever, rash, nausea and vomiting, whereas DHF of increased vascular permeability, hemorrhagic manifestation, and in the case of DSS, shock (91).

There are four genetically diverse dengue virus serotypes (DENV 1-4), which maintain similar ecology and pathogenicity (41). Across serotypes there is about 35-40% difference at the amino acid level, whereas viruses within each serotype have approximately 3% divergence (36). Infection provides long-lived homologous protection to reinfection from that same serotype, and temporary heterologous immunity to other serotypes (17; 72).

Primary infections are usually relatively mild but can still cause DF and sometimes DHF. Secondary infections tend to exhibit higher disease severity (75; 31). In fact, it is estimated individuals experiencing a secondary infection are in danger for acute illness by more than six-fold compared to those experiencing their primary infection (12; 39). Almost all of DHF/DSS cases occur during secondary infections (35). Post-secondary infections are the mildest ones and are mostly asymptomatic. It is believed that sufficient cross-protective immunity has been achieved by two different DENV infections to reduce disease severity in

tertiary and quaternary infections (35). Disease severity is also age-dependent with young children being at higher risk for vascular leakage (34; 25). The increased risk for disease severity in secondary infections is attributed to antibody-dependent enhancement (ADE) (40; 35), although this hypothesis is not supported by all studies (71; 49). This enhanced viral replication during secondary infections is moderated mainly by preexisting and non-neutralizing or subneutralizing antibodies to E and prM antigens that enhance access of virions with bound antibodies to FcγR-brearing cells (93; 40; 69). Such cells would not have been effectively infected in the absence of antibodies (54). Even though the phenomenon of ADE is well-documented in vitro, evidence for its impact on human disease remains circumstantial (37; 38; 44; 33; 34; 13).

Dengue control and prevention consists of vector control efforts. Dichlorodiphenyltricholoethane (DDT) was among the first chemicals, and the most widely used, which targeted adult stages of *Aedes* mosquitoes, accomplishing significant reductions in population sizes. However, vector resistance to DDT emerged, which lead the re-emergence of dengue in the 1960s. Although more insecticides are now available, they have a important environmental impact, such as contamination and bioactivation of toxins, which can also impose risks to human health. Alternative methods for vector control include biological control, such as the release of transgenic vectors and environmental management, such as the provision of safe water, covering and screening of water containers and reduction of human-mosquito contact by using screens on doors and windows (18).

Another promising method for controlling dengue and other arboviruses is the use of *Wolbachia* (52; 10). More specifically, *Wolbachia* has been shown to dramatically reduce the vector competence of traninsfected *Aedes aegypti* mosquitoes for dengue. Bian *et al.* demonstrated that *Wolbachia* inhibits viral replication in *Aedes aegypti*. In addition, viral transmission potential of *Wolbachia*-infected mosquitoes was significantly reduced compared to wild-type mosquitos (10). Progress made in the *Aedes aegypti-Wolbachia* system spans the development of *Wolbachia* as a novel control strategy for dengue to its deployment and ongoing evaluation of its impact on dengue transmission.

Considering that no specific treatment for dengue exists and that current interventions

are used to treat symptoms such as blood transfusions to replace lost blood in DHF and control the vector, vaccine development appears to be the only long-term epidemiologically effective method for dengue elimination.

1.2 Overview of Current Dengue Vaccines

1.2.1 Sanofi-Pasteur vaccine (Dengvaxia)

In the early 2000's Sanofi-Pasteur developed Dengvaxia, a recombinant live attenuated chimeric yellow-fever-dengue virus tetravalent vaccine (CYD-TDV). This vaccine consists of four recombinant live attenuated chimeric viral vaccines, each based on the yellow fever vaccine (YFV) backbone (80). The genes that encode for the PrM and E proteins in the YFV are replaced by those from the DENV serotypes for each of the chimeric viral vaccine to create a dengue vaccine (89). Preclinical trials demonstrated that all vaccine candidates were genotypically and phenotypically safe, not likely to return to virulence or infect mosquitoes. Phase IIb trials aimed at determining the vaccine's effectiveness in protecting against symptomatic dengue. Overall, the vaccine did not pose any safety concerns and had strong neutralizing antibody responses to all four serotypes. Regardless, according to IIb trials, vaccine efficacy differed by serotype. The efficacy for DENV-4 was 90.0%, DENV-3 was 81.9%, and DENV-1 was 61.2%. The results for DENV-2 were alarmingly low with an efficacy of only 3.5% (73).

Phase III trials were conducted to evaluate the vaccine's efficacy in decreasing symptomatic and virologically-confirmed dengue cases. These trials were conducted in Southeast Asia and Latin America. The study in Asia reported highest efficacy against DENV-3 (78.4%) and 4 (75.3%), intermediate against DENV-1 (50%) and lowest for DENV-2 (35%). Overall efficacy against virologically-confirmed dengue was 56.5%. Additionally, efficacy was higher in children of age 12-14 years, followed by those of 6-11 years and 2-5 years (14). Similarly, the study in Latin America concluded that general efficacy against virologically-confirmed dengue was 60%. In this case, highest efficacy was reported for DENV-4 (77.8%), followed by DENV-3 (74%), DENV-1 (50.3%) and finally by DENV-2 (42.3%) (86). In both

studies the vaccine was particularly successful against severe dengue disease, dengue hemorrhagic fever and hospitalizations (14; 86).

Dengvaxia is the only licensed dengue vaccine. So far it has been approved in 19 countries with mass immunization campaigns first taking place in the Philippines and Brazil in 2016 and 2017. However, severe controversy regarding its efficacy was prompted by the death of a 10-year old girl in the Philippines which was allegedly attributed to vaccination. The result of the incident was the termination of school-based vaccination programs in the Philippines (32). The main concern is that the vaccine may increase the risk for severe disease in children naïve to dengue virus. Although this is a valid argument, the effectiveness of dengue vaccination should be considered under a general epidemiologic context. The vaccine is recommended in highly endemic countries only, where 90% of the population are likely to be infected with dengue virus by adolescence. For the remaining 10% risk for severe disease remains relatively low (two cases per 1000 individuals) (62).

Dengvaxia is an imperfect vaccine and cannot prevent infection in the long-term. It acts as a silent infection (23; 22), which poses epidemiological risks. If Dengvaxia is administered to a naive individual, it acts as a primary infection, so when that person is naturally infected for the fist time, this infection acts as a secondary one. On the contrary, to individuals who have already had a primary infection vaccination acts a silent secondary infection, so subsequent natural infections will act as post-secondary.

1.2.2 Other dengue vaccine candidates

There are multiple dengue vaccines under development, both in pre-clinical and clinical trials. For an overview of potential dengue vaccines refer to (30; 82; 83; 87; 50; 88; 89; 90). Of these candidates, TAK-003 developed by Takeda Inc successfully completed phase II clinical trials. Results of the study demonstrated that TAK-003 induced sustained antibody responses against all four serotypes of dengue virus, regardless of previous dengue exposure and dosing schedule and that children and adolescents who received TAK-003 had a relative risk of symptomatic dengue of 0.29 compared to children and adolescents in the

placebo control group (74). Phase III trials started in 2017 in eight dengue-endemic countries to evaluate the efficacy of two doses of TAK-003 administered three months apart (78). TAK-003 is expected to obtain license four years after Dengvaxia, and is speculated to be more successful due to its vaccine design: it consists of an attenuated DENV-2 strain and three chimeric viruses containing the prM and E protein genes of DENV-1, -3, and -4 (60).

None of the current dengue vaccines considered is perfect, in that all have shown that natural infection can still occur following vaccination. While imperfect vaccines can reduce both incidence and disease, they can also put selection pressure on pathogens to evolve. Here, we address this possibility in the context of the licensed Sanofi Pasteur Dengvaxia vaccine.

1.3 Disease Severity Evolution and Potential Vaccine Effects

The impact of pathogens on hosts is of important concern. That impact is determined in part by the pathogen's genetic makeup and is therefore subject to selection.

Virulence can be defined as disease severity as measured by reductions in host fitness following infection (66). There are different hypotheses that aim to explain how selection shapes the varying levels of virulence that are exhibited by different pathogens or different strains of the same pathogen. For a review of such theories refer to (66). Here we will focus on the most widely accepted theory.

Initially proposed by Anderson and May (4), this idea stems from the trade-off between transmission rate and and parasite-induced mortality, as expressed by the basic reproductive number, R_0. The R_0 expression for a simple SIR model is

$$R_0 = \frac{\beta S}{\mu + \nu + \gamma}$$

where β is the transmission rate, S is the number of susceptibles in the population, μ is the background mortality rate, ν is the parasite-induced mortality rate and γ is the host recovery. Based on this expression, the number of secondary cases that a single case generates, and can be thought of as parasite fitness, is the product of the rate at which new

infections occur (βS) and the duration of infection $(\mu + \nu + \gamma)^{(-1)}$ (11). Therefore, parasite fitness is increased with higher transmission rates β and by prolonging the duration of infection though decreasing ν. The classically described transmission-virulence trade-off theory assumes that a parasite cannot achieve both simultaneously. When this trade-off occurs between β and ν, increasing transmission rate means that the pathogen is replicating faster, which requires exploiting the host more, which in cases of extreme virulence can lead to host death (increase in ν). Experimental work seems to support such predictions. Read *et al.* (67) found that immunization of chickens against Marek's disease enhances the fitness of more virulent strains and can promote the emergence of strains that are more likely to cause death in unvaccinated hosts.

Another potential trade-off happens between β and γ. Increasing the transmission rate requires exploiting the host more and that can eventually lead to prolonged host recovery. In this case, an increase in β is correlated with an increase in γ. This is known as the transmission-clearance trade-off. This trade-off has been experimentally demonstrated in a malaria mouse model. Mackinnon *et al.* (51) demonstrated that serial passage of malaria parasites increased disease severity (measured as the the minimum red blood cell density in lines of *P. chaubadi*) in both naive and immunized mice, but significantly more in immunized mice.

The transmission-clearance trade-off has been empirically demonstrated in dengue (8). In the context of dengue, disease severity, meaning the manifestation of DHF/DSS, is highly correlated with peak viral load (84). Quantitative analysis indicates that viral transmission potential is maximized at intermediate levels of viral replication and depends on dengue's epidemiological context (8). Specifically, primary infections, although they generally result in less severe dengue disease, select for strains with higher pathogenicity relative to secondary infections (8). Dengvaxia, acting as a silent natural infection, changes the fraction of the population that experience primary, secondary (or secondary-like) infections, thus altering the epidemiological context in which dengue transmits. This in turn should impact pathogenesis-associated selection pressures on the virus.

Chapter 2

Methods

To examine the potential effect of Dengvaxia vaccination on dengue disease severity evolution, we develop a nested, multi-scale model of viral replication and transmission. The model includes deterministic within-host dynamics, which differ by host infection status and the pathogenesis-associated phenotype of a viral strain. The model also includes epidemiological dynamics simulated through an individual-based model in a dengue-endemic context. The within-host and epidemiological model are linked through the transmission probability from humans to mosquitoes: R_0 depends on within-host dynamics and onward transmission to mosquitoes and back to humans. By introducing vaccination into the population, we examine whether a vaccine that acts as a silent infection, like Dengvaxia, would select for strains that cause higher or lower disease severity.

2.1 Within-Host Model

Transmission of dengue from humans to mosquitoes through a blood meal depends on the the viral load of the infected host: the higher the viral load, the greater the probability of transmission from an infected human to a susceptible mosquito (58). Here we use a within-host model to simulate the viral dynamics within a human host experiencing either a primary or a secondary infection.

Our within-host model is adopted by Ben-Shachar & Koelle (8) and is described by the following system of differential equations:

$$dX/dt = -\beta XV \tag{2.1}$$

$$dY/dt = \beta XV - \alpha NY - \delta_T YT \tag{2.2}$$

$$dV/dt = \omega Y - \kappa V \tag{2.3}$$

$$dN/dt = qY - d_N N \tag{2.4}$$

$$dT/dt = q_T YT \tag{2.5}$$

where X is the number of uninfected cells, Y is the number of infected cells, V is the the free-virus concentration, N is the number of natural killer (NK) cells, and T is the number of T-cells.

The number of uninfected cells is reduced by infection caused by free-viruses at rate β. The number of infected cells increases due to new infections and decreases by clearance due to NK cells and T-cells at rates α and δ_T respectively. Free virus concentration increases through production of free-virus at rate ω and decreases due to free virus clearance at rate κ. NK cells are produced at rate q and decay at rate d_N. T-cells increase in numbers due to their interaction with infected cells at rate q_T.

We assume that T-cells are important in clearing infected cells in secondary infections, but not in primary ones. We also assume that all four serotypes follow the same within-host dynamics. Parameterizations and initial conditions values are taken from Ben-Shachar & Koelle (8). Disease severity in the context of dengue is DHF/DSS manifestation and it correlated with peak viral load. For this reason strains that cause elevated disease severity

TABLE 2.1: Within-Host Model Parameter Values

Param	Description	Value	Units
β_{PI}	viral infectivity rate (primary)	$3.11 * 10^{-10}$	(genome copies/ml)$^{-1}$ day $^{-1}$
β_{SI}	viral infectivity rate (secondary)	$3.95 * 10^{-10}$	(genome copies/ml)$^{-1}$ day $^{-1}$
α	innate IR clearance rate	0.001	day $^{-1}$
κ	viral clearance rate	5	day $^{-1}$
q_T	T-cell activation rate	$4.84 * 10^{-5}$	(cells/ml)$^{-1}$ day $^{-1}$
q_{PI}	innate IR activation rate	0.23	day $^{-1}$
q_{SI}	innate IR activation rate	0	day $^{-1}$
δ_T	T-cell clearance rate	10^{-6}	day $^{-1}$
ω_L	viral reproduction rate (low)	$0.5 * 10^4$	(genome copies/cell)$^{-1}$ day $^{-1}$
ω_H	viral reproduction rate(high)	$2.3 * 10^4$	(genome copies/cell)$^{-1}$ day $^{-1}$
d_N	NK cell decay rate	0	day $^{-1}$
X_0	initial uninfected cells	10^7	cells/ml
Y_0	initial infected cells	0	cells/ml
V_0	initial viral load	10^3	genome copies/ml
N_0	initial NK cells	0	cells/ml
T_0	initial T-cells (primary)	0	cells/ml
T_0	initial T-cells (secondary)	10^5	cells/ml

have a different replication rate, ω. Higher replication rates, lead to higher peak viral load and therefore to higher risk of developing severe dengue disease. Figure 2.1 a depicts peak viral loads for primary and secondary infections for both types of dengue strains.

2.2 Epidemiological Model

Population-level dynamics are simulated through an individual-based model that consists of 60,000 humans and 48,000 mosquitoes (Fig. 2.2). We assume that mosquito lifespan is exponentially distributed with mean 7 days. The transmission probability from human to mosquito is determined based on an individual's viral load (Fig. 2.1). Once a mosquito is infected with one of the four dengue serotypes, it does not clear the infection. We set the extrinsic incubation period (EIP), i.e. the amount of time since a mosquito gets infected until it is able to transmit lasts 7 days (65; 81). We further assume that mosquitoes cannot be coinfected with multiple dengue serotypes. For each mosquito simulated, we keep track of its age, the serotype it is infected with and the time since infection. Specific mosquito

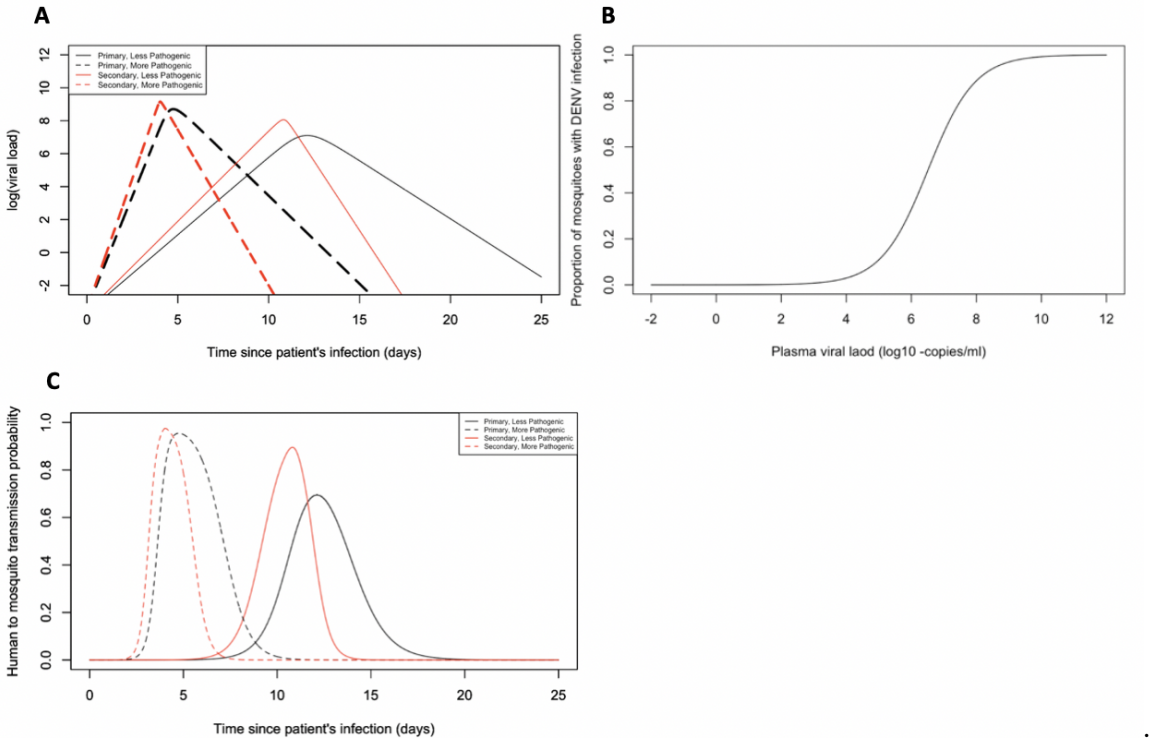

FIGURE 2.1: **Within-host dynamics and their effect on transmission to mosquitoes.** A) Within host viral dynamics for primary and secondary infections for less and more pathogenic strains. More pathogenic strains are associated with higher peak viral loads. B) Proportion of mosquitoes that become infected upon blood meal as a function of an individual's viral load. C) Human-to-mosquito transmission probability over the course of an infection for primary and secondary infections for less and more pathogenic strains.

within-host dynamics, other than duration of latency, are not taken into consideration.

Human population size remains constant throughout simulations. This means that the birth rate equals to the death rate. To maintain any epidemic, it is important to replenish the susceptible population. For that reason we set the human lifespan to 35 years, to match the birth rate. We vaccinate children at the age of nine, with an 80% coverage rate. We model vaccination as a non-circulating fifth serotype. We simulate primary and secondary infections and assume they last 25 days. Since post-secondary infections are assumed to be asymptomatic and not transmissible we omit them for simplicity and assume that individuals are immune to dengue after their secondary infection. After a primary infection, humans gain permanent homologous protection and a half-year cross-protection. Infection with a heterologous serotype during cross-protection results in seroconversion and the individual

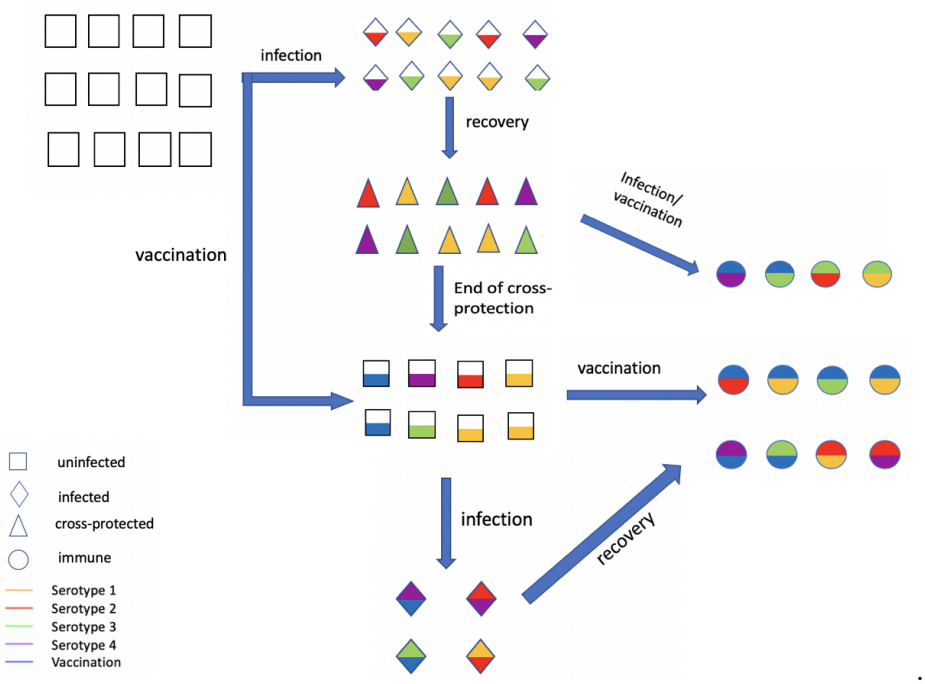

FIGURE 2.2: **Epidemiological, individual-based model.** Individuals of different categories are modeled as different shapes and different serotypes are indicated by different colors. Because vaccination acts as a non-contagious, asymptomatic infection, it is modeled as a fifth non-circulating serotype. Colors in the bottom half of rhombi and squares indicate the serotype of primary infection, whereas the colors of the top half represent the serotype of secondary infection.

is not symptomatic or infectious (56). We assume that humans and mosquitoes mix homogeneously and that individuals infected with different serotypes transmit similarly. We do not account for seasonal effects (Table 2.2).

2.3 Interface Between Epidemiological Model and Within-Host Dynamics

Individual viral load determines the transmission probability of dengue from an infected individual to a susceptible mosquito when the mosquito receives a blood meal. To determine the transmission potential we rely on a study (58), where susceptible mosquitoes

fed on hospitalized dengue patients. Host viremia was determined to be the strongest covariate explaining human-to-mosquito infection. The authors quantified the relationship between host viral load and human-to-mosquito transmission probability using a logistic regression. We use this relationship to couple the dynamics of the within-host model $V(\tau)$ with the transmission probability from an infected human to a susceptible mosquito $p(V(\tau)) = \frac{1}{1+e^{-(\epsilon_0+\epsilon_1 V(\tau))}}$ (8). We use $\epsilon_0 = -9.04$ and $\epsilon_1 = 1.39$ (Figure 2.1B). Using this function we obtain the probability that a susceptible mosquito becomes infected after a blood meal from an infected patient, given the patient's time since infection for primary and secondary, wild-type and mutant serotype infections. (Figure 2.1 C). The area under the curve is equal to $\frac{T_H}{\mu_H+\gamma_H}$, where T_H is the human-to-mosquito transmission probability, μ_H is the human death rate and γ_H is human recovery rate.

2.4 Parameter Values and Initial Conditions

In our model we simulate a high transmission setting, $R_0 \approx 5$ (23). Using R_0 we determine the remaining parameters of the model. According to Keeling and Rohani ((43) page 138), for a vector-borne infection, R_0 is given by the equation

$$R_0 = \frac{b^2 T_H T_M N_M}{\mu_M(\gamma_H + \mu_H)N_H} \tag{2.6}$$

where b is the mosquito biting rate, T_H is the (assumed constant) transmission probability from human to mosquito, T_M is similarly the transmission probability from mosquito to human, μ_M and μ_H are the mosquito and human death rates respectively, γ_H is the constant human recovery rate and N_M and N_H are the mosquito and human population sizes.

We define $b = 0.5$ bites per day, which is consistent with previous estimates (55; 19; 29). T_H does not remain constant because it depends on the patient's viral load. If we integrate the area under the curve in Fig. 2.1C we obtain the expression $\frac{T_M}{\gamma_H+\mu_H}$. For primary infections the area under the curve is 3.01 and 3.50 for the less and more pathogenic strains respectively and for secondary infections it is 2.78 and 2.34 for the less and more pathogenic strains respectively. We have that $\mu_M = \frac{1}{8.75}$ per day, define $T_M = 0.95$ and maintain a ratio

TABLE 2.2: Epidemiological Model Parameter Values

Parameter	Description	Value	Unit
y	extrinsic incubation period	7	days
v	vaccination coverage	0.8	per year
h	period of cross protection	183	days
m	number of migrant mosquitoes	2	per strain per year
b	biting rate	0.5	per day
γ_H	human recovery rate	$\frac{1}{25}$	days
μ_H	human death rate	$\frac{1}{35}$	per year
μ_M	mosquito death rate	$\frac{1}{8.75}$	per day
T_H	human to mosquito transmission rate	estimated	
T_M	mosquito to human transmission rate	0.745	
N_H	human population size	$60,000$	humans
N_M	mosquito population size	$48,000$	mosquitoes

of the populations $\frac{N_M}{N_H} = 1.25$ to obtain $R_0 = 5.01$ for primary infections. We assume that R_0 is the same for all four serotypes.

Human and mosquito population sizes are determined somewhat arbitrarily. Our aim is to have population sizes that are large enough to sustain an epidemic, despite being constrained by computational power. We determine $N_H = 60,000$ and $N_M = 48,000$. To stabilize dynamics, we introduce a low migration rate of two infected mosquitoes per year for each serotype.

To investigate how vaccination affects evolution of pathogenicity, we run four different simulations. In the first simulation we start with an unvaccinated population, where only the less pathogenic strains circulate. We introduce the more pathogenic strains and hypothesize that they will not be able to invade the population. For the second simulation, only the more pathogenic strains circulate in the unvaccinated population and we introduce the less pathogenic ones. We predict that they will be able to invade the population. For the third and fourth simulations we have a vaccinated population. At time zero we implement a catch-up vaccination campaign where 80% of those who are nine years old and older get vaccinated, and then each year 80% of 9-year old are vaccinated. Only the less pathogenic stains circulate in the in the third simulation and we introduce the more pathogenic ones. The contrary happens in the fourth simulation. We predict that more pathogenic strains will be able to invade the vaccinated population in the third simulation, whereas those lower

TABLE 2.3: List of Model Simulations

Simulation	Vaccination Status	Circulating Strains	Strains Introduced
A	Unvaccinated	Low pathogenesis	High pathogenesis
B	Unvaccinated	High pathogenesis	Low pathogenesis
C	Vaccinated	Low pathogenesis	High pathogenesis
D	Vaccinated	High pathogenesis	Low pathogenesis

pathogenesis will not be able to reach high frequencies in the fourth simulation (Table 2.3). In all cases we assume that infection with the less or more pathogenic strain of a serotype confers life-long protection to the more or less pathogenic strain of the same serotype.

We aim to start simulations near the steady state. According to Keeling and Rohani ((43), pages 27-29), for a single-strain model, at equilibrium $\frac{\hat{S}}{N} = \frac{1}{R_0}$, $\frac{\hat{I}}{N} = \frac{\mu_H(1-\frac{1}{R_0})}{\gamma_H}$ and $\frac{\hat{R}}{N} = 1 - \frac{1}{R_0} - \frac{\mu_H(1-\frac{1}{R_0})}{\gamma_H}$. These expressions can be used as a rough approximation for endemic conditions in our model, yielding $(\hat{S}, \hat{I}, \hat{R}) = (11,973, 94, 47,933)$ for each serotype. We start our simulations with 94 infected individuals for each of the four dengue serotypes. Given that an individual could have experienced a primary only or a primary and a secondary infection and that the probability of having been infected increases with age, we use a probabilistic function to decide which individuals will be infected and how many infection they have had. For each serotype, the probability of having already been infected by age t years is given by $y(t) = 1 - e^{\lambda t}$, where λ is the force of infection. For an endemic disease, where homogeneous mixing and a rectangular population distribution are assumed, as in this case, $\lambda = \frac{1}{A} = \frac{1}{\frac{L}{R}} = \frac{1}{A}$, where A is the average age of infection and L is the human lifespan. To determine the initial number of infected mosquitoes, we know that the number of mosquitoes infected by an infectious human is $\frac{bT_H N_M}{(\gamma_H+\mu_H)N_H}$. Multiplying this number by $\hat{I}$, yields 114 infected mosquitoes with each serotype.

Chapter 3

Results

All four simulations are characterized by high levels of stochasticity and large epidemics that fade out. For example, in Fig3.3B strain 3 of high pathogenicity remains at very low frequency throughout the simulation and causes a large outbreak at the end. Most primary infections occur to individuals younger than 9 years of age (Fig. 3.1C, Fig. 3.3C, Fig. 3.5C, Fig. 3.7C), whereas almost all secondary infections are manifested in indivduals older than 9 years(Fig. 3.1D, Fig. 3.3D, Fig. 3.5D, Fig. 3.7D), which is consistent with an endemic setting. Populations where highly pathogenic strains initially circulate generate considerably higher numbers of dengue infections, compared to populations where low pathogenic strains are initially present. Vaccination reduces greatly the number of infections, with higher gains obtained in simulation D.

In both simulations of unvaccinated populations, higher virulence strains persist in the population. In simulation A, the total number of cases caused by low pathogenesis strains is almost equal to that caused by high pathogenesis strains, although their frequencies differ at different times. On the contrary, in simulation B, highly pathogenic strains cause almost three times as many infections during the 15-year run of their coexistance. Dengue seroprevalence in 9-year olds (SP9) starts of high at around 90% and around year 10, reaches a very low minimum, probably due to the depletion of susceptibles and increases again to around 60% (Fig. 3.2C, Fig. 3.4C). In addition, most infections are secondary in the beginning and by around year 15, primary infections slightly outnumber secondary ones (Fig. 3.2B, Fig. 3.4B).

Similarly, in vaccinated populations low pathogenicity strains reach higher frequencies than lower pathogenicity ones. In simulation C, the difference between the two is only approximately $3,000$ cases over the 15 years of their coexistance, whereas in simulation D low pathogenicity strains cause twice as many infections as high pathogenicity ones. Secondary infections are at slightly higher proportion than primary ones, until approximately year 25 when primary infections begin to outnumber secondary ones (Fig. 3.6B, Fig. 3.8B). SP9 follows a similar trend to the unvaccinated populations, but vaccination raises the percentage to around 90% (Fig. 3.6C). Despite the positive effects of vaccination in reducing dengue cases, still a large number of individuals ($10,000$) before infected after vaccination (Fig. 3.6C, Fig. 3.8D). Such infections are primary but behave as secondary ones, raising the risk for DHF/DSS.

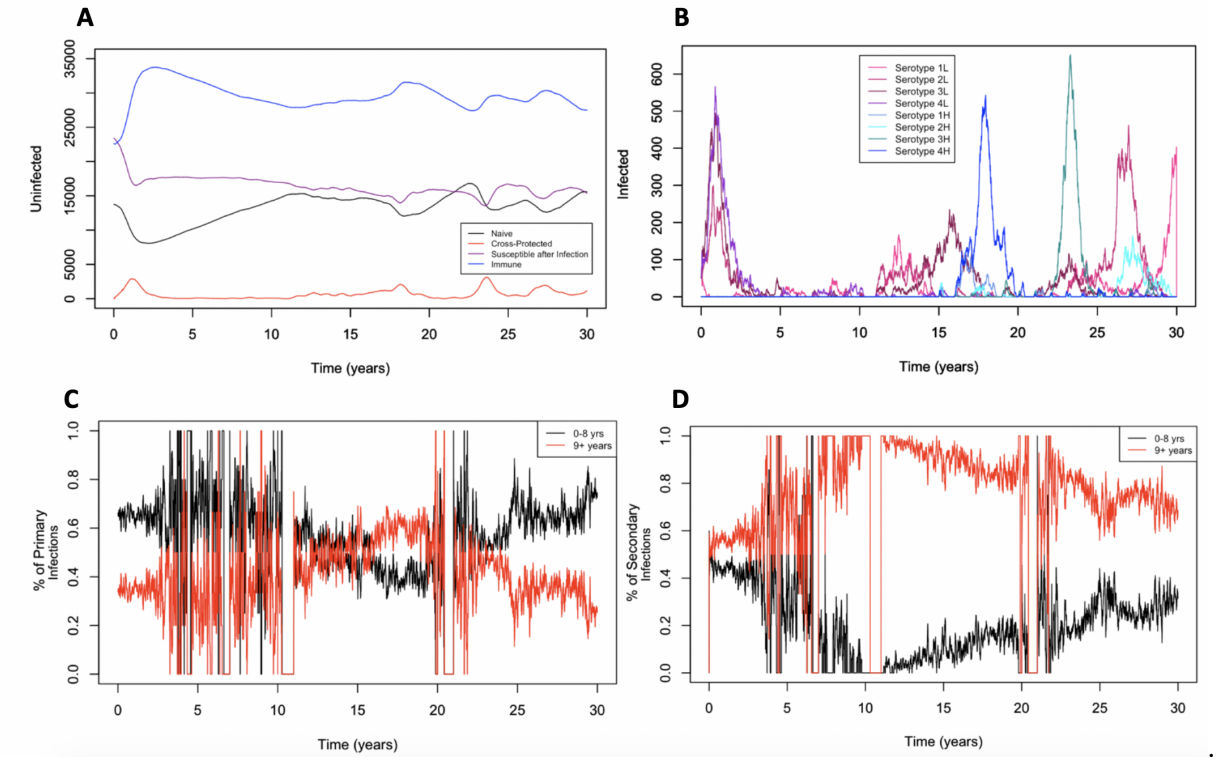

FIGURE 3.1: **Dynamics of Simulation** *A*. A) Uninfected population over time. B) Frequency of each strain over time. C) Age-class distribution of primary infections. D) Age-class distribution of secondary infections.

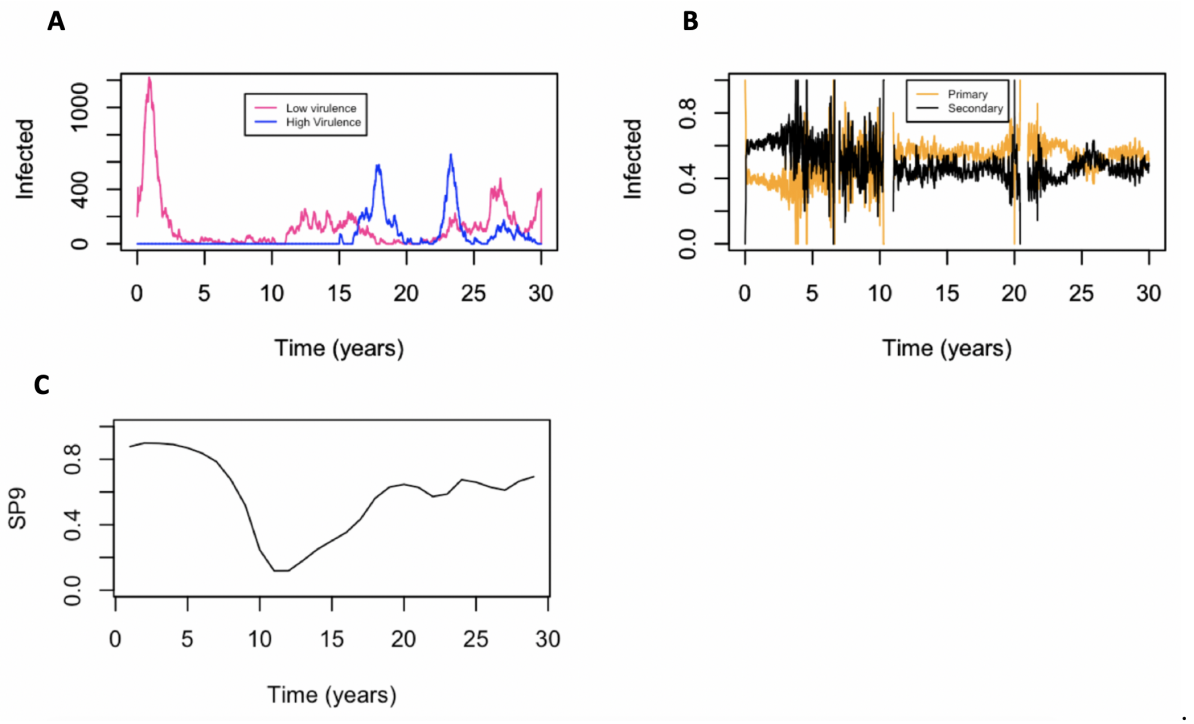

FIGURE 3.2: **Dynamics of Simulation** *A*. A) Frequency of all lower virulence and higher virulence strains. B) Proportion of primary and secondary infections. C) Percentage of 9 year-old children that are seropositive to dengue.

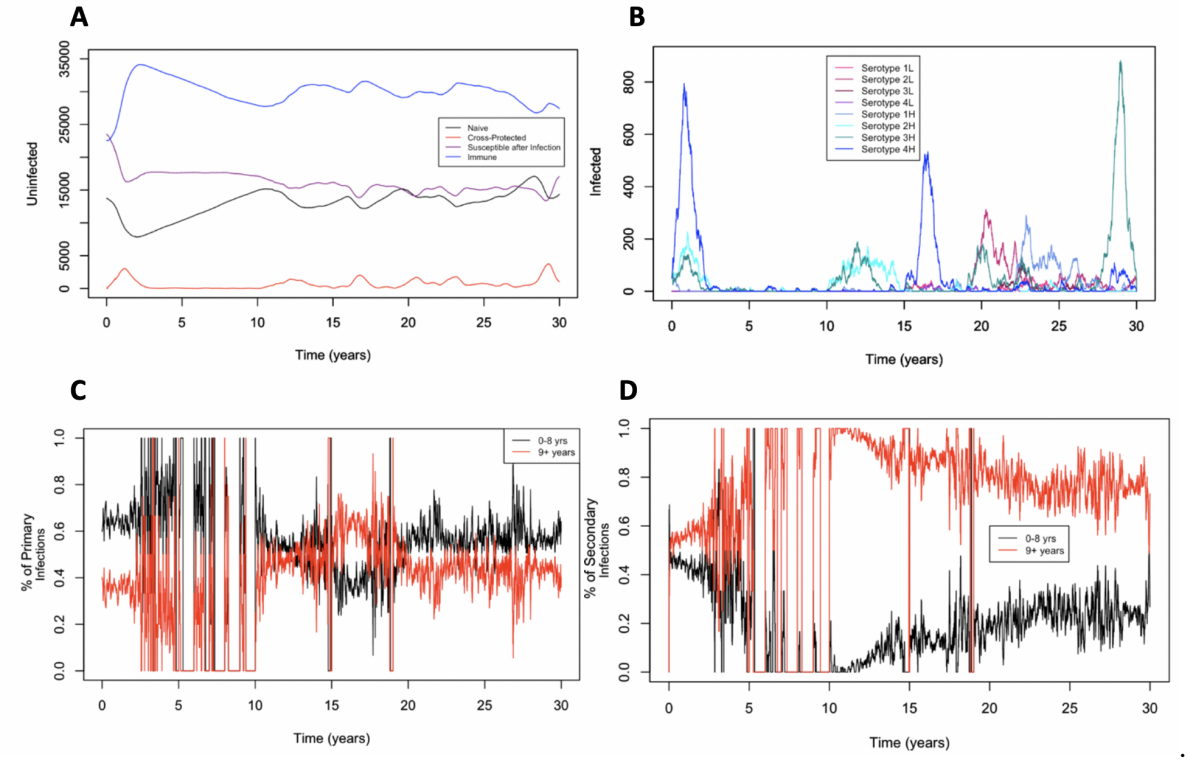

FIGURE 3.3: **Dynamics of Simulation** B**.** A) Uninfected population over time. B) Frequency of each strain over time. C) Age-class distribution of primary infections. D) Age-class distribution of secondary infections.

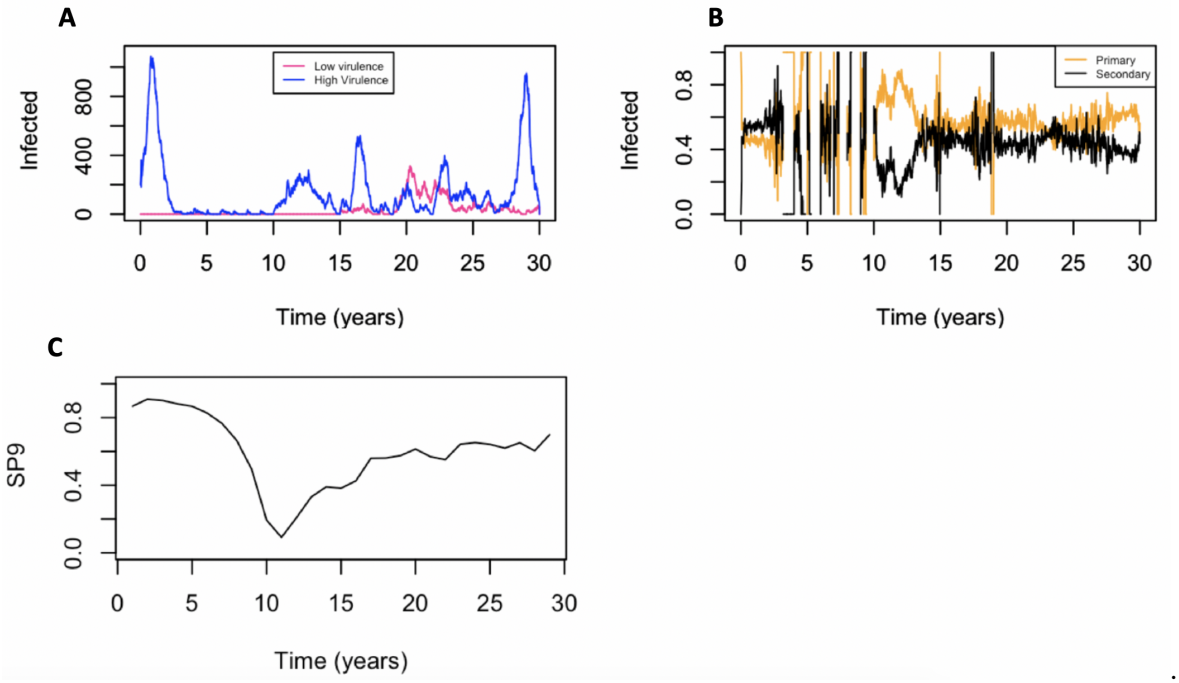

FIGURE 3.4: **Dynamics of Simulation** *B***.** A) Frequency of all lower virulence and higher virulence strains. B) Proportion of primary and secondary infections. C) Percentage of 9 year-old children that are seropositive to dengue.

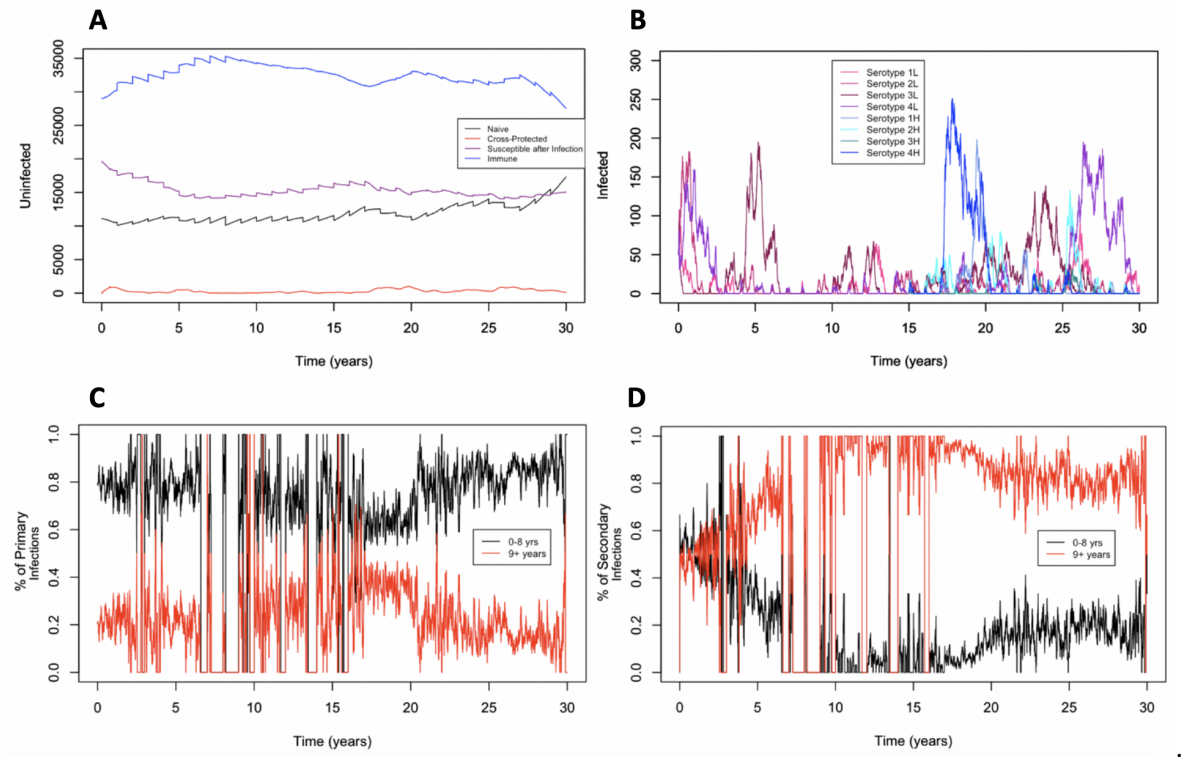

FIGURE 3.5: **Dynamics of Simulation** C**.** A) Uninfected population over time. B) Frequency of each strain over time. C) Age-class distribution of primary infections. D) Age-class distribution of secondary infections.

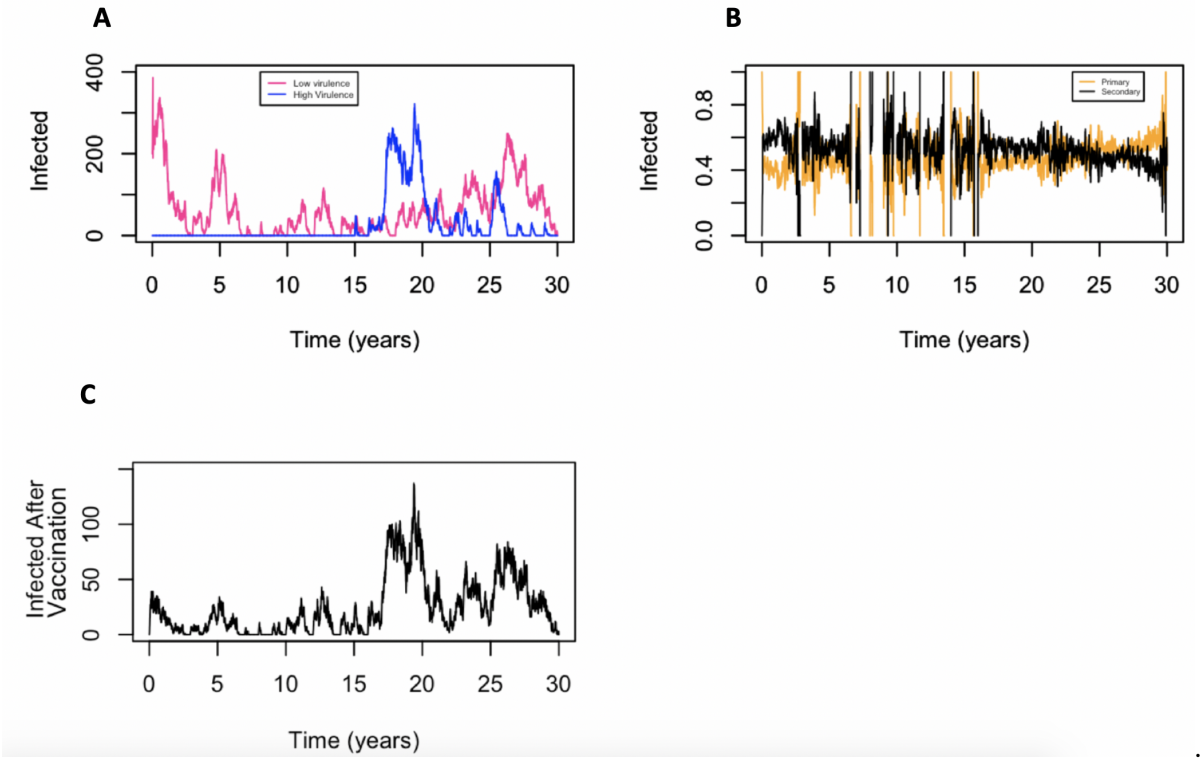

FIGURE 3.6: **Dynamics of Simulation** C**.** A) Frequency of all lower virulence and higher virulence strains. B) Proportion of primary and secondary infections. C) Number of individuals infected after vaccination. These natural primary infections are secondary-like.

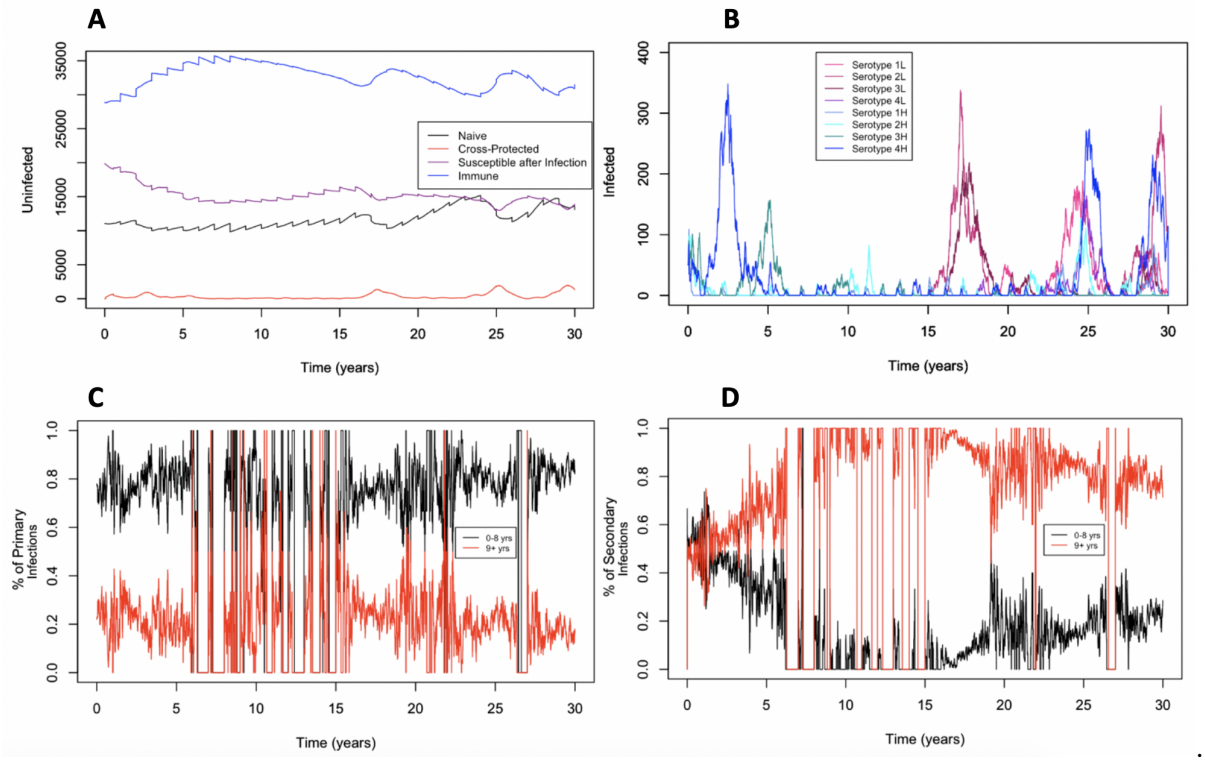

FIGURE 3.7: **Dynamics of Simulation** D**.** A) Uninfected population over time. B) Frequency of each strain over time. C) Age-class distribution of primary infections. D) Age-class distribution of secondary infections.

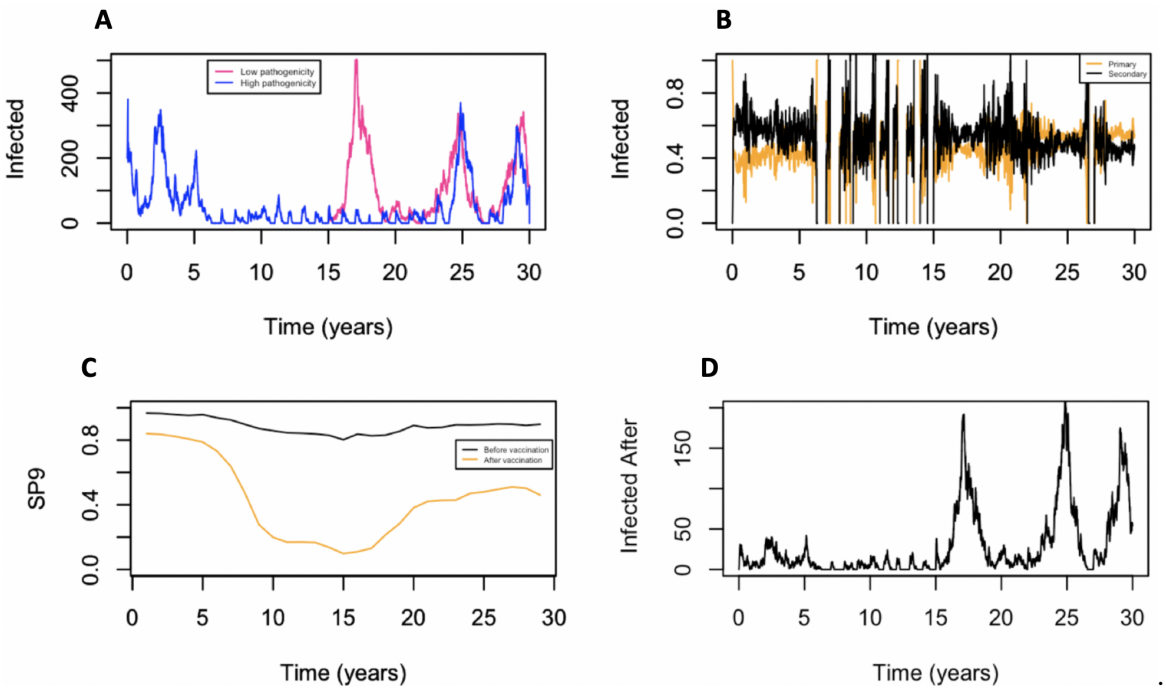

FIGURE 3.8: **Dynamics of Simulation** *D*. A) Frequency of all lower virulence and higher virulence strains. B) Proportion of primary and secondary infections. C)Percentage of 9 year-old children that are seropositive to dengue before and after vaccination. D) Number of individuals infected after vaccination. These natural primary infections are secondary-like.

Chapter 4

Discussion

In this body of work we explored the effects of vaccination on dengue pathogenicity evolution by developing a nested multi-scale model of within-host and epidemiological dynamics. We implement this model through simulations of both unvaccinated and vaccinated populations where dengue strains of lower or higher pathogenicity circulate and introduce strains of opposite pathogenicity. We find that in unvaccinated populations high pathogenicity strains reach higher frequencies, whereas in vaccinated populations low pathogenicity strains maintain greater frequency. Interestingly, in all four simulation low and high pathogenicity strains coexist, with no type of strain being led to competitive extinction. It appears that the selective advantage of each type of strain is not strong enough.

Introduction of newly imported strains requires pre-existing diversity in the replication phenotype of dengue virus. Within-host mosquito dengue dynamics provide suitable conditions for dengue variants to arise and for genetic diversity to persist at the metapopulation level. Even though there are population bottlenecks during mosquito infection (65), dengue, as an RNA virus, has a high mutation rate and during replication in the salivary glands this genetic diversity is replenished by *de novo* mutations (46; 47). This means that there are opportunities for dengue variants, and in this case highly pathogenic variants to arise. The question that we are interested in is whether vaccination poses enough of a selective pressure for these variants to rise in frequency and persist in the population.

In this model we made a number of assumptions that greatly influence the results.

The most important is the exclusion of post-secondary infections. Post-secondary infections were not incorporated in this analysis due to low risk of apparent disease and high rates of cross-reactive antibodies for this group (59). Still there is no conclusive evidence about viremia of post-secondary infections (59). Even though post-secondary infections are asymptomatic, they could significantly impact transmission dynamics, considering that the majority of mosquito infections are derived from asymptomatic humans (16; 79). That of course would depend on the infected individual's viral load over the course of an infection (58): if viremia during post-secondary infections is high enough to result in mosquito transmission that should, in turn, affect human transmission.

Additional assumptions are made in regards to the mosquito part of the epidemiological model. Mosquito dengue dynamics are largely unknown (65). Yet coinfection with multiple dengue serotypes seems not to happen in mosquitoes (or humans (61)). In addition, we assume that there are no differences in behavior between infected and uninfected mosquitoes. There is evidence that dengue infection affects *Aedes aegypti* feeding behavior. Infected mosquitoes have a significantly longer time required for feeding, as well as time spent probing compared to uninfected mosquitoes (64). The increased time needed for infected mosquitoes to obtain a blood meal may affect the efficacy of *Aedes aegypti* as a vector to dengue virus (64).

Several factors that influence transmission heterogeneity were excluded from our modeling framework. To begin with, we assume that the force of infection is constant across time and dengue serotypes. Instead, transmission varies seasonally and from year to year (7; 53; 57; 70; 76). There is also evidence for variation in the force of infection across dengue serotypes (68). In addition, we assume homogeneous mixing of humans. Yet, it appears that spatial dyanamics are an important driver of dengue dynamics. According to Stoddard *et al.*, human house-to-house movement plays a central role in defining individual infection risk, local patterns of incidence and heterogeneity in rates of DENV transmission in Iquitos, Peru (77).

Apart from these single sources of heterogeneity, multiple factors could have synergistic impacts on DENV transmission (85). For example, disease severity might be positively

associated with viral load (84), which should lead to increased mosquito infectiousness. In addition, due to fever and other symptoms, it could be expected that mosquitoes are more attracted to severely diseased individuals and more likely to successfully obtain a blood meal from them. On the contrary, a negative coupling between human mobility and disease severity could reduce the cumulative number of human-mosquito contacts during the infectious period by reducing human movement due to the severity symptoms (85). The interplay between such factors greatly influences transmission dynamics and potentially affects the efficacy of dengue control policies and vaccination strategies, especially when vaccination allows for breakthrough infections (63).

The greatest limitation of this work is the complexity of the model. Initial work consisted of developing an ordinary differential equation model, based on the Duke model in Flasche *et al.* (23). However, that model was very intricate in its implementation and, instead, we decided to switch to an individual-based model, which was highly more straightforward. Still, the individual-based model presented major computational intensity. That intensity determined the size of the human and the mosquito populations, as well as the time frame for which the simulations were run. Although the population sizes allow the epidemic to persist, there is still a lot of stochasticity and unstable dynamics among individual strains. In many cases, a single strain takes over, while others remain at very low frequencies for a short period of time. In addition, we notice that certain strains go extinct and are reintroduced through migration. The goal of immigration was to stabilize dynamics, but instead allows strains to circulate again in the population. This stochasticity could be influencing the relative frequencies of lower pathogenicity compared to higher pathogenicity strains. For example, in Simulation B, strain 2H remains at very low frequencies or is even lost throughout the run, whereas strain 3H starts off at low frequencies and toward the end of the run creates a large epidemic (Fig. **??**B).

These limitations inform possible directions for future work. It would be interesting to explore the outcomes of simulations containing larger human and mosquito population sizes for a time frame that spans at least 50 years. Such modifications could potentially reduce the aforementioned stochasticity that we observe in the current dynamics. It would

also be imperative to create replicates of these simulations. This would confirm whether the results we report here are repeatable or have been obtained due to chance. Further, to investigate whether the selective pressure is strong enough, we should simulate dynamics with different within-host viral replication rates (ω) that would yield greater differences in the transmission potentials of the two types of strains. In addition, it would be beneficial to perform a sensitivity analysis. Identifying the key parameters that are a major influence in the model, can reveal information about the dynamics and possibly inform better vaccination policies.

Other possible future work includes incorporating more realistic aspects of a dengue epidemic. For example, we could incorporate differences among DENV serotypes, such as that DENV-2 is the most pathenogenic (24). Even though such additions make the model more realistic, they also add complexity and computational intensity, which we do not believe would alter considerably the dynamics that we observe. Finally, we could establish a different value for R_0 for our simulations. R_0 determines the epidemiological context in which dengue transmits, and even though a value of $R_0 = 5$ has been used for modeling dengue epidemics in high transmission settings (23), there are estimates that range from 3-36, depending on the method of estimation and area of data collection (15; 20; 21; 42; 56).

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