Inapparent and Vertically Transmitted Infections in Two Host-Virus Systems

Submitted by Martin David Grunnill to the University of Exeter

as a thesis for the degree of

Doctor of Philosophy in Biological Sciences

In October 2015

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……..

Acknowledgements

This PhD would not of happened without NERC giving me the funding, the University of Exeter providing the facilities to carry out the research and the supervision (given with patient supportive advice) of Prof Mike Boots. I would like to thank Dr Katherine Roberts and Paweł Sierciński for crucial advice on DNA extraction, storage and PCRs, Thomas Holding for advice on coding in general and the much needed weekend use of his computer to run simulations, Dr Ben Ashby for advice on Matlab, Steven Sharpe and Drew Wilson for advice on *Plodia interpunctella* and help in maintaining colonies of them.

Nicos Antzoulatos, Lewis Campbell and Richard Woods it has been a great and fun time living with you at 90 Killigrew St. The Jacobs Ladder pub quiz team, the post-grad society, times spent in the ant room you have provided a much needed break from PhD work. Other friends I have made in the 3.5 years of PhD based in Cornwall, there have been lots of you and as such there is not enough room to mention here. Cornwall based friends it has been great time getting to know you and I will miss you.

Oli Padget it has been great having phone conversations with you, about each other's science and speculating over such matters as to whether or not psychopathy (being selfish and incapable of empathy, not necessarily a serial killer) was a negatively frequency selected evolutionary trait.

Chris Rutherford, Lucy Gettings, Simon Stead, Becca Oddman Stonehouse, Miles Baillie thank you for keeping me sane when writing this thesis and proof reading the odd chapter.

Of course no ridiculously long thank you speech or acknowledgements goes without thanking family. Mum, Dad and Grandparents you have been very supportive over the years and I doubt I would be submitting this thesis without that support.

Abstract

Despite the advances made since the advent of germ theory, infectious diseases still wreak havoc on human societies, not only affecting us directly but impacting the crops and livestock upon which we rely. Infectious diseases also have dramatic effects on wildlife ecology. Therefore research into infectious diseases could not only directly lead to the improvement and saving of human lives, but aid in food security and the conservation of many wildlife species. Of vital importance in understanding the ecology of infectious diseases are the mechanisms by which they persist in host populations. One possible mechanism is vertical transmission: the transmission of a pathogen from a parent to its offspring as a result of the process of host reproduction. Another possible mechanism is inapparant infections, where an infected host does not display symptoms. Focusing on dengue fever and the *Plodia interpunctella* granulovirus laboratory system, this PhD thesis looks at the role these two mechanisms play on the persistence of two viral infections and their ecology. Regarding the *Plodia interpunctella* granulovirus (PiGV) low host food quality led to greater detection of vertically transmitted inapparant PiGV, but did not lead to its activation to an apparent form. Host inbreeding did not lead to vertically transmitted inapparant PiGV's activation, nor had an effect on its vertical transmission. The vertical infection rate of PiGV was very low. I would therefore suggest that it may be better to use an insect virus system with a higher rate of vertical infection in future research into vertically transmitting inapparent infections. Regarding dengue virus I conclude that vertical transmission is not likely to play a role in the persistence of this virus. However modelling work found that inapparent infections could provide dengue viruses with a means of persistence and should be subject to further research.

Contents Table

Table of Figures

Table of Tables

Table of Equations

Author's declaration and publications arisen from this PhD

All the work reported in this thesis is my own, conducted under the supervision of Professor Mike Boots with advice obtained from him, Dr Katherine Roberts, Thomas Holding, Paweł Sierciński, Dr Ben Ashby, Steven Sharpe and Drew Wilson. Chapter 4 details my work within a group project within Professor Mike Boots' laboratory group. Dr Katherine Roberts, Steven Sharpe and Drew Willson produced the inbred and outbred *Plodia interpunctella* populations. Dr Katherine Roberts then conducted a PiGV dose response experiment on the first generation of those *Plodia interpunctella* populations. Some of the *Plodia interpunctella* surviving that experiment where then used to produce a second generation for the experiment detailed in Chapter 4.

At the time of submission a shortened version of Chapter 5 has been accepted for publication in the Journal of Medical Entomology.

Definitions of terms and acronyms specifically coined within this PhD Thesis

Acronyms

Insect specimen caught in the field as an egg, larvae or pupae and Raised to Adulthood in the Laboratory (RAL)

Chapter 1: Introduction

1.1 General introduction

Even today with all the advances in technology and understanding made since Koch's postulates gave clarity to germ theory in 1890 (Goering et al. 2008a), infectious diseases still wreak havoc on human societies. HIV and diarrheal diseases were each responsible for 1.5 million deaths in 2012, making them the sixth and seventh global cause of death that year (WHO 2012a). The WHO using the measure Disability Adjusted Life Years (DALY), which combines the number of years lost from the standard life expectancy and years lived with disability (WHO 2015e), estimate that 430,000,000 years of healthy life were lost due to infectious disease in 2012 (WHO 2012b).

Infectious diseases have often shaped human history, through the sheer scale of lives lost in epidemics. The Black Death of the 1340s, caused by *Yersinia pestis*, is thought to have killed 25 million people in Europe (Goering et al. 2008e). In England alone, approximately 35% of the population may have died in the space of two and a half years (Goering et al. 2008e). This dramatic loss of life is thought to have either brought about or hasten the end of feudalism (Fasulo 2008). Similarly the introduction of disease from Europe to the Americas, aided in the European conquest of the Americas (Diamond 1997). For example nearly half the population of the Aztecs died due to the arrival of smallpox in 1520, aiding the comparably unaffected troops of Hernán Cortés in the conquest of the Aztecs (Diamond 1997). Often accompanying wars and other such political upheavals, infectious diseases can cause similar morbidities and mortalities as the events themselves. For instance the epidemic of typhus accompanying the chaos in the aftermath of the Russian revolution was

responsible for 30 million cases of illness and 3 million deaths in European Russia alone (Fasulo 2008). Supposedly regarding the control of the body louse, the vector of typhus, Lenin speculated "Either socialism will defeat the louse or the louse will defeat socialism".

Moving to the future there are several ways in which infectious diseases may become an increasingly important cause of morbidity and mortality. The opening of the 2011 annual report of the UK chief medical officer, highlighted that in the absence of new antibiotics or other treatments the growth of antibiotic resistance could lead to minor surgery and routine infections becoming high risk (Davies 2011). Furthermore in 2013 the same UK chief medical officer went on to publically urge the UK government to bring up the issue at the G8, suggesting that antibiotic resistance should be ranked alongside terrorism as a threat to the nation (Walsh 2013). It has also been debated that climate change may well increase the burden and geographical range of many vector borne diseases (Lambrechts et al. 2010; Fan et al. 2014; Campbell-lendrum et al. 2015). This is not only due to the increased global distribution of the vectors of these diseases (M. Service 2012b; Lambrechts et al. 2010; WHO 2015b) but also the changes in extrinsic incubation period within the vector that is associated with temperature change (M. Service 2012a; Fan et al. 2014; Campbell-lendrum et al. 2015), as well as the effects of temperature and climatic factors on the life cycles of these vectors (M. Service 2012a; M. Service 2012b; Campbell-lendrum et al. 2015).

Infectious diseases not only affect us directly but impact the crops and livestock upon which we rely. *Phytophthora infestans* the causative agent of the Irish potato famine of the 1840s (which led to the death of a million people and emigration of another million people (Turner 2005)) is reported to cost the EU economy more than €1 billion per year (Haverkroft et al. 2008). In the semi-arid regions of Africa, infections

caused by parasites of the genus *Trypanosoma* for centuries has prevented an area larger than the size of the United States from being used for large scale cattle rearing (Roberts & Janovy 2006). If these *Trypanosoma* diseases were to be eliminated it is estimated that it would generate $2.5 billion over the next twenty years (Shaw et al. 2014). Recent outbreaks of livestock diseases in the UK have caused tremendous costs to the economy. Figures by the UK Audit Office estimated the cost of the Foot and Mouth epidemic of 2001 at £3 billion to the UK government and £5 billion to the private sector (National Audit Office 2002). A freedom of information request revealed that the UK's Department for Environment Food and Rural Affairs (DEFRA) spent more than £26 million in compensation and animal slaughter in the programme to control bovine TB, for the tax year 2012-2013 (DEFRA TB Programme 2013).

On a more positive note there has been growing research and a market in the use of infectious diseases as biopesticides in the control of crop pests (Wilson et al. 2013). Whilst these biopesticides have the advantages of higher target specificity, a capacity for secondary cycling and transgenerational transmission over traditional pesticides, they have proved so far to have had varying efficacy, a slower speed of kill and greater environmental sensitivity (Wilson et al. 2013).

Infectious diseases not only have dramatic effects on human communities but on populations of wildlife. Often having effects on an animal's ecology that at face value do not make intuitive sense until further study reveals the underlying mechanics. A study of cowpox infections in bank voles on the Wirral peninsula, North West England found that in the summer months an infection with cowpox increased survival at both the individual and population level, a similar affect was found in wood mice at the population level (Telfer et al. 2002). It was later found that both female wood mice and bank voles infected with cowpox are more likely to delay maturation and therefore

reproduction, suggesting that these rodents maximise their overall fitness by delaying reproduction in order to transfer resources to fight the cowpox infection (Telfer et al. 2005). This delay in reproduction associated with cowpox infection could lead to an increase in survival over the summer months as the rodent would not be playing a role within the breeding season which has its own risks and strains on resources (Telfer et al. 2002; Telfer et al. 2005).

More notably infectious diseases can cause dramatic wildlife population declines. Infectious diseases have been linked to the decline of many amphibian species, to levels of critical endangerment, even when the environments in which these amphibians live is protected (Stuart et al. 2004; Wake & Vredenburg 2008). The infectious agent most linked to this decline is the chytrid fungus *Batrachochytrium dendrobatidis* (Stuart et al. 2004; Wake & Vredenburg 2008), which has rapidly expanded across the continents capable of sustaining amphibians (Wake & Vredenburg 2008; Olson et al. 2013). Many of the viruses found in the honey bee species *Apis mellifera*, some of which are linked to the damage caused by the *Varroa* mite, have been demonstrated to infect many wild pollinating insects (Manley et al. 2015). Meaning that these viruses pose a possible risk to the continued pollination of not only many crop species but the pollination of wild plants that form the basis of many ecological communities (Manley et al. 2015).

Therefore for the reasons outlined above research into infectious diseases could not only directly lead to the improvement and saving of human lives but aid in food security and the conservation of many wildlife species. Of vital importance in understanding the ecology of infectious diseases are the mechanisms by which they persist.

1.2 Persistence of infectious agents within host populations

In order for an infectious agent to invade a population events that allow an infectious agent to transmit from an infected host to a susceptible host must on average occur at least once before an infected host dies or clears an infection through immunity (Anderson & May 1991a). Similarly if these transmission events occur in proportion to host population size, as an infectious agent uses up the pool of susceptible hosts it may run out of susceptible hosts to infect, unless enough susceptible hosts are quickly added to the population through births or immigration (Anderson & May 1991b; Keeling & Rohani 2008c). Therefore for a given combined birth and immigration rate there is a critical community size (CCS) below which an infectious agent could not persist in a population (Anderson & May 1991b; Keeling & Rohani 2008c).

The most widely known work on critical community size was that of Bartlett's in the late 50s and early 60s (Grenfell 1997), who estimated the CCS required for measles to persist in a US city being between 250,000-300,000 (Bartlett 1960). This and other work on measles demonstrated that measles was maintained in populations above this CCS by being introduced from these larger urban areas to villages or towns, where upon measles would spread through the population, use up the pool of susceptible hosts and then become extinct (Grenfell 1997). After a period of time births would increase the pool of susceptible hosts in these villages or towns, meaning that measles could once again be reintroduced by someone infected from a city or town over the CCS (Grenfell 1997). The patchiness of resources leading to different thresholds in which an organism can survive gives rise to what is called metapopulation dynamics (Grenfell 1997). In the case of infectious agents hosts can be seen as such a resource (Grenfell 1997). The example of measles can further illustrate how infectious agents are maintained through metapopulation dynamics by the natural experiment that was the

UK mass vaccination programme of the late 60s to the late 80s (Grenfell 1997). It was expected that there would be an increase to measles' CCS, due to 60% of those been born susceptible to measles quickly acquiring immunity through vaccination Surprisingly this did not occur (Grenfell 1997). Before the vaccination programme epidemics of measles were synchronised between the major population centres (Grenfell 1997). However, vaccinations led to desynchronicity in measles incidence between those population centres (Grenfell 1997). Such asynchronies can aid disease persistence via different patches of population acting as reservoirs of infection for each other. A disease becomes extinct in one patch only to be later reseeded from another patch where the disease is about to use up its susceptible population, and so on (Grenfell 1997). This metapopulation dynamic in measles persistence was ended when the UK vaccination rose to 90% in the late 80s (Grenfell 1997).

An infectious disease being reseeded via metapopulation dynamics is not the only mechanism by which an infectious disease may persist. Furthermore reseeding via metapopulation dynamics in many situations would not on its own cause a disease to persist. Factors such as large seasonal fluctuations in transmission or little connectivity between sparse populations of hosts that are all below the CCS, may prevent metapopulation dynamics from playing a role. This PhD looks at two other mechanisms by which infectious disease may persist, focusing on a moth virus laboratory system and a mosquito borne viral infection of humans.

1.3 Vertical transmission of an infectious agent and its role in disease persistence

Most pathogens transmit through horizontal transmission between hosts of the same or different generations. Vertical transmission is specifically where a pathogen is transmitted from parent to offspring as a result of the process of host reproduction, such as through an infected egg (transovarial transmission), the surface of an egg (transovum

transmission), sperm, seminal fluid, placenta, reproductive tract or breast milk. Vertical transmission provides a pathogen with a mechanism of persisting across many generations. An example of a retrovirus that infects humans and is thought to persist through vertical transmission is Human T-cell Lymphotropic Virus type 1 (HTLV-1) (Goering et al. 2008g; Goering et al. 2008c). HTLV-1 is common to certain islands in the Caribbean and Japanese archipelago where 5-15% of the population is thought to be infected (Goering et al. 2008c). HTLV-1 is mainly transmitted vertically through breast milk with some minor horizontal transmission through sexual intercourse and intravenous drug use (Goering et al. 2008c). The major issue with HTLV-1 is the 5% risk of developing T-cell leukaemia, which has a high and rapid mortality rate (Goering et al. 2008c).

Through the insertion of their genome into the host genome, in the form of a provirus, retroviruses can be transmitted vertically if they infect germline cells (Goering et al. 2008g; Haig 2012). There is an indication of the widespread past vertical transmission of many retroviruses, from the long history of mammalian genomes containing ancestral non-functioning retrovirus genomes (Goering et al. 2008g; Haig 2012). It has been suggested that many parts of these non-functioning retrovirus genomes have been co-opted in the evolution of the placenta (Haig 2012). In a like fashion vertically transmitting lysogenic bacteriophage often cause many bacterial strains to become pathogenic through the encoding of pathogenic genes from the integrated prophage genome (Campbell 2003).

Vertical transmission is not restricted to viral pathogens. For example the intracellular symbiotic bacteria of the genus *Wolbachia* are maintained through vertical transmission in populations of their host and depending on circumstances can either

have a parasitic or mutualistic relationship with their hosts (terrestrial arthropods and filarial nematodes) (Salunkhe et al. 2014).

1.4 Inapparent (asymptomatic) infections and their role in disease persistence

Inapparent infections where to the eye of researchers, veterinary and medical practitioners, an infected host does not display symptoms can be a common occurrence for many pathogens. Such infections are often described as asymptomatic infections, with the infecting pathogen being able to horizontally transmit to other hosts. When a host does not clear an infection through immunity (chronic infection) and is asymptomatic, it can act as a persisting reservoir of infection, continuously transmitting an infection horizontally (Goering et al. 2008f). A classic example of this is Mary Mallon, A.K.A Typhoid Mary (Goering et al. 2008f). Upon infection with *Salmonella typhi* a human develops typhoid fever, after recovering from typhoid fever 1-3% of the population develop a chronic asymptomatic infection, continually transmitting *S. typhi* through faeces and urine (Goering et al. 2008b). From 1901-1914 Marry Mallon through her job as a cook and her chronic asymptomatic *S. typhi* infection is thought to have caused 200 cases of typhoid fever, despite frequent warnings from the US authorities not to be employed as a cook (Goering et al. 2008f). This led to two spells of incarceration, the second of which was from 1914 to 1938 when she died (Goering et al. 2008f).

In a like manner, even when chronic infections do not occur if a large proportion of infections are asymptomatic, a pathogen can be said to persist through the population of hosts that are asymptomatically infected acting as a reservoir of infection. Polio virus remains endemic to only a few countries, where it persists through a large proportion of asymptomatic infections (72%) acting as a reservoir (Hamborsky et al. 2015). Within many animal species infection with certain pathogens is often asymptomatic and thus

these asymptomatic infections can act as a reservoir. The already mentioned cowpox infection presents as an asymptomatic infection within wood mice and bank voles with an infectious period of 4 weeks (Telfer et al. 2005). Another member of the Poxviridae family squirrel parapoxvirus causes no signs of infection for the vast majority of infections in its natural host grey squirrels (Sainsbury et al. 2000).

1.5 Inapparent (covert) infections and their role in disease persistence

Inapparent infections can come in the form of covert infections. This is where upon infection a host becomes either overtly infected becoming symptomatic leading to the host transmitting the infection horizontally, or the host becomes covertly infected, were the host does not display symptoms and does not transmit the infection horizontally but may vertically transmit the covert infection. In some forms of this type of infection a host first becomes overtly infected followed by being covertly infected. This includes many viruses of the family Herpesviridae, such as varicella-zoster virus (the causative agent of chickenpox and shingles in its activated form), herpes simplex 1 and 2 (causative agents of both oral and genital herpes) (Goering et al. 2008d). A key feature of covert infections is that at some point the infection may switch to an overt form (activation), leading to the horizontal transmission of the infectious agent (Sorrell et al. 2009). Covert infections can occur in two forms. In the persistent form the virus is actively translating proteins and replicating its genome but does not cause observable symptoms. In the latent form a viral infection likewise does not cause observable symptoms but the translation of proteins is lacking or reduced and the replication of its genome is absent (Hughes et al. 1997; Bonsall et al. 2005; Goering et al. 2008d; Sorrell et al. 2009). The circumstances under which a covert infection could be selected and allow a pathogen to persist were hypothesised by Sorrell et al. (2009), using mathematical models. Sorrell et al. (2009) predicted that highly fecund hosts that go

through fluctuating population densities would select for an infectious agent using a covert strategy for persistence. Sorrell et al. (2009) goes on to point to a few studies which found insects covertly infected with baculoviruses of which there are many examples dating back to the 1950s (Steinhaus 1958; Steinhaus & Dineen 1960; Jaques 1962; Longworth & Cunningham 1968; Etzel 1976; Biever & Wilkinson 1978; Jurkovíčová 1979; Fuxa & Richter 1992; Hughes et al. 1997; Fuxa et al. 1999; Cooper et al. 2003; Burden et al. 2003; Burden et al. 2006; Vilaplana et al. 2008; Vilaplana et al. 2010; Murillo et al. 2011).

1.6 Baculoviruses, *Plodia interpunctella* and its baculovirus *Plodia interpunctella* granulovirus (PiGV)

Baculoviruses are a widely occurring infection within lepidopteran species (Cory & Myers 2003). Many of the host lepidopteran species of baculoviruses are agricultural pests, as such the use of baculoviruses as biopesticides has been and is being actively researched (Smith & Rivers 1956; Grzywacz et al. 2013). Baculoviruses are DNA based viruses which are divided into two groups; nuclear polyhedrosis viruses (NPVs) and granuloviruses (GVs) (Cory & Myers 2003). Infection by baculoviruses commonly occurs when larvae consume proteinaceous structures known as occlusion bodies (OBs). OBs contain either a single virus in the case of GVs or multiple viruses in the case of NPVs (Cory & Myers 2003). Horizontal transmission in the overt form of infection occurs when the host body tissue is converted into millions of OBs, causing the death of the host and subsequent release of OBs (Cory & Myers 2003).

In the laboratory the baculovirus of the Indian meal moth (*Plodia interpunctella*), *Plodia interpunctella* granulovirus (PIGV) has provided a useful tool for exploring host-pathogen dynamics (Boots & Begon 1993; S. M. Sait et al. 1994b; S. M. Sait et al. 1994a; Boots & Mealor 2007). *P. interpunctella* is a notable pest of stored

grains, nuts and fruits (Mohandass et al. 2007). Found in every habitable continent this pest causes not only direct economic costs through lost product but indirect costs through consumer complaints, quality loss and the control of this pest (Mohandass et al. 2007). Depending on diet, temperature and humidity the duration of the life cycle of *P. interpunctella* can vary considerably (Mohandass et al. 2007). The laboratory group I work within achieves a generation time of 35-40 days for *P. interpunctella*, provided *P. interpunctella* is maintained on a mixture 200g Ready Brek (porridge), 120g bran, 80g ground rice, 80g brewer's yeast, 0.8g sorbic acid, 0.8g methyl paraben, 100 ml organic honey and 100 ml glycerol, at 27 (+/-1) °C on 16/8 hour light/dark cycle. Other academics using *P. interpunctella* as a model system have achieved similar generation times (Begon et al. 1996; Bjornstad et al. 1998). The typical experimental procedure for infecting *P. interpunctella* with PiGV is to place several of the third instar larvae on a petri dish along with small droplets of the dosing solution made of 75ml green food dye, 25ml distilled water and 5g sucrose along with the required concentration of PiGV (depending on the type of experiment). The larvae given time will then feed on the dosing solution. Larvae are considered dosed when the green food dye is clearly visible within the third instar larvae. The ease of maintaining *P. interpunctella* stocks in the laboratory, quick generation time, easy storage of PiGV (-20°C i.e. a domestic freezer), simplicity of infecting *P. interpunctella* with PiGV make this a useful model system for exploring the ecology of host-pathogen dynamics.

1.7 Dengue viruses and the illnesses they cause

Since cases of dengue haemorrhagic fever (DHF) started appearing the Philippines and Thailand in the 1950s, illnesses caused by the four serotypes of dengue viruses, have grown to be a leading cause of childhood illness and death within Latin America and most Asian countries (WHO 2015b). Dengue viruses are positive-stranded

RNA viruses of the *Flavivirus* genus (Guzman et al. 2010; Rodenhuis-Zybert et al. 2010), with mosquitoes of the genus *Aedes* acting as a vector (M. Service 2012b). *Aedes aegypti* is the principal vector of dengue viruses, but also *Aedes albopictus* may have an important role in acting as a vector of dengue viruses (M. Service 2012b; Lambrechts et al. 2010). *Ae. albopictus* has increased its range to include 29 states of the USA, 15 European countries, Australia and New Zealand (M. Service 2012b; Lambrechts et al. 2010; WHO 2015b). With the increasing spread of *Ae. albopictus* there has been a growing concern that there could be an increasing risk of outbreaks of dengue fever in these countries (Lambrechts et al. 2010). A literature review and meta-analyses of the vector competency of *Ae. albopictus* conducted by Lambrechts et al. (2010) demonstrates that as things currently stand *Ae. albopictus* is an inefficient dengue vector. However, Lambrechts et al. (2010) point that other arthropod-borne viruses (arboviruses) have adapted to alternative vectors and the expansion of *Ae. albopictus* to islands of the Indian Ocean, Central Africa and Italy did lead to outbreaks of chikungunya.

Dengue viruses are grouped into four immunological serotypes (DENV-1, DENV-2, DENV-3 and DENV-4), (Guzman et al. 2010; Rodenhuis-Zybert et al. 2010). Infection with one serotype can lead to asymptomatic infection through to dengue fever (DF), a severe flu like illness that seldom causes death (Guzman et al. 2010; Andraud et al. 2012; Grange et al. 2014; WHO 2015b). Upon recovery from infection with a dengue serotype an individual is homotypically immune to that serotype for life (Guzman et al. 2010; Rodenhuis-Zybert et al. 2010; Andraud et al. 2012). However there is approximately only 1-3 years of heterotypic immunity to other dengue serotypes (Reich et al. 2013). After this period of cross protective immunity is over, secondary infection with another serotype puts a person at risk of developing DHF (Guzman et al.

2010; Rodenhuis-Zybert et al. 2010; Andraud et al. 2012; WHO 2015b). DHF can lead to dengue shock syndrome (DSS), both of which are life threatening. Many group DHF and DSS into severe dengue, which has been estimated to cause 500,000 cases of illness, mostly in children and have a mortality rate of 2.5% (WHO 2015b).

1.8 Summary of the proceeding chapters

In Chapter 2 for two reasons I test whether food quality can cause the activation of covert vertically transmitted PiGV in *P. interpunctella*. The first reason being that the activation of a virus from a covert vertically transmitted state to an overt horizontally transmitted state would be advantageous to the virus at high host densities. Such a potential cue for detecting a host's density may well be the drop in the resource quality associated with increased host density. The second reason being that many studies of insects demonstrate an association between poor diet quality and a loss of immune function. I then build on this work by further developing the molecular methods to screen for the presence of the genome of covertly infecting PiGV within *P. interpunctella* larvae. The larvae from Chapter 2 were then tested for covert PiGV using these methods.

In Chapter 3 as inbreeding has been demonstrated to have varying effects on different measures of immune function in insects. I use the molecular techniques developed in the previous chapter to examine whether there are differences in covert vertical transmitted PiGV infections within 3 inbred populations of *P. interpunctella* and 2 outbred populations of *P. interpunctella.*

In Chapter 4 as the role of vertical transmission of dengue viruses in mosquitoes on the epidemiology of dengue fever has been debated (Angel and Joshi 2008; Joshi et al. 2002; Rosen et al. 1983; Adams and Boots 2010; Pinheiro et al. 2005; Zeidler et al. 2008) and a significant amount of work is still published on this subject (Martínez et al.

2014; Sanchez-Rodríguez et al. 2014; Yang et al. 2014; Espinosa et al. 2014). I review the literature on vertical transmission and discuss its role in dengue's epidemiological persistence and control.

In Chapter 5, considering that recent reviews have found asymptomatic dengue viral infections to be common, I adapt two frequency dependent SIR type models that include an asymptomatic class (SAIR models), to dengue virus dynamics. I then use the models to test the role asymptomatic dengue virus infections on the epidemic success and persistence of dengue viruses, as well as the population left at risk of developing DHF.

The models from Chapter 5 lacked the explicit inclusion of mosquitoes. The length of time an infecting dengue virus takes to produce an infectious mosquito host may affect the speed at which an epidemic progresses or uses up the newly born susceptible population; this in turn could lead to an epidemic becoming endemic. For these reasons in Chapter 6 I modified the two models from Chapter 5 to explicitly include mosquitoes in dengue's transmission.

The discussion sections of chapters 6 and 7 highlighted the ways in which stochasticity may affect the ecology and persistence of dengue viruses. Therefore in Chapter 7 one of the SAIR models is modelled stochastically in both the frequency transmission dependent and mosquito transmission dependent forms in order to ascertain whether stochasticity could affect any of the insights into dengue's epidemiology gained in the previous two chapters.

Finally in Chapter 8 I start by summarising the key findings of this PhD thesis, going on to point to wider conclusions as well as future avenues of research concerning inapparent and vertically transmitted infections in arboviruses and insect viruses. As discussed above, inapparent infections whether in the form of covert or asymptomatic

infections could allow a pathogen to persist by providing a reservoir of infected host, whose role would be at first sight hidden from researchers, medics, vets and conservationist (see Section 1.4-5). Similarly, vertical transmission could provide a pathogen with a persisting infecting reservoir across generations (see Section 1.3). The main aim of this thesis therefore is to see if broad trends or parallels in how inapparent and vertically transmitted infections relate to pathogen persistence can be found by looking at these forms of infection in dengue viruses and PiGV. The last section of the discussion chapter returns to this theme by putting what this thesis has found out about pathogen persistence through looking at inapparent and vertically transmitted infections in these two viruses in a broader ecological context.

Chapter 2: The impact of host food resource level on vertically transmitted covert PiGV within its host *Plodia interpunctella*

Abstract

Covert baculovirus infections can be transmitted vertically within their Lepidopteran hosts; this may be how such pathogens persist in fluctuating host populations. Quality of the host's food resources could impact covert infections in a number of ways. Baculoviruses may be directly selected to switch to an overt form, when the host is on a poor resource quality since the host is less likely to survive, or as low resource quality could be a cue for high host density. Many studies of insects have demonstrated that diet of poor quality can lead to a loss of immune function. Therefore, I examined the role of *Plodia interpunctella's* food resource quality on the vertical transmission of covert baculovirus infections and their activation to overt infections. I found that lower resources increased the likelihood of vertical transmitted covert infections being detected. However, I found no covert to overt activation across any resource environment in more than 1774 challenged individuals. These results are novel in that they show that poor resources increase individual host's risk of vertically transmitted covert infection. However due to the low number of covert infections that occurred I cannot conclude anything of the role of resources on activation of covert infection.

2.1 Introduction

Baculoviruses are a widely occurring infection within Lepidopteran species (Cory & Myers 2003). In the laboratory baculovirus Lepidopteran systems have provided a useful tool for exploring host-pathogen dynamics (Boots & Begon 1993; S. M. Sait et al. 1994b; S. M. Sait et al. 1994a; Boots & Mealor 2007) and because many

host Lepidopteron species are agricultural pests consequently the use of baculoviruses as bio-pesticides has been and is being actively researched (Smith & Rivers 1956; Grzywacz et al. 2013). Baculoviruses are DNA based viruses which are divided into two groups nucleopolyhedroviruses (NPVs) and granuloviruses (GVs) (Cory & Myers 2003). Infection of baculoviruses commonly occurs when larvae consume proteinaceous structures known as occlusion bodies (OBs). OBs contain either a single virus in the case of GVs or multiple viruses in the case of NPVs (Cory & Myers 2003). Horizontal transmission of the overt form, occurs when the host body tissue is converted into millions of OBs, causing the death of the host and subsequent release of OBs (Cory & Myers 2003).

Alternatively baculovirus infections can exist in a covert form, where the host does not suffer from observable symptoms but there is the possibility of vertical transmission and potentially reversion to an overtly infected state. Such covert forms of baculovirus infections have been found to be widespread within Lepidopteran species (Steinhaus 1958; Steinhaus & Dineen 1960; Jaques 1962; Longworth & Cunningham 1968; Etzel 1976; Biever & Wilkinson 1978; Jurkovíčová 1979; Hughes et al. 1993; Hughes et al. 1997; Fuxa et al. 1999; Cooper et al. 2003; Burden et al. 2003; Burden et al. 2006; Vilaplana et al. 2008; Vilaplana et al. 2010; Murillo et al. 2011; Myers et al. 2011). Covert forms of viral infection have been found in other host-virus systems such as the temperate bacteriophage Lambda of *Escherichia coli* (Madigan et al. 2003) and chronic asymptomatic infections in Humans, such as Herpes simplex 1 and 2 (Goering et al. 2008d). Covert baculovirus infections can be transmitted vertically within their Lepidopteran hosts and it has been suggested that the vertical transmission of viruses in a covert form may be how the pathogen persists in the host when overt horizontal

transmission rates vary, for example population fluctuations (Burden et al. 2003; Boots et al. 2003; Sorrell et al. 2009).

The activation of a virus from a covert vertically transmitted state to an overt horizontally transmitted state would under certain circumstances be advantageous to the virus. In particular a number of studies have demonstrated that covert baculovirus infections in insects can be activated to an overt state through the hosts infection with another baculovirus with the potential for superinfection (Longworth & Cunningham 1968; Jurkovíčová 1979; Hughes et al. 1993; Cooper et al. 2003; Burden et al. 2003). Secondly if the host is in a high population density, then it may be advantageous to the virus to switch to an overt state and transmit horizontally to the other hosts. . Steinhaus (1958) explored the role of crowding as a stress factor, causing increased infectious disease mortality in several lepidopteran species, none of which were orally infected with baculoviruses. Steinhaus (1958) found an increase in mortality for all causes with increased crowding. However, Steinhaus (1958) the statistical analyses was purely descriptive, not comparative and as such there is disagreement as to whether or not increased crowding led to increased baculovirus mortality (Jaques 1962; David & Gardiner 1965). Jaques (1962) found that both orally infected and non-lab infected control *Trichoplusia ni* had higher rates of TnNPV mortality in higher densities. However, as with Steinhaus (1958) statistical analyses was purely descriptive, not comparative. David & Gardiner (1965) found conflicting results in the effect of population density on the incidence of GV mortality in susceptible and resistant *Pieris brassicae* lines. Fuxa et al. (1999) found that the where greater activation rates and subsequent mortality rates from two baculoviruses, a NPV and CPV at higher densities of the host, *Trichoplusia ni* larvae. One potential cue for a virus detecting a host's density may well be the drop in the resource quality associated with increased host

density. Moreover the virus may be directly selected to switch to an overt form, when the host is on a poor resource quality since the host is less likely to survive.

Many studies of insects have demonstrated that diet of poor quality can lead to a loss of immune function (Myers et al. 2011; Triggs & Knell 2012). It should be noted however that this loss of immune function does not always lead to an increased susceptibility to horizontally transmitted baculovirus infection (Boots & Begon 1994; Boots 2000; McVean et al. 2002). Two previous studies have noted food resources effect on activation of covert baculovirus infection in control specimens that had not been dosed with baculovirus. Jaques (1962) looked at the effects of minimal diet and starvation on TnNPV mortality in orally infected and non-lab infected control *Trichoplusia ni* larvae. Whilst none of the control *T. ni* larvae supplied excess food or a subsistence diet died from TnNPV some of those that where starved did, which suggests that starvation caused the activation of covert TnNPV. However, (Jaques 1962) the statistical analyses again is purely descriptive and no statistical comparisons were made. Similarly a study of western tent caterpillar (*Malacosoma pluviale califonicum*) and food levels effect on its susceptibility NPV (Myers et al. 2011), noted that two larval controls (not dosed with virus in the lab) died from overt infection. However both were from two separate experiments; one was raised on normal food, whereas one was raised on half-food. Therefore it would be of interest to see if food resource level could affect the detection of vertically acquired covert PiGV infection, through molecular techniques and not being purely reliant on the activation of covert PiGV.

Covert infections can occur in two forms. Either a viral infection that is actively translating proteins and/or replicating its genome, but does not course observable symptoms, here referred to as persistent infection. Alternatively a latent viral infection

that also does not cause observable symptoms, but is defined by a lack of these processes. (Hughes et al. 1997; Bonsall et al. 2005).

Burden et al. (2002) demonstrated that the baculovirus (PiGV) of surviving *Plodia interpunctella* was vertically transmitted by both sexes to offspring of either sex, causing a covert sub lethal infection that was actively transcribing the late stage granulin gene (therefore it was a persistent infection not a latent infection). Burden et al. (2002) found a high rate of vertical transmission to the offspring (60-80%), although the sample sizes of offspring where small. For the reasons outlined above, I tested whether PiGV can be vertically transmitted in *P. interpunctella* and produce an overt infection in the offspring *P. interpunctella*. I then tested whether food quality can affect the activation of covert PiGV in the larval offspring of *P. interpunctella* that survived PiGV infection. I also further develop the molecular techniques used by Burden et al. (2002) to detect the level of covert PiGV in *P. interpunctella* larvae fed on the two different resource levels, whose parents had survived PiGV infection.

2.2 Materials and methods

2.2.1 Insects and virus

A stock population of *P. interpunctella* which had being maintained at University of Exeter and University of Sheffield, for several years, was used for these experiments. Each generation of this stock had been maintained on a diet of 150g of the following mixture: 200g Ready Brek (porridge), 120g bran, 80g ground rice, 80g brewer's yeast, 0.8g sorbic acid, 0.8g methyl paraben, 100 ml organic honey and 100 ml glycerol. This stock population was maintained at 27 (+/-1) °C on 16/8 hour light/dark

cycle. Similarly the PiGV used was from a stock which had being maintained at -20°C at University of Exeter and University of Sheffield, for several years.

2.2.2 Experimental design

Eight repeats of the following methodology were carried out. The first generation of *P. interpunctella* were taken from the stock population. At third instar 200 individuals were dosed with a solution containing 75ml green food dye, 25ml distilled water and 5g sucrose, as a control. Another 200 third instar larvae were dosed with a solution of 95% (75ml green food dye, 25ml distilled water and 5g sucrose) and 5% virus solution. (NB in the third repeat only 100 larvae were dosed with the control or viral solution, similarly only 75 larvae of the 4th repeat were dosed with the control or viral solution). Dosing occurred via placing several of the larvae on a petri dish along with small droplets of the dosing solution. The larvae given time would then feed on the dosing solution. Larvae were considered dosed when the green food dye was clearly visible within the third instar larvae. All dosed individuals were placed within a well of a 5 by 5 well petri dishes and supplied with enough food mixture (see above) to see them through to pupation. Once these larvae reached fifth instar they were then separated according to gender, by means of a clearly visible spot on males.

Table 2.1: Factorial design under which the first generation of *P. interpunctella* where placed, to breed and lay eggs.

Gender	Male Infected	Female Infected	Control Male	Control Female	Food type
Percentage of adults	25%	25%	0%	0%	Normal quality food
	0%	0%	25%	25%	Normal quality food
	25%	0%	0%	25%	Normal quality food
	0%	25%	25%	0%	Normal quality food
	25%	25%	0%	0%	Low quality food
	0%	0%	25%	25%	Low quality food
	25%	0%	0%	25%	Low quality food
	0%	25%	25%	0%	Low quality food

Once the surviving larvae had reached adulthood they were put in tubs of low and normal quality food according to the factorial design in Table 2.1. The Normal quality food was that used to maintain the lab stock (see earlier). The low quality food was made up from 150g of the following mixture: 100g Ready Brek (porridge), 60g bran, 40g ground rice, 200g methyl cellulose, 80g brewer's yeast, 0.8g sorbic acid, 0.8g methyl paraben, 100 ml organic honey and 100 ml glycerol. Once the resulting 2nd generation of *P. interpunctella* had become 3rd instar larvae. Up to 100 individuals were sampled and placed in an individual well of a 5 by 5 well dish and supplied with enough normal or low quality food to survive to 5th instar larvae. This was carried out to prevent cannibalism between the *P. interpunctella* larvae. When the 2nd generation larvae had become 5th instar larvae they were checked for overt infection by distinctive difference in colour from healthy individuals, (white pale as opposed to the healthy yellow with pink tinges) and then stored individually in 100% ethanol for late molecular analyses.

2.2.3 Molecular analyses

2.2.3.1 DNA extraction

Individual larvae where left to dry from ethanol, frozen in liquid nitrogen and ground using a pestle. The resulting solid was then made into a suspension, by adding 500µl Tris EDTA pH 7.6. 50 µl of this vortexed suspension, which had 150 µl of stirring 5% Chelex suspension added to it. The remaining 450 µl was then frozen at -20 °C should reanalyses need to be conducted. This was vortexed and incubated at 99 °C for 15 minutes. This was vortexed again and put in centrifuge for half an hour at 4 (+/- 2) °C and 13000 rpm. 1 µl of the supernatant of this underwent PCR analysis for CO1 lepidopteran mitochondrial gene and 1 µl of the supernatant underwent PCR analysis for the PiGV granulin gene.

2.2.3.2 PCR of CO1 lepidopteron mitochondrial gene

In order to ascertain whether the preservation of DNA through storing samples in ethanol was successful, a PCR analyses for the host's CO1 Lepidopteron Mitochondrial Gene was carried out. The PCR methodology for the host's CO1 lepidopteron mitochondrial gene was derived from Emery et al. (2009). For each set of samples a master mix was made up of the following solution multiplied up to provide enough master mix for all the samples. The 9 µl of master mix used in reactions comprised of 6.15 µl sterilized distilled water, 1 µl 10x PCR buffer (Qiagen), 0.2 µl dNTP (Qiagen), 0.6 µl $MgCl_2$ (Qiagen), 0.5 µl CO1 LEP F1 primer (5'-ATTCAACCAATCATAAAGATATTGG-3'), 0.5 µl CO1 LEP R1 primer (5'-TAAACTTCTGGATGTCCAAAAAATCA-3') and 0.05 µl Taq DNA polymerase (Qiagen). 1 µl of the sample was then added to the 9 µl master mix. This then underwent a PCR reaction comprising of one cycle of 3 minutes at 94°C, followed by 35 cycles of 1 minute at 94°C, 1 minute at 53°C, and 1 minute at 72°C, with a final step of 10 minutes at 72°C. 5 µl of this was then visualised by gel electrophoresis, using 1.2% agarose and RedSafe.

2.2.3.3 PCR of PiGV granulin gene

The PCR methodology for the PiGV's granulin gene was derived from Burden et al. (2002). For each set of samples a master mix was made up of the following solution multiplied up to provide enough master mix for all the samples. The 9 µl of master mix used in reactions comprised of 6.35 µl sterilized distilled water, 1 µl 10x PCR buffer (Qiagen), 0.2 µl dNTP (Qiagen), 0.4 µl $MgCl_2$ (Qiagen), 0.5 µl GRAN 5' F1 primer (5'-ACAATGAAGCTGGTGTGCAACTGGAGCG-3'), 0.5 µl GRAN 3' R1 primer (5'-TACGTCGGGTGCGAATTCCTTGATCTTG-3') and 0.05 µl Taq DNA polymerase (Qiagen). 1 µl of the sample was then added to the 9 µl master mix. This

then underwent a PCR reaction comprising of one cycle of 2 minutes at 94°C, 1 minute at 65°C and 30 seconds at 72°C, followed by 31 cycles of 30 seconds at 94°C, 1 minute at 63°C and 30 seconds at 72°C, with a final step of 10 minutes at 72°C. 5 µl of this was then visualised by gel electrophoresis, using 1.2% agarose and RedSafe.

2.2.3.4 Molecular analyses sampling method and controls

The second generation larvae were analysed in blocks, with roughly equal numbers of each treatment. This was done to determine if there is any variation in the molecular analyses in detecting PiGV infection. For every DNA extraction and PCR the following were analysed as controls, sterile distilled water, non-PiGV infected 5th instar larvae from the stock population, PiGV infected 5th instar larvae and pure stock virus solution.

2.2.4 Statistical methods

The blocks larvae were analysed in molecularly were ordered one after the other. Therefore it could be argued as to whether or not block was a fixed effect or random effect. As such the data was analysed twice with block as a fixed effect and then as a random effect. To analyse the data with block as a fixed effect, R 3.1.1's generalized linear model function was used to analyse the data, with a binomial error structure. The GLM from the package "brglm" was used to fit the model Proportion of Larvae Infected with PiGV explained by Block plus Food Quality plus Parental Treatment.

R 3.1.1's glmer function from the package lme4 was used to construct GLMMs for explaining the variance within the proportion of larvae infected with PiGV, with a binomial error structure and now block as a random effect. The ANOVA feature in the "lme4" package is based upon model simplification by removal of none significant terms, until reaching the Minimum Adequate Model (MAM). Explanatory variables

where then removed and tested for significance using ANOVA. If a term was found to be significant it was kept in the model, if not it was dropped. This process was done until the MAM was reached.

2.3 Results

Many of the first generation *P. interpunctella* died either from PiGV infection or other factors. Leaving a limited number of female and male *P. interpunctella* from which to breed a second generation of larvae.

2.3.1 Molecular analyses of the second generation larvae

Only the 1st repeat was analysed molecularly due to a lack of sample size covering all treatments and sample viability in the other repeats. In total 434/477 of the samples were positive for the CO1 Lepidopteron Mitochondrial Gene. The samples that were not positive for this host gene were excluded from further analysis, because their DNA had clearly degraded. Due to their high number of samples, the larvae where analysed in six blocks, with samples from each treatment being spread as evenly as possible across each block.

Samples found positive for PiGV were double checked by being reanalysed at a later date, purified and PCR amplified. The PCR amplified PiGV granulin gene product was sent to University of Sheffield, Core Genomic Facility, FU27/28 Medical School for sequencing. Unfortunately 3 samples had degraded. However the PCR amplified PiGV granulin gene product of the other 11 samples showed high degrees of similarity to the granulin genes of highly related GV. At the time of writing the PiGV genome has not been published and is therefore unavailable for comparison. Furthermore the laboratory stock populations of *P. interpunctella* have been maintained for decades

without signs of GV infection. This suggests that the molecular techniques used in this study only detected the PiGV granulin gene only.

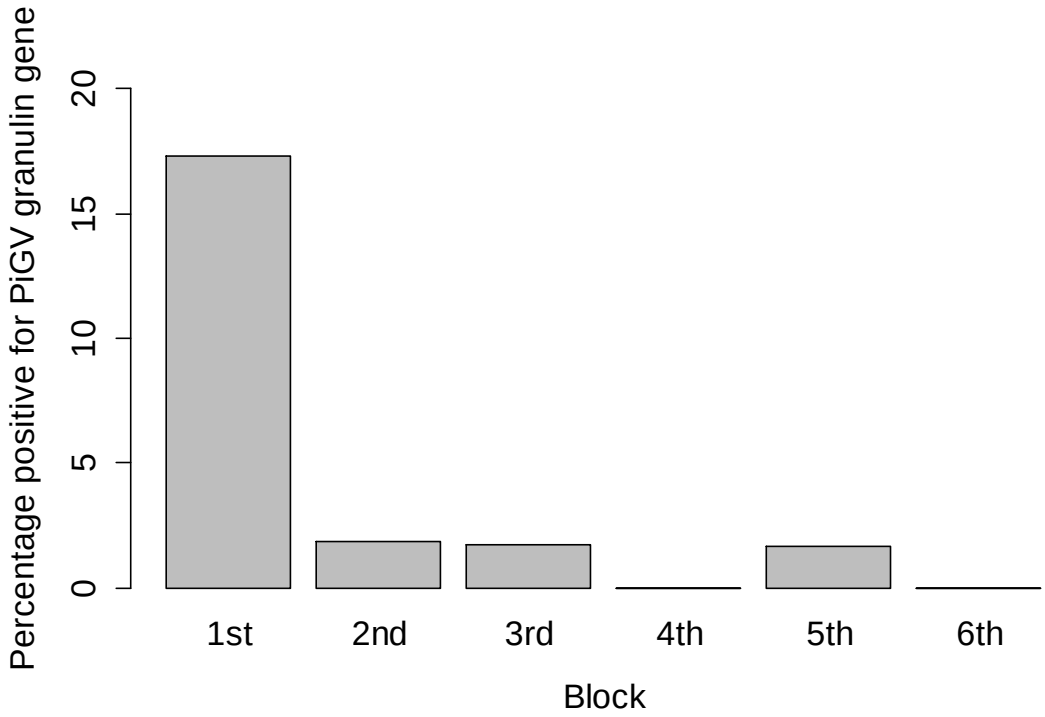


Figure 2.1: Percentage of larvae from first experimental repeat that were positive for PiGV granulin gene by molecular analyses, given that they were positive for CO1 Lepidopteron mitochondrial gene and raised on low quality food. Arranged by the Block they were analysed in.

None of the second generation larvae that were raised on the higher quality food was positive for the PiGV granulin gene, but 3.5% of the second generation larvae raised on lower quality food were found to be positive for the PiGV granulin gene. It can be seen from Figure 2.1 that there is a higher percentage of larvae infected with PiGV in the 1st block, suggesting a block effect. I unexpectedly found that a single infected larva was found in the control treatment. There was no obvious effect of the different parental treatments on the second generation larvae's PiGV infection status in the other treatments (See Figure 2.2). It is therefore unsurprising that fitting GLMs to

the results found that only block and food quality were significant factors in explaining the proportion of larvae infected with PiGV (Block χ^2=25.2 DF=5 p=0.00013, Food χ^2=6.39 DF=1 p=0.0114 and Parental Treatment χ^2=4.95 DF=3 p=0.175). Likewise when block was fitted as a random effect GLMM analyses to the data found the MAM to be the food larvae where raised on (χ^2=6.34 DF=1 p=0.0118).

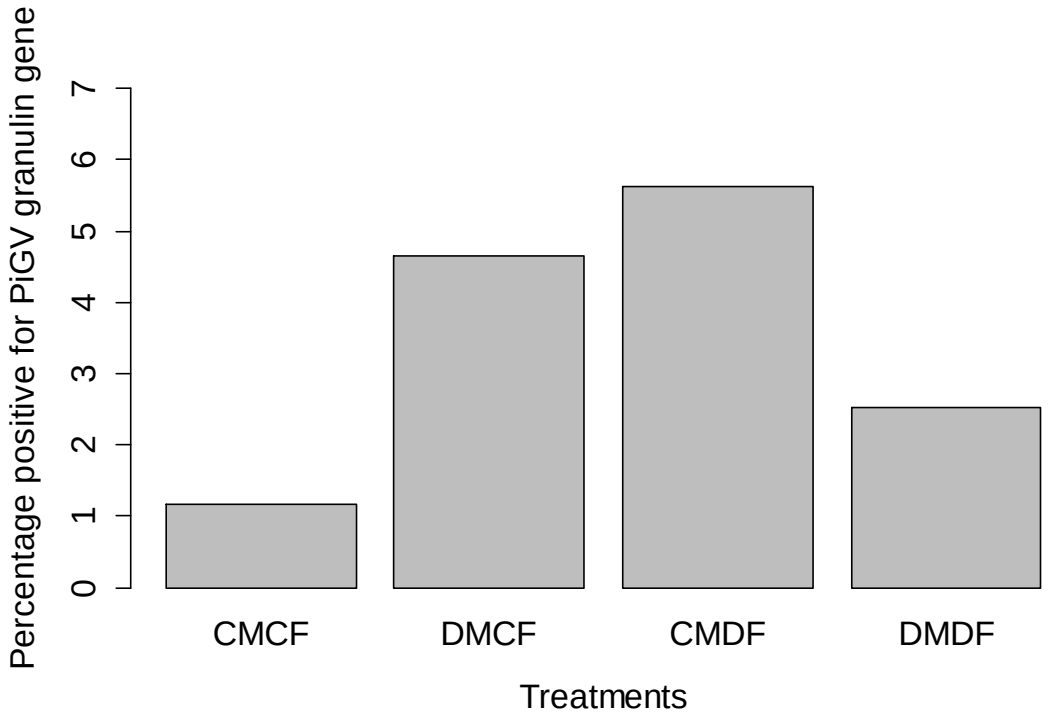


Figure 2.2: Percentage of larvae from first experimental repeat that were positive for PiGV granulin gene by molecular analyses, given that they were positive for CO1 Lepidopteron mitochondrial gene and raised on low quality food. Arranged by parental treatment. (CMCF=control male parent and control female perant, DMCF = dosed male parent and control female, CMDF control male and dosed female, DMDF dosed male and dosed female).

Since PiGV infection was only detected in larvae raised on low quality, I analysed this data set separately to see if there was an effect of parental treatment. A GLM of Proportion of Larvae Infected with PiGV explained by block plus Parental Treatment, with binomial errors was fitted on the data for larvae that were raised upon

low quality food only. An ANOVA of this model shows that only block was significant and therefore there is no effect of parental treatment even on the low quality food (Block χ^2=25.5 DF=5 p=0.00011 and Parental Treatment χ^2=4.95 DF=3 p=0.175). Likewise GLMM analyses of only the low quality food data set, with block as a random effect, found that the effect of parental treatment was not significance when compared to the null model (χ^2=4.6609 DF=3 p=0.198). Again therefore there is a clear effect of food quality but not parental treatment.

2.3.2 Observations of overt PiGV infection

In all of the repeats of this experiment and across all treatments, no overt infection was observed in any of the fifth instar larvae of the second generation (1774 of which a parent had been dosed with PiGV, 955 of which neither parent had been dosed with PiGV). What is worth noting is that none of the 995 offspring of a dosed parent raised on low quality food showed signs of overt infection, thereby suggesting that the low quality food used does not cause activation of PiGV.

2.4 Discussion

The fact that none of the 995 larvae from PiGV treatments, raised on low quality food showed overt signs of PiGV infection, suggests that the lower quality food does not stress the larvae enough to cause the activation of PiGV. Also as none of the1774 larvae showed overt signs of PiGV infection from parents infected with PiGV, this experiment did not show any signs of vertical transmission of PiGV leading to an overt infection, unlike in SfNPV (Fuxa & Richter 1991). That being said I showed that food resource quality was a significant explanatory factor for vertical transmission of PiGV, whereas parental viral treatment was not (as suggested by Figure 2.2). As far as I know this is the only study to use molecular techniques to see if lower food quality can have

an effect in detecting vertically transmitted covert baculovirus infection. Two previous studies had noted food resources effect on activation of covert baculovirus infection, in control specimens that had not been dosed with baculovirus (Jaques 1962; Myers et al. 2011). In the case of Jaques (1962) after being dosed with an NPV *Trihoplusia ni* where either provided with excess food, a subsistence diet or starved. The controls not dosed with NPV but starved for 3 days suffered an NPV mortality of 3%, NPV mortality for those controls starved for 4 days was 5-6%. None of the controls on other diets that suffered NPV mortality (Jaques 1962). Jaques (1962) provide no statistical comparisons of these results meaning it is unwise to draw conclusions. Myers et al. (2011) found that 1 *Malacosoma pluviale californicum* larva died from its NPV from the half food feeding regime, being a control larva neither it nor its parents had been dosed with the NPV. Myers et al. (2011) also noted that one larva out of 159, of the full feeding regime, from the parental generation died from the NPV. This larva had been collected from the field as an egg, which was surface sterilised, meaning that the NPV infection would have to of been acquired vertically (Myers et al. 2011). The small sample size and conflicting results similarly mean that is unwise to draw conclusion from Myers et al. (2011) work on this.

Burden et al. (2002) found a higher vertical transmission rate for PiGV (60-80%) then was found in these experiments. The difference in the proportion of second generation larvae infected with PiGV between this work and Burden et al. (2002), may be due to the fact that Burden et al. (2002) used different strains of *P. interpunctella* and PiGV as well as a different food resource. Also Burden et al. (2002) dosed the parental generation at 5th instar, in this experiment the parental generation were dosed as 3rd instar larvae. This suggests that larval instar may have an effect on vertical transmission of PiGV and other baculorviruses. Fuxa & Richter (1991) suggested that fifth instar

Spodoptera frugiperida dosed with two strains of SfNPV had higher levels of SfNPV in their offspring, compared to those dosed as third instars. However Fuxa & Richter (1991) did not make any statistical comparison to validate their claims. Sait et al. (1994) studying dose response effects of horizontally transmitted PiGV in the five instars of *P. interpunctella*, found that PiGV mortality increased with dose, but decreased with *P. interpunctella* age. Furthermore Sait et al. (1994) found no PiGV mortality in fifth instars. If it is the case that late instars are less likely to succumb to baculovirus infection but vertically transmit the infection to their offspring, then this may suggest that vertical transmission occurs when a host can subdue a baculovirus infection but not clear it.

In all the various statistical tests parental treatment did not have a significant effect on the proportion of offspring infected with PiGV. These results echo Burden et al. (2002) who also found no obvious differences in covert PiGV infection rate in the larvae of a PiGV infected mother and father. However, the unusual samples comparing 5 pools of 5 larvae whose mothers were dosed with the 5 pools of 5 larvae whose fathers were dosed or 10 individual larvae from 1 egg batch of a dosed father with 10 individual larvae from 1 egg batch of a dosed mother, mean that a statistical analyses of Burden et al. (2002) work on this is invalid due to reasons of statistical resolution, small sample size and pseudoreplication.

Measuring an invertebrate's susceptibility to an infectious agent, involves the measurement of the outcome of a combination of three processes. The first is the ability of the infectious agent in infecting the host. The second is the host ability to clear the infection. Thirdly the host's ability to suppress the infection. Previous work on the effect of food resource on *P. interpunctella's* susceptibility to PiGV (as measured by mortality), suggested that food resource level either had no effect (Boots & Begon

1994) or that lower food quality decreased susceptibility to PiGV (McVean et al. 2002). Both Boots & Begon (1994) and McVean et al. (2002) raised *P. interpunctella* larvae on the same resource level before and after oral infection with PiGV. With these two experimental set ups it is difficult untie whether resource level is affecting the host, firstly in terms of letting the infectious agent infect, secondly the host ability to clear the infection or thirdly the hosts ability to suppress the infection. When considering that in this experimental set up the effect of food resource level could only have taken place once a second generation *P. interpunctella* would of have hatched and therefore after *P. interpunctella* had been infected, this suggests that decreased food resource level reduced the second generation *P. interpunctella's* ability to suppress or ultimately clear PiGV infection. Further experimentation would be needed to untie effect of food resource on these three processes in the infection of baculoviruses. Injection of PiGV into *P. interpunctella* may provide a possible solution, as the infection route would not be affected by any changes in *P. interpunctella* caused by food resource level.

Myers et al. (2011) study and this study only used two resource levels, whereas Jaques (1962), had three. In order to fully ascertain whether lower food quality can cause the activation of latent baculovirus infection further experimentation is needed. A greater range of food qualities needs to be tested since it may well be the case that it is only starvation or near starvation levels that cause activation of baculorviruses. Whilst Jaques (1962) inadvertently looked at this. Jaques (1962) experimental set up was designed to look at the role of food resource level (up to starvation) on susceptibility to GV, not food resource level (up to starvation) on activation of covert GV. Similarly, more recently starvation effect on activation of a baculovirus may have been inadvertently tested. As previously mentioned Fuxa et al. (1999) found that there were greater activation rates and subsequent mortality rates from NPV and CPV at higher

densities. However at the higher densities the *T. ni* 5th instar larvae and pupal weight was lower and at the highest density *T. ni* larvae began to cannibalise each other. This suggests that Fuxa et al. (1999) may not have controlled for resource limitation and/or starvation at the higher densities and that these two factors may well be having an effect at the high densities of *T. ni* larvae, in causing the activation of NPV and CPV. Fuxa et al. (1999) also do not take into account the possibility of NPV and CPV activation through an increased dose occurring via cannibalism of latently infected *T. ni* larvae.

There is a suggestion that an unusual host diet, which leads to a general weakening of the host, may cause the activation of a covert baculovirus. David & Gardiner (1965) fed different strains of *Pieris brassicae* food that they were not habituated to. The Canary Islands strain of *Pi. brassicae* in the wild eat nasturtium. In the laboratory when fed cabbage there was a higher activation rates of PbGV, then on nasturtium. However with a long term laboratory strain of *Pi. Brassicae* that had been maintained on cabbage, activation rates of PbGV were higher on cabbage than nasturtium for the lab strain of PbGV. More recently Ilyinykh et al. (2013) in experiments with the gypsy moth (*Lymantria dispar*) from Khabarovsk and Novosibirsk, Russia and its NPV (LdNPV), found that they had to abandon feeding the Khabarovsk population on branches of the birch *Betula pendula* and instead feed them on their native Mongolian oak *Quercus mongolica.* All of the Khabarovsk population died, many of which were diagnosed with an NPV infection (28+/-3%), whereas none the Novosibirsk population that fed on their native birch *B. pendula* showed any signs of infection or suffered such a high mortality rate. It should be noted that Steinhaus & Dineen (1960) fed GV infected *Peridroma margaristo* the unusual diet of plantain, this did not induce activation of *Pe. margaristo's* GV, however most larvae died through other causes.

It may also be the case that latent baculoviuses are not activated by low food quality in general, but rather by a lack or deficiency of a specific nutrient that an unusual diet might cause. Furthermore the higher rate of vertically transmitted covert PiGV infection found in second generation larvae raised on lower quality food may also not be caused by low food quality in general, but such a lack or deficiency of a specific nutrient. David & Taylor (1977) found that sucrose deficient diets could lead to a greater susceptibility of *Pieris brassicae* to its GV. Biever & Wilkinson (1978) suggested that *Pieris rapae* fed on a dehydrated diet had a greater mortality rate due to its GV compared to those on a normal diet. However Biever & Wilkinson (1978) only used descriptive statistics in his data analysis, not comparative. A number of studies have demonstrated that low protein levels can affect an invertebrate's immune function and susceptibility to infection (Lee et al. 2006; DeGrandi-Hoffman et al. 2010; Povey et al. 2013). Such a loss in an invertebrate immune function could lead to the covert PiGV infection not being cleared by the insects immune system, as such studying the effect of different protein and carbohydrate ratios on vertically acquired covert baculovirus infections needs exploration. Lee et al. (2006) and Povey et al. (2013) also demonstrated that when given a choice of protein: carbohydrate ratio in the diet, the larvae infected with a baculovirus that did not succumb to infection ate more protein. It would be interesting to know whether the larvae that did not succumb to the baculovirus infection just suppressed the baculovirus infection into a covert state or cleared the infection. This would shed light as to whether or not a covert baculovirus infection was mediated by the virus or the insect's immune system supressing the infection but not clearing it. On this point, what might be of interest is that (Fuxa et al. 1992) discovered a high degree of non-infectious SfNPV OBs that contained no virus in adult *S. frugiperda* whose parents had survived SfNPV infection. However this could be related

to the fact baculoviruses produce no overt infection in adults and unrelated to vertical transmission of baculoviruses.

There is also the question of whether or not the surviving larvae would transmit any covert infection on to their offspring, i.e. does parental food resource level effect the vertical transmission of covert baculoviruses. On this subject a study by Boots & Roberts (2012) found that lower quality food in maternal parents increased measures of *P. interpunctalla's* immune function and decreased susceptibility to orally acquired PiGV.

Another process that leads to the activation of a latent or overt baculovirus may be an infection with another agent. Many previous studies have demonstrated the activation of a latent baculovirus through infection with another baculovirus (Longworth & Cunningham 1968; Jurkovíčová 1979; Hughes et al. 1993; Cooper et al. 2003; Burden et al. 2003). Curiously Jurkovíčová (1979) found that not only were there higher infection rate of NPV particles in *Adoxophyes orana* and *Barathra brassicae* when they were dosed by the others NPV but that there higher infection rates for *B. brassicae* by its NPV when fed an extract from *A. orana*. This suggests that the activation of a covert baculovirus infection within a host may not be caused by a foreign baculovirus in of itself but by a substance embedded in the OB of the foreign baculovirus from its previous host. Further research to validate this point would be needed.

To my knowledge no research has been published on whether or not latent baculoviruses can be activated through infection with viruses with RNA based genomes, bacteria, fungi, protozoan, filarial parasite or parasatoid wasps. Baculoviruses infect many crop pests and field studies demonstrate that latent baculoviruses can be present in a large percentage of these pest species populations. Consequently the

potential use of infectious agents as bio-pesticides that lead to activation of latent baculoviruses could have an added advantage of causing increased mortality from the now activated baculoviruses, on top of their own mortality rates.

Considering that only the 1st repeat of the experiment was analysed molecularly, extrapolating this finding to the other repeats from this study would suggest that the number of second generation larvae with covert PiGV was around 22, 18 and 11 for those whose mother, father or both parents had both been dosed with PiGV respectively. This unfortunately represents a small number of samples from which a covert PiGV infection could activate to an overt PiGV infection. If the effect of diet on activation of vertically transmitted covert baculovirus infection was to be further explored, it may be better to use an insect baculovirus system with a higher rate of vertical transmission.

Fuxa & Richter (1991) were able to select for an increased rate of vertical transmission of SfNPV through isolation SfNPV in the host pupae of *Spodoptera frugiperda* produced by parents who had survived SfNPV infection. Of interest to this study is that both the wild type and selected strains of SfNPV produced overt and covert infections within the offspring of orally infected *S. frugiperda* (Fuxa & Richter 1991). It is of note that further experimentation by Fuxa & Richter (1992) followed transgenerational mortality of SfNPV overt infections up to the F5 and F7 generations for the wild-type and selected strains of SfNPV. This demonstrates that the vertical transmission of baculoviruses can be selected for. The circumstances under which a vertically transmitted baculovirus would be selected for in nature were hypothesised by Sorrell et al. (2009). Through mathematical simulations they suggested that vertically transmitted covert viral infection would be promoted in highly fecund hosts that go through fluctuating population densities, as found in many insect baculovirus systems. Sorrell et al. (2009) pointed to work such as Burden et al. (2003), suggesting that the

high rates of covert vertically transmitting viruses seen in many insect systems are not solely explained by the lower rates of covert vertically transmitting viruses seen in Sorrell et al. (2009) mathematical simulations. Sorrell et al. (2009) suggests that therefore the high rates of covert viral infection seen in some field studies of insect populations may be mediated by an interaction between the host and the virus not the virus alone. One such interaction would be the host's immunity suppressing the viral infection but not clearing it. Reduced host immune function through poor diet could cause such a process. If this is the case then outbreaks in overt baculoviruses at high host populations could be explained through the activation of covert baculovirus infection, when host immune function is decreased sufficiently through poor food quality, brought about by high host density. Further investigation of the effect of food resource on covert baculovirus infection or mathematical simulation, could shed light on the validity of this hypothesis.

In conclusion I found a higher rate of vertically transmitted covert PiGV in *P. interpunctella* raised on low quality food, however only two food resource levels were used in this study and there was a low rate of vertical transmission of PiGV. Therefore in order to shed further light on the effect of food resource on the activation and vertical transmission of covert baculovirus, a greater range of food resources, different protein: carbohydrate levels and unusual food resources need to be utilised in experiments similar to this one. Burden et al. (2002) found higher rates of vertical transmission from 5^{th} instar infected *P. interpunctella*, as opposed the 3^{rd} instar *P. interpunctella* infected in this study, this would suggest a need for research surrounding the effects of parental host instar at point of infection on the vertical transmission of covert baculoviruses. Added to this Sait et al. (1994) finding that PiGV mortality increased with dose, but decreased with *P. interpunctella* age would suggest a need for research into the effect of

infective dose of parental generation on the vertical transmission of covert baculovirus. A number of studies have demonstrated the activation of a latent baculovirus through infection with another baculovirus (Longworth & Cunningham 1968; Jurkovíčová 1979; Hughes et al. 1993; Cooper et al. 2003; Burden et al. 2003), but to my knowledge research on the role of other infectious agents in causing activation of covert baculovirus infections is lacking. Lastly, due to the low rate of vertical transmission of covert baculoviruses seen in this study, using an insect baculovirus system with a higher rate of vertical transmission and activation of covert baculovirus should be considered in further research.

Chapter 3: The effect of host inbreeding on the vertical transmission of PiGV within its host *Plodia interpunctella*

Abstract

The deleterious effects of inbreeding have been demonstrated consistently upon a variety of traits within many animal and plant species. However, studies have found varying effects of inbreeding on different measures of immune function in insects. No studies have looked at the effect of host inbreeding on a covert vertical transmitted virus in insects, or the covert pathogens of insects in general. Therefore, using the molecular techniques developed in the previous chapter, this chapter examines whether there are differences in covert vertically transmitted PiGV infections within 3 inbred populations of *P. interpunctella* and 2 outbred populations of *P. interpunctella.* There was no significant difference in the PiGV infection rates in the larvae of adult *P. interpunctella* from these different populations. This was true when the populations were compared individually or grouped together as larger inbred and outbred populations. Further to this none of the covert PiGV infection switched to an overt form, suggesting that host inbreeding had no effect on covert vertically transmitted PiGV infections. However, it should be noted that these findings could be due to the low rate of vertical transmission of PiGV leading to a small sample size.

3.1 Introduction

The deleterious effects of inbreeding has been demonstrated consistently upon many traits within many animal and plant species (Charlesworth 1987; Keller & Waller 2002). In birds the deleterious effect of inbreeding has been found in hatching rates, survival and reproductive success (Keller & Waller 2002). Similarly in mammals reductions in survival, birth weight and reproductive success due to inbreeding have

been found (Keller & Waller 2002). Within insects many studies have demonstrated inbreeding's deleterious effects. Roff (1998) and Roff & DeRose (2001) showed decreased growth and fecundity in the cricket *Gryllus firmus*. In the ant *Formica exsecta* Haag-Liautard et al. (2009) showed that inbreeding decreased a colony's production of queens and survival. Mattey et al. (2013) found decreased hatching rates and survival in the inbred offspring of the burying beetle *Nicrophorus vespilloides*. The inbred parents of *Ni. vespilloides* also had lower hatching success and survival rates in raising outbred offspring. Such inbreeding depression is postulated to occur via two main processes. Inbreeding causes a greater number of homozygotes to be produced in a population, firstly this increases the chance of a deleterious trait being expressed if is recessive or partially recessive. The fact that deleterious mutations occur through time exacerbates this process (Charlesworth 1987; Keller & Waller 2002). Secondly, if there is heterozygote advantage, this is less likely to be expressed (Charlesworth 1987; Keller & Waller 2002).

A study of soay sheep *Ovis aries* from the St. Kilda archipelago found that inbreeding increased susceptibility to gastrointestinal parasites (Coltman et al. 1999). Not only this Coltman et al. (1999) found that this increased susceptibility led to decreased overwinter survival. Similarly Ilmonen et al. (2008) found that full-sibling inbreeding in the house mouse *Mux musculus domesticus* led to increased susceptibility and mortality to *Salmonella enterica*. Infection with *S. enterica* also led to a further reduction in fitness in male first-cousin inbred mice, as measured by reproductive success (Ilmonen et al. 2008). Several studies have investigated inbreeding effects on measures of immune function in insects. Lee et al. (2012) found that heterozygosis did not affect encapsulation or phenoloxidase (PO) activity of honey bees *Apis mellifera*. Vitikainen & Sundström (2010) found no effect of inbreeding on the encapsulation

response of worker ants *Formica exsecta*, but did find an increased encapsulation response in the gynes (new reproductive females) of *F. exsecta*. Vitikainen & Sundström, (2010) suggest that this may be down to inbreeding acting as a physiological stressor leading to increased immune activity. Gerloff et al. (2003) found no effect of inbreeding on encapsulation response in the bumble-bee *Bombus terrestis*. Similarly Whitehorn et al. (2011) found no effect of decreased genetic diversity on encapsulation and PO level in *Bombus muscorum* but did find increased prevalence of the gut parasite *Crithidia bombi*. Calleri et al. (2006) found no effect of inbreeding on encapsulation rate of the termite *Zootermopsis angusticollis* but found that whilst isolated inbred and outbred termites had no difference in susceptibility to conidia fungus, the grouped outbred termites were more likely to survive then the inbred grouped termites (no such findings were found with a bacterial agent). Calleri et al. (2006) therefore suggest that inbreeding detrimentally affects social behavioural mechanisms of disease resistance but not physiological derived immunity in social insects.

Relatively few studies have explored the effect of inbreeding on the susceptibility and immune function in non-social insects. Rantala & Roff (2007) found no effect of inbreeding on the immune function of the cricket *Gryllus firums*, as measured by lytic activity and encapsulation rate. Drayton & Jennions (2011) found no effect of inbreeding on immune function in terms of lysozyme-like activity but increased hymocyte activity in the cricket *Teleogryllus commodus*. Stevens et al. (1997) found an overall increased prevalence of the parasite *Hymenpolepis diminuta* in inbred female *Tribolium castaneum* but the opposite being true for males and there being no effect of inbreeding on the intensity of infection. However there was a great deal of variation with regards to *H. diminuta* prevalence and intensity among the different

inbred populations *Tribolium castaneum*, leading Stevens et al. (1997) to suggest that generalisations over his results cannot be made.

As such the limited data suggests that there may not be a strong effect of inbreeding on the physiological immunity of insects to infectious disease. This is despite the presumption that a host's genetic diversity is important for resistance to pathogens. As suggested by theoretical and empirical work on the selective pressure caused by pathogens in the evolution and maintenance of sexual reproduction (Neiman & Koskella 2009). No studies have looked at the effect of host inbreeding on a covert vertical transmitted virus in insects, or to my knowledge the covert pathogens of insects in general. A covert infection being an infection that produces no signs of symptoms, does not horizontally transmit the infection but may be activated (switch) to an overt symptomatic state, which does horizontally transmit the infection. The *Plodia interpunctella* PiGV (baculovirus) system has been a useful system for studying host-pathogen dynamics (Boots & Begon 1993; S. M. Sait et al. 1994b; S. M. Sait et al. 1994a; Boots & Mealor 2007). Furthermore it has been demonstrated that vertical transmission of covert PiGV can occur within *P. interpunctella* (see Chapter 2 and the work of Burden et al. (2002)). Using the molecular techniques developed in Chapter 2, this chapter examines whether there are differences in covert vertical transmitted PiGV infections within 3 inbred populations of *P. interpunctella* and 2 outbred populations of *P. interpunctella*.

3.2 Materials and methods

3.2.1 Establishment of populations

In order to examine the role of inbreeding on transmission of the virus, four new populations of *P. interpunctella* were established (3 Inbred and 1 more outbred known

as Kernow) by Dr Katherine Roberts, Steven Sharpe and Drew Willson. This experiment used a population known as the "Liverpool" strain as the standard outbred population. The "Liverpool" strain has been maintained for several years in the University of Exeter and the University of Sheffield. All the populations were maintained on a diet of 150g "Normal food", comprising of the following: 200g Ready Brek (porridge), 120g bran, 80g ground rice, 80g brewer's yeast, 0.8g sorbic acid, 0.8g methyl paraben, 100 ml organic honey and 100 ml glycerol. The populations were maintained at 27 (+/-1) °C on a 16/8 hour light/dark cycle.

A new "Kernow" strain was created in the following manner: 6 tubs of 25 males of the Liverpool strain and 25 females of the Dundee strain (which had been maintained for several years in the University of Exeter and the University of Sheffield), were set up to breed to form the LD strain. At the same time 6 tubs of 25 males of the Dundee strain and 25 females of the Liverpool strain were set up to breed to form the DL strain. 8 tubs of 25 males of the LD strain and 25 females of the DL strain were then set up to breed to form the UK strain. At the same time 8 tubs of 25 males of the DL strain and 25 females of the LD strain were then set up to breed to form the UK strain. The *P. interpunctella* of both sets of UK strain were then bred with a strain of *P. interpunctella* from the US Department of Agriculture (USDA). 8 tubs of 25 males of the UK strain and 25 females of the USDA strain were then set up to breed to form the UK*USDA strain. At the same time 8 tubs of 25 males of the USDA strain and 25 females of the UK strain, were then set up to breed to a form the USDA*UK strain. 8 tubs of 25 males of the UK*USDA strain and 25 females of the USDA*UK strain and 8 tubs of 25 males of the USDA*UK strain and 25 females of the UK*USDA strain were then set up to breed to form the Kernow strain. The presumption being that the Kernow strain would therefore be more outbred than the Liverpool strain.

Four inbred strains were created by inbreeding from the Liverpool strain for 16 generations in the following manner: Four pots containing a randomly selected male and female 5th instar larvae and 20g of the standard food were maintained at 27 (+/-1) °C on 16/8 hour light/dark cycle. Once the subsequent offspring had become 5th instar larvae a randomly selected male and a randomly selected female (between all four pots) was then placed into another four pots containing the same amount of standard food and maintained in the same way for production of the next generation. At each generation any larvae not used to create the next generation were placed in a larger pot along with their food, which was topped up with standard food to 200g and maintained at 27 (+/-1) °C on 16/8 hour light/dark cycle for use in experiments. Unfortunately one of the inbred populations (Inbred 1) did not survive the inbreeding process leaving three populations to experiment on.

The PiGV used was from a stock which had being maintained at -20°C at University of Exeter.

3.2.2 Experimental design

Between 125 and 130 third instar of each of the five populations were dosed with a 75ml green food dye, 24ml distilled water, 1ml of virus solution and 5g sucrose solution giving a 10^{-2} dose of PiGV. This dose was chosen as a compromise between giving a high enough PiGV dose so that many of the larvae would transmit PiGV vertically but not a high enough dose to kill the majority of the parental generation, thus rendering a small sample size of offspring. Dosing was carried out using our standard bioassay technique. 3rd instar larvae were placed on a petri dish, small 2-8µl droplets of the dosing solution were then placed in front of them. The larvae given time would feed on the dosing solution. Larvae could be seen to be dosed when the green food dye was clearly visible within the third instar larvae. All dosed individuals were placed within a

well of a 5 by 5 well petri dish and supplied with enough standard food media (see above) to see them through to pupation, and maintained at 27 (+/-1) °C on a 16/8 hour light/dark cycle. The larvae were checked for overt infection by distinctive difference in colour from healthy individuals, (white and pale, as opposed to the healthy yellow with pink tinges).

The surviving larvae were then allowed to pupate. After pupation the adults of the same strain were allowed to mate and to lay eggs on 150g of a lower quality food. Low quality food is made from the following mixture: 100g Ready Brek (porridge), 60g bran, 40g ground rice, 200g methyl cellulose, 80g brewer's yeast, 0.8g sorbic acid, 0.8g methyl paraben, 100 ml organic honey and 100 ml glycerol. The low quality food was chosen, as Chapter 2 suggested that vertical transmission of covert PiGV was more likely to be seen in *P. interpunctella* that had been raised on a lower food resource. *P. interpunctella* adults were maintained at 27 (+/-1) °C on a 16/8 hour light/dark cycle. Between 9-12 days after being put upon low quality food to breed and lay eggs, the adult 1st generation *P. interpunctella* were removed, so as to prevent their remains from being eaten by their offspring. When the 2nd generation larvae had become 5th instar larvae they were checked for overt infection. The 2nd generation larvae were then stored individually in 95% ethanol for later molecular analyses.

The 1st generation *P. interpunctella* emerged from pupae at different times due to them not all being the same age or dosed at the same time. This allowed for the creation of three blocks of the experiment.

3.2.3 Molecular analyses

3.2.3.1 DNA extraction

Individual larvae were left to dry from ethanol and ground using a pestle. The resulting solid was then made into a suspension by adding 500µl Tris EDTA pH 7.6. 25 µl of this vortexed suspension had 75 µl of stirring 5% Chelex suspension added to it. The remaining 475 µl was then frozen at -20 °C should reanalysis need to be conducted. This was then vortexed and incubated at 99 °C for 15 minutes, after which it was then put in centrifuge for half an hour at 4 (+/- 2) °C and 13000 rpm. 1 µl of the supernatant of this underwent PCR analysis for CO1 lepidopteran mitochondrial gene and 1 µl of the supernatant underwent PCR analysis for the PiGV granulin gene.

3.2.3.2 PCR of CO1 lepidopteran mitochondrial gene

In order to ascertain that the DNA in the sample had not degraded in storage, a PCR analyses for the host's CO1 lepidopteran mitochondrial gene was carried out. The PCR methodology for the host's CO1 lepidopteran mitochondrial gene was derived from Emery et al. (2009). For each set of samples a master mix was made up of the following solution multiplied up to provide enough master mix for all the samples. The 9 µl of master mix used in reactions comprised of 6.15 µl sterilized distilled water, 1 µl 10x PCR buffer (Qiagen), 0.2 µl dNTP (Qiagen), 0.6 µl $MgCl_2$ (Qiagen), 0.5 µl CO1 LEP F1 primer (5'-ATTCAACCAATCATAAAGATATTGG-3'), 0.5 µl CO1 LEP R1 primer (5'-TAAACTTCTGGATGTCCAAAAAATCA-3') and 0.05 µl Taq DNA polymerase (Qiagen). 1 µl of the sample was then added to the 9 µl master mix. This then underwent a PCR reaction comprising of one cycle of 3 minutes at 94°C, followed by 35 cycles of 1 minute at 94°C, 1 minute at 53°C, and 1 minute at 72°C, with a final

step of 10 minutes at 72°C. 5 µl of this was then visualised by gel electrophoresis, using 1.2% agarose and RedSafe.

3.2.3.3 PCR of PiGV granulin gene

The PCR methodology for the PiGV's granulin gene was derived from (Burden et al. 2002). For each set of samples a master mix was made up of the following solution multiplied up to provide enough master mix for all the samples. The 9 µl of master mix used in reactions comprised of 6.35 µl sterilized distilled water, 1 µl 10x PCR buffer (Qiagen), 0.2 µl dNTP (Qiagen), 0.4 µl $MgCl_2$ (Qiagen), 0.5 µl GRAN 5' F1 primer (5'-ACAATGAAGCTGGTGTGCAACTGGAGCG-3'), 0.5 µl GRAN 3' R1 primer (5'-TACGTCGGGTGCGAATTCCTTGATCTTG-3') and 0.05 µl Taq DNA polymerase (Qiagen). 1 µl of the sample was then added to the 9 µl master mix. This then underwent a PCR reaction comprising of one cycle of 2 minutes at 94°C, 1 minute at 65°C and 30 sec at 72°C, followed by 31 cycles of 30 sec at 94°C, 1 minute at 63°C and 30 sec at 72°C, with a final step of 10 minutes at 72°C. 5 µl of this was then visualised by gel electrophoresis, using 1.2% agarose and RedSafe.

3.2.3.4 Molecular analyses sampling method and controls

Each of the blocks were analysed in batches with roughly equal numbers of moth larvae from each line. This was done so as to determine if there is any variation in the molecular analyses in detecting PiGV infection. For every PCR I also analysed as controls, (1) sterile distilled water and (2) PiGV infected 5^{th} instar larvae.

3.2.4 Statistical methods

Block and batch larvae were molecularly analysed in were carried out in an order. Therefore block and batch larvae were molecularly analysed in could be considered as fixed effects or as random effects. Considering this the data was analysed

in two ways. Firstly with block and batch larvae were molecularly analysed in as fixed effects and then again with them as random effects. Both times this was done using binomial error structures. The first statistical analysis of the data was done using the R package "Bias reduction in Binomial-response GLMs" (BRGLM). The ANOVA feature in the BRGLM package is based upon model simplification by removal of none significant terms until reaching the Minimum Adequate Model (MAM).

The second statistical analyses of the data was done using R 3.1.1's glmer function from the package lme4, to construct GLMMs the proportion of larvae infected with PiGV explained by population of *P. interpunctella*.

As comparing each of the inbred and outbred populations of *P. interpunctella* separately could be seen as pseudoreplication, GLM and GLMM analysis, as described above, was applied to the data with the Liverpool and Kernow populations grouped as outbred and Inbred population 2, 3 and 4 grouped as inbred.

3.3 Results

3.3.1 Molecular analyses of larvae and comparison between the different *P. interpunctella* populations

As in the previous chapter none of the second generation 2950 larvae showed signs of overt infection (regardless of being from the outbred or inbred population).

Table 3.1 Parental *P. interpunctella* generation that were bred from to produce the second generation larvae

Block/repeat	Kernow	Liverpool	Inbred 2	Inbred 3	Inbred 4	Total
1	17	12	10	22	13	74
2	24	20	20	22	13	99
3	16	12	24	20	10	82
Total	57	44	54	64	36	255

Table 3.2: Number of *P. interpunctella* 5th larvae in second generation

Block	Kernow	Liverpool	Inbred 2	Inbred 3	Inbred 4	Total
1st	200	200	200	200	200	1000
2nd	200	200	200	200	23	823
3rd	200	200	200	200	327	1127
Total	600	600	600	600	550	2950

Table 3.3 Number of *P. interpunctella* 5th larvae in second generation positive for CO1 mitochondrial DNA

Block	Kernow	Liverpool	Inbred 2	Inbred 3	Inbred 4	Total
1st	106	189	172	174	182	823
2nd	125	180	190	152	16	663
3rd	193	192	185	163	309	1042
Total	424	561	547	489	507	2528

Table 3.4: Number of *P. interpunctella* 5th larvae in second generation positive for PiGV granulin DNA

Block	Kernow	Liverpool	Inbred 2	Inbred 3	Inbred 4	Total
1st	2	2	0	0	0	4
2nd	0	0	0	2	0	2
3rd	0	0	0	0	0	0
Total	2	2	0	2	0	6

2528/2948 of the samples were positive for the CO1 lepidopteran mitochondrial gene, with the samples that were not positive for the host gene being excluded from further analysis since the DNA had clearly degraded (see Tables 3.2-3). Note two samples had to be discarded due to them being accidentally knocked onto the floor. Due to the large number of samples, the molecular analysis was conducted in 32 batches. Samples from the 1st block were analysed within batches 1-11, each batch containing

roughly equal numbers of samples from each population. Samples from the 2nd block were analysed within batches 12-20, each batch containing roughly equal numbers of samples from each population and so on with the 3rd block. From the low percentage of larvae positive for PiGV granulin gene, Figure 3.1 suggests that the overall level of vertical transmission of PiGV is quite low. There was no sign PiGV granulin gene in Inbred lines 2 and 4, or in the 3rd block. Many of the batches also showed no signs of PiGV granulin gene. BRGLM analyses of Proportion of Larvae Infected with PiGV explained by experimental block, batch larvae was analysed in and population of *P. interpunctella* found the null model to be the MAM. (ANOVA comparison of the BRGLM of proportion of larvae infected with PiGV explained by block + batch larvae was analysed in + population of *P. interpunctella* to the block + population of *P. interpunctella* model had 29 degrees of freedom, a χ^2=12.293 and p= 0.9972. ANOVA comparison of the BRGLM of proportion of larvae infected with PiGV explained by block + population of *P. interpunctella* to the block model had 4 degrees of freedom, a χ^2= 3.6359 and p= 0.4575. ANOVA comparison of BRGLM of proportion of larvae infected with PiGV explained by block to the null model had 2 degrees of freedom, a χ^2=5.5813 and p=0.06138). Likewise ANOVA of the GLMM of proportion of larvae infected with PiGV explained by population of *P. interpunctella*, with experimental block and batch larvae was analysed molecularly in, as random effects found the model none significant compared to the null model (4 degrees of freedom, a χ^2= 6.1978 and p= 0.1849). Note due to issues of non-convergence with other settings the R's glmer function had the control set with the optimizer ="optimx" and optCtrl=list(method="L-BFGS-B").

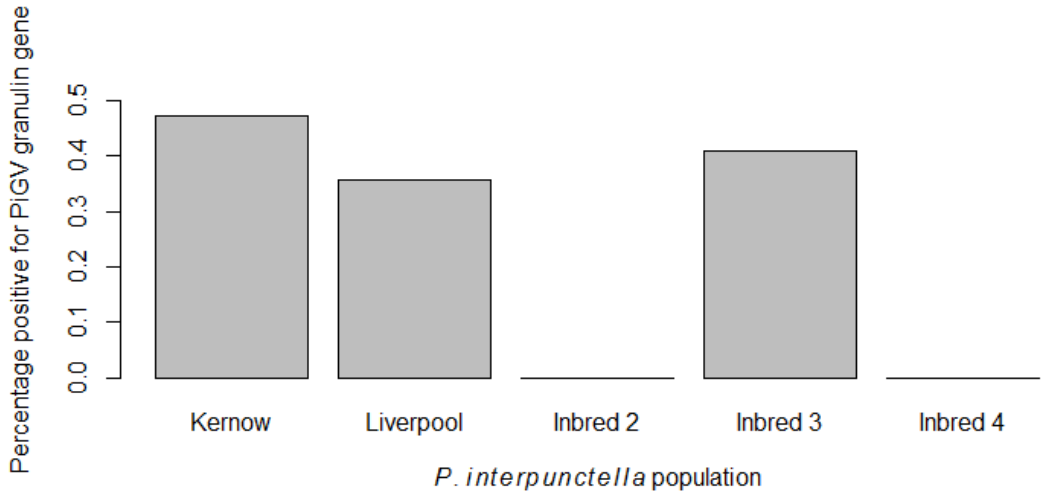


Figure 3.1 Percentage of larvae that were positive for PiGV granulin gene by molecular analyses, given that they were positive for mitochondrial DNA, arranged by *P. interpunctella* population. Raw data available in Tables 3.3-4.

3.3.2 Comparison the *P. interpunctella* populations grouped together as inbred and outbred populations

Table 3.5 Number of *P. interpunctella* 5th larvae in second generation

Block	Outbred	Inbred	Total
1st	400	600	1000
2nd	400	423	823
3rd	400	727	1127
Total	1200	1750	2950

Table 3.6: Number of *P. interpunctella* 5th larvae in second generation positive for CO1 mitochondrial DNA

Block	Outbred	Inbred	Total
1st	295	528	823
2nd	305	358	663
3rd	385	657	1042
Total	985	1543	2528

Table 3.7: Number of *P. interpunctella* 5th larvae in second generation positive for PiGV granulin gene

Block	Outbred	Inbred	Total
1st	4	0	4
2nd	0	2	2
3rd	0	0	0
Total	4	2	6

Figure 3.2 suggest there is little difference in PiGV infection rates between the different *P. interpunctella* population when grouped together as either outbred (Kernow and Liverpool populations) or inbred (Inbred populations 2-4). BRGLM analyses of proportion of larvae infected with PiGV explained by experimental block, batch larvae was molecularly analysed in and population of *P. interpunctella* found that the null was the MAM. (ANOVA of the BRGLM of proportion of larvae infected with PiGV explained by block + batch larvae was molecularly analysed in + population of *P. interpunctella* compared to the block + population of *P. interpunctella* model had 29 degrees of freedom, a χ^2= 10.547 and p= 0.9993. ANOVA of the BRGLM of proportion of larvae infected with PiGV explained by block + population of *P. interpunctella* compared to the block model had 1 degree of freedom, a χ^2= 1.6289 and p=0.2019. ANOVA of BRGLM of proportion of larvae infected with PiGV explained by block compared to the null model had 2 degrees of freedom, a χ^2=5.5813 and p=0.06138). Similarly ANOVA of the GLMM of proportion of larvae infected with PiGV explained by population of *P. interpunctella*, with experimental block and batch larvae was molecularly analysed in as random effects, compared to the null equivalent finds no significant difference (1 degree of freedom, a χ^2= 1.7638 and p= 0.1842).

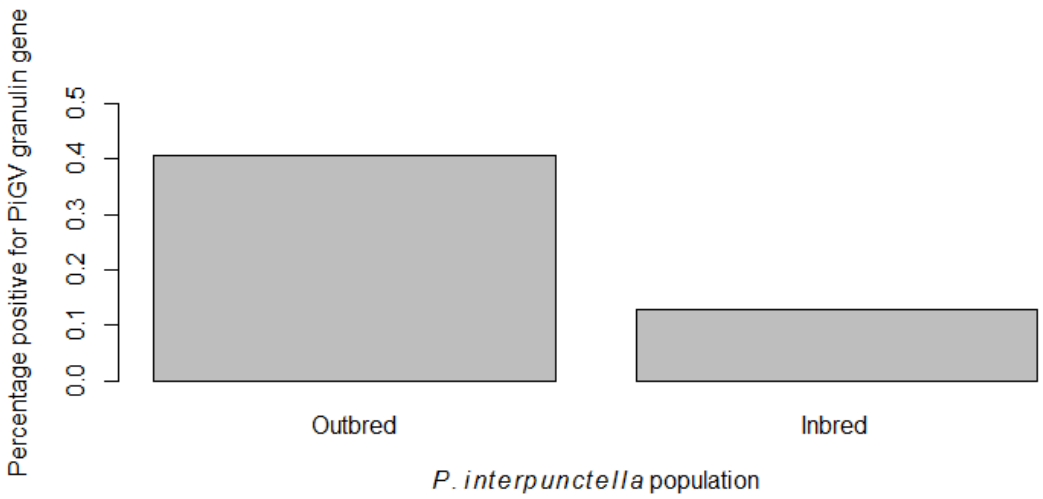


Figure 3.2. Percentage of larvae that were positive for PiGV granulin gene by molecular analyses, given that they were positive for mitochondrial DNA, arranged by *P. interpunctella* population. Raw data available in Tables 3.6-7.

3.4 Discussion

Assuming that larvae positive for PiGV granulin gene were infected with PiGV. I found no evidence that inbreeding affected the vertical transmission of the virus or it's activation from a covert to overt state. This would seem to confirm the findings of others who also found no effect of inbreeding on insect immune function (Lee et al. 2012; Gerloff et al. 2003; Rantala & Roff 2007). Considering that other studies found that inbreeding increased susceptibility to infection (Whitehorn et al. 2011) or increased immune function (Drayton & Jennions 2011). It may be the case that host inbreeding's effect on immune function and susceptibility may be specific to a host or infectious agent. This may even reflect an insect hosts ecological niche down to its role within a social insect system (Vitikainen & Sundström 2010). Vitikainen & Sundström (2010) suggest that queen *F. exsecta* life history leads to an immune system that needs to be up and down regulated at certain times, whereas a worker *F. exsecta* immune system is

constantly active due to pathogen exposure. Inbreeding could affect the timing of queen *F. exsecta* immune system's up regulation (Vitikainen & Sundström 2010). Hence Vitikainen & Sundström (2010) found no effect of inbreeding on the encapsulation response of worker ants *F. exsecta* but an increased encapsulation response in the gynes of *F. exsecta*.

Whilst the sample size in terms of the number of larvae analysed is large, the lack in significance of population of *P. interpunctella* on PiGV infection rate, as seen by the various statistical tests, may be interpreted to be down to the sample size in terms of PiGV infected larvae (only 6), being too low to detect an effect of host inbreeding on PiGV infection rate. The low PiGV infection rate as seen in Figure 3.1-2 would seem to indicate this. To further illustrate this I used the proportion of larvae infected from the Kernow population (0.0047), Liverpool population (0.0036) and Inbred 3 population (0.0041) with R's power.prop.test function. Comparing the proportions (each combination of two of the proportions) under the two way, 0.05 significance and 0.9 power level setting, produces crude estimates of sample sizes needed to find significant differences in these proportions of infection of between 65309 – 290800 of each population sampled. These are unfeasibly large sample sizes for such an experimental setup. For this reason it may be worth looking at repeating this experiment in different insect baculovirus system with a greater vertical transmission rate, such as SfNPV in *Spodoptera frugiperda* (Fuxa & Richter 1992).

I also found that none of the 1750 second generation larvae that were from inbred lines showed any signs of overt infection, thus suggesting that inbreeding does not have an effect on activation of covert baculovirus. Further causes of activation need to be investigated in order to determine the processes that lead to activation of baculoviruses in nature. Chapter 2 in comparing two qualities of food resources found

no activation of covert PiGV on either food resource. It was suggested that a greater range of food resource levels up to starvation needs to be tested, pointing to studies by Jaques (1962) and Fuxa et al. (1999), which may have inadvertently found that starvation led to the activation of covert baculoviruses (see Chapter 2). Covert baculoviruses may be activated by a lack or deficiency of a specific nutrient rather than low food levels in general (see Chapter 2). David & Taylor (1977) found that sucrose deficient diets could lead to a greater susceptibility of *Pieris brassicae* to its GV and a number of studies have demonstrated that low protein levels can affect an invertebrate's immune function and susceptibility to infection (Lee et al. 2006; DeGrandi-Hoffman et al. 2010; Povey et al. 2013). Two studies suggest that unusual or non-native host diets could lead to activation of a baculovirus (David & Gardiner 1965; Ilyinykh et al. 2013). A number of studies have found that covert baculoviruses can be activated through infection with another baculovirus (Longworth & Cunningham 1968; Jurkovíčová 1979; Hughes et al. 1993; Cooper et al. 2003; Burden et al. 2003).

In conclusion, whilst this study found that inbreeding had no significant effect on the vertical transmission of PiGV in *P. interpunctella* or its activation from a covert to overt state, this could be down to the low rate of vertical transmission leading to a low sample in terms of PiGV infected larvae. Further study of other insect baculovirus systems with much higher vertical transmission rates may be needed to verify the effect of inbreeding on the vertical transmission of baculoviruses or their activation from a covert to overt state. Repeating this experiment or carrying out a similar experiment in a different insect baculovirus laboratory system, which has a much high vertical transmission rate, such as SfNPV in *S. frugiperda*, would be a good first step. Other studies have found differing results regarding the effect of insect inbreeding on pathogen susceptibility and immunity. Comparing the outcome of such an experiment to

the wider literature may therefore reveal that the effects of inbreeding on an insect's immunity and susceptibility to a pathogen are specific to that insect, its ecological niche or the pathogen.

Chapter 4: How important is vertical transmission of dengue viruses by mosquitoes (Diptera: Culicidae)?

Abstract

Vertical transmission of dengue viruses by mosquitoes was discovered at the end of the late 1970s and has been suggested to be a means by which these viruses persist. However, it is unclear how widespread it is in nature and its importance in the epidemiology of this disease is still debated. Here, I review the literature on vertical transmission and discuss its role in dengue's epidemiology and control. I conclude that given the number of studies that failed to find evidence of vertical transmission, as well as mathematical models and its mechanistic basis, it is unlikely that vertical transmission is important for the epidemiological persistence of dengue viruses. A combination of asymptomatic infection in humans and movement of people are likely to be more important determinants of dengue's persistence. I argue, however, that there may be some need for further research into the prevalence of dengue viruses in desiccated, as well as diapausing eggs and the role of horizontal transmission through larval cannibalism.

4.1 Introduction

Since the 1950s the incidence of illness caused by dengue viruses has increased 30 fold (Nathan et al. 2009). Over 40% of the world's population are at risk, mostly in the urbanised tropics and subtropics and the WHO estimates that there are between 50-100 million cases of dengue viral illnesses per year (WHO 2015b). Furthermore, recently Bhatt et al. (2013) have estimated that there are 390 million human dengue virus infections a year, causing 96 million cases of illnesses a year. As such the dengue virus has emerged as one of the world's major public health problems and there is

therefore a pressing need to understand the mechanisms by which it persists in populations.

Dengue viruses are positive sense RNA viruses of the Flavivirus genus, categorised into four closely related serotypes (DENV-1, DENV-2, DENV-3 and DENV-4). Infection with a serotype leads to lifelong immunity; however subsequent infection with a differing serotype is a major risk factor in the more severe forms of dengue viral illnesses (dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS)). Hospitalisation decreases the mortality of these severe forms of dengue viral illnesses from more than 20% to less than 1% (WHO 2015b).

The urbanized *Aedes aegypti* (L.) is the principle DENV vector, with the more rural *Ae. albopictus* (Skuse) acting as a secondary vector in most regions (M. Service 2012b). However, a meta-analyses of laboratory and natural experiments by Lambrechts et al. (2010) suggests *Ae. albopictus* is an inefficient vector of dengue virus. Both mosquitoes breed in small containers of water and their eggs are able to withstand periods of desiccation (M. Service 2012b; WHO 2015d). The eggs of many *Aedes* species, including some strains of *Ae. albopictus*, undergo periods of diapause, but the eggs of many other *Aedes* species such as *Ae. aegypti* are incapable of diapause (M. Service 2012b; WHO 2015d).

It is also known that the vertical transmission of dengue virus can occur in both of these key vectors (Rosen et al. 1983; Khin and Than 1983; Hull et al. 1984). As such there has been much interest and some debate concerning the epidemiological role of vertical transmission of dengue virus within mosquito populations (Angel and Joshi 2008; Joshi et al. 2002; Rosen et al. 1983; Adams and Boots 2010; Pinheiro et al. 2005; Zeidler et al. 2008). A significant amount of work is still published on this subject (Martínez et al. 2014; Sanchez-Rodríguez et al. 2014; Yang et al. 2014; Espinosa et al.

2014) but a review looking solely at vertical transmission of dengue virus is lacking. Furthermore it has been suggested that the monitoring of larvae *Aedes* mosquitoes for dengue virus could be used to predict dengue fever epidemics (Chow et al. 1998; Lee & Rohani 2005). There is clearly therefore a need to examine the role that vertical transmission plays in the epidemiology of the dengue virus.

4.2 Early work on vertical transmission of dengue virus

The earliest experiments exploring the possibility of vertical transmission of dengue virus within mosquitoes date back to the 1920s and 30s (Siler et al. 1926; Simmons et al. 1931). Two studies involved feeding *Ae. aegypti* on a dengue virus infected patient. The offspring of these *Ae. aegypti* were then fed on susceptible volunteers who were then monitored for signs of infection. The parental generation of mosquitoes were determined as to being infected by feeding them on susceptible volunteers, who were also monitored for signs of infection. No infection occurred from the offspring generation and therefore these experiments suggested that vertical transmission of dengue virus was not possible.

The next mention of the possibility of vertical infection, is a citation to unpublished work in a short report by Rosen et al. (1978) that suggests that transovarial transmission of dengue virus had occurred within *Ae. albopictus* from Hawaii, under laboratory conditions, however information on this is scant. Then in 1979 Kubersk, using fluorescent antibody staining of *Ae. albopictus* infected with DENV-2 found the presence of DENV-2 antigens in the tissues surrounding the eggs and oviducts, although not within under-developed eggs or spermatheca (Kuberski 1979). This finding gave further impetus to experimental studies on vertical transmission leading to Jousset, (1981) showing that a DENV-2 virus strain could be vertically transmitted in *Ae. aegypti* (see Table 4.1). Furthermore, mosquitoes from five distinct geographical

regions (Queensland, Hannoi, Lagos, Guadalope and Burkina Faso) were used in the experiments suggesting that this was a widespread phenomenon.

Next Rosen et al. (1983) demonstrated again in another lab study that all four serotypes of dengue virus were capable of vertical transmission in *Ae. albopictus*, (see Table 4.1). Only DENV-1 was demonstrated to vertically transmit within *Ae. aegypti*. This led them to suggest that vertical transmission within *Ae. albopictus* may assist in the persistence of dengue virus. Rosen et al. (1983) also tested how well three strains of vertically acquired DENV-1 survived through the life cycle of *Ae. albopictus*. However no statistics were carried out to verify if vertically acquired DENV-1 was significantly decreased in the pupae or adults, compared to the larval stages. They also found that many of the geographical strains of the dengue virus serotypes were not vertically transmitted. Following the introduction *Ae. albopictus* into South America, Mitchell & Miller (1990) infected 3 Brazilian strains of *Ae. albopictus* with DENV-1 and DENV-4 and also found evidence of vertical transmission (see Table 4.1).

Gubler et al., (1985) showed experimentally that vertical transmission of DENV-1 in *Ae. mediovittatus* was possible (see Table 4.2) and suggested that this uncommon vector for the horizontal transmission of dengue virus could be helping maintain the virus through vertical transmission. Later, experiments on mosquitoes of the *Ae. scutellaris* group found low or zero rates of vertical transmission for DENV-1-4 (Freier & Rosen 1987). However, at about the same time in similar experiments on *Ae. mediovittatus* infected with DENV-1-3 much higher rates of vertical transmission were seen (Freier & Rosen 1988) (see Table 4.2) and de Souza & Freier (1991) reported vertical transmission of both larvae and pupae of a Panamanian strain of the largely sylvatic *Haemgogus equinus* mosquito from intrathorcical DENV-1 infection (see Table

4.2). It was clear therefore from these early laboratory studies that vertical transmission was possible in a wide range of vectors.

In the first field study Khin & Than (1983) detected DENV-2 within *Ae. aegypti* that had been caught in the field as larvae. A proportion of the larvae were raised to adults in the lab; others were raised to fourth instar larvae. They found DENV-2 within the larvae and the male adults, although no dengue virus was found in the adult females (see Table 4.3). Next Hull et al. (1984), caught *Ae. aegypti*, as well as larvae (using ovitraps) and raised them to adults in the lab. No dengue virus was found in the adults raised from larvae but DENV-4 was found in one pool of adults raised from eggs (see Table 4.3). As such there was the emergence of some evidence of vertical transmission in the field as well as in the laboratory.

Nevertheless two further papers at this time cast doubt on the importance of vertical transmission in the field. In an extensive field study on *Ae. aegypti* and *Ae. albopictus* in Bangkok no vertical transmission was detected in and around the houses of DHF cases (Watts et al. 1985) (see Table 5.4b). Then a field study in Kelang, Malaysia also found no evidence of vertical transmission of dengue virus in *Ae. aegypti* and *Ae. albopictus*, from around the homes of dengue fever (DF), as well as DHF cases (Ramalingam et al. 1986) (see Table 5.4).

As such by the start of the 1990s there was a very mixed set of results from the laboratory that showed vertical transmission occurred but it was dependent on the route of infection, the strain of dengue virus and both the species and the genotype of the mosquito vector. In addition the very limited number of field observations showed that it occurred but cast doubt on the role that vertical transmission may play in the persistence of dengue virus. There was however enough interest to encourage further

studies that took advantage of alternative approaches, including using novel dengue virus screening techniques.

4.3 The measurement of vertical transmission of dengue virus

It is important to understand that in the laboratory experiments reviewed in this study there are three ways of measuring vertical transmission. The first is the vertical transmission rate (VTR), which is defined as the proportion of infected parents that produce at least one infected offspring. The second is the filial infection rate (FIR) that is defined as the proportion of infected progeny produced from infected parents, given that vertical transmission has occurred. The third is the vertical infection rate (VIR) which is the VTR multiplied by the FIR.

In many of the field surveys and laboratory studies reviewed in this study the sample size of specimens is so great and due to limited laboratory resources, specimens were analyzed in pools or groups. The simplest way of calculating the infection rate (IR) for pools of mosquitoes is the Minimum Infection Rate (MIR) (listed in the per 1000s), which is the number of pools positive for infection divided by the total number of individual specimens tested. A few of the surveys reviewed here use the more statistically powerful Maximum Likelihood Estimator (MLE). It should be noted that pool size can affect the accuracy and the range of possible infection rates estimated. For example with 5 out of 200 mosquitos positive for an infectious agent, if analyzed individually this would produce an MIR of 25/1000, analyzed in pools of 10 the MIR could range from 5-25/1000, analyzed in pools of 50 the MIR could range from 5-20/1000 and analyzed in pools of 100 the MIR could range from 5-10/1000.

MLE was devised by Chiang and Reeves (1962) and improved by Walter et al. (1980) and Le (1981) and is generally seen as an improvement upon MIR (Katholi & Unnasch 2006; Gu et al. 2003; Gu & Novak 2004). At a high infection rate and a larger

pool size MIR's assumption of a positive pool representing only one infected individual can lead to an inaccurate estimate of infection rate; MLE relaxes this assumption (Katholi & Unnasch 2006; Gu et al. 2003; Gu & Novak 2004). Gu et al. (2004) demonstrated that varying the pool size combined with MLE led to a more accurate measure of infection rate. However, at lower rates of infection (as seen in this review) there is likely to be very little difference between MIR and MLE (Gu et al. 2003). One study by Le Goff et al. (2011) calculates MIR and the more statistically based method of True Infection Rate (TIR). TIR like MLE does use a maximum likelihood procedure. As can be seen in Table 4.3, Le Goff et al. (2011) found a very similar infection rate when calculating MIR and TIR, for DENV-1 and DENV-3 in adult *Ae. aegypti* caught as larvae. Similarly, where possible I calculated the MIR for the few studies that calculated infection rate using MLE, for example, Chen et al. (2010) and Das et al. (2013). Chen et al. (2010) found a MLE for adult female *Ae. aegypti* of 0.97/1000 and I calculated a similar MIR of 0.97/1000 (see Table 5.4b). Likewise Das et al. (2013) found MLEs of 8.92/1000, 6.09/1000 and 2.63/1000 for DENV-2 infected female adult *Ae. albopictus*, *Ae. albopictus* pupae and *Ae. aegypti* pupae, respectively. I calculated MIR of 8.9/1000, 5.8/1000 and 2.6/1000 for DENV-2 infected female adult *Ae. albopictus*, *Ae. albopictus* pupae and *Ae. aegypti* pupae, respectively (see Table 5.6). These findings would seem to support the assertion of Gu et al. (2003), that there is likely to be very little difference between MIR and MLE when infection rates are low and when pool sizes are similar.

Despite the key issues highlighted in the main body of the text, the ease of carrying out more modern dengue virus detection techniques has led to a burgeoning literature on various aspects of vertical transmission of dengue virus. Laboratory and field studies find generally low rates of vertical transmission (MLEs and MIRs of less

than 10/1000) with MIRs and MLEs typically ranging from less than single digits to low double digits per 1000 (see Table 4.1-7). The VTR in *Ae. albopictus* and *Ae. aegypti*, when not selected for ranges from 0-41.2% and the FIR is less than 5% (see Table 4.1). VTR and FIR in uncommon dengue vectors ranges greatly from 12.4-94.7% and 0.1-20.3% respectively (see Table 4.2).

4.4 Key issues with field and laboratory studies

In the early-mid 1990s RT-PCR and ELISA began to be used to detect dengue viruses instead of the previously used immunological techniques. The immunological techniques required greater man power and sometimes the use of *Toxomrhynchites amboinensis* or *Aedes* mosquito colonies to amplify dengue viruses before the mosquitoes were stained using either indirect or direct fluorescent antibody techniques (IFAT or DFAT) (Kuberski & Rosen 1977). In some cases the squashed heads of the sample specimens were directly examined for dengue viruses using IFAT or DFAT. Some studies used peroxidase-antiperoxidase staining (PAP) or compliment fixation tests. Serufo et al. in 1993 was the first to detect vertical transmission of dengue viruses using molecular techniques in a field study. Other than finding DENV-1 in 2 pools of *Ae. albopictus* larvae collected from car tyres in Campos Altos (Brazil), information on this study is scant and it may not have gone through the peer review process. Older techniques such as amplification followed by IFAT were still being used as late as 2012 (Martins et al. 2012).

Rohani et al. (2007) is the only study to compare the sensitivity of different methods of analysing larval or adult male *Aedes* for dengue virus infections. They compared RT-PCR in detecting dengue viruses in field caught *Ae. aegypti* and *Ae. albopictus* (from several locations across Malaysia) with C6/36 *Ae. albopictus* cell culture amplification, followed by PAP staining (see Table 4.5). Rohani et al. (2007)

suggest that the latter method is most sensitive. However, no statistical evaluation was presented and the sampling could be seen as flawed with unequal amounts of larvae from specific sample sites being tested through the different screening techniques. This led to 2,250 larval *Ae. aegypti* and 2,130 larval *Ae. albopictus* being analyzed in pools of 10 through immunological techniques in sites where vertical transmission was found compared to only 990 larval *Ae. aegypti* and 780 larval *Ae. albopictus* being analyzed in pools of 10 through RT-PCR in sites where vertical transmission was found (Rohani et al. 2007). A better way to compare these two methodologies would be to use both methods to analyse the same homogenised pools of larvae.

Many studies do not mention using positive or negative controls in their RT-PCR or ELISA screening for dengue virus (Table 4.1-7). If positive controls were not used there is a danger of false negative results. Similarly false positive results caused by contamination could occur if negative controls were omitted from the screening process. Several experiments used dengue virus that were passed many times through unusual hosts or *Aedes* cell lines (see Table 4.1-3 and Table 5.7) which may have affected the wider inferences that can be drawn from such studies. Chen et al. (2003) found that passage through a mammalian cell line caused higher nucleotide and amino acid changes in two DENV-2 genes than passage in *Ae. albopictus* cell lines or alternating passage through both of these cell lines (it should be noted that this difference was not assessed statistically).

Two further cautionary notes should be made about interpretations made when using RT-PCR followed by gel electrophoresis as a diagnostic tool for dengue virus isolation. Firstly careful consideration of primers should be made so as to avoid amplification of related Flaviviruses leading to false positives. Ideally viral RNA detected by RT-PCR should be followed by confirmation through sequencing, viral

antigen detection through IFA or other such techniques to avoid such false positives. Many of the studies reviewed in this chapter did not take either of these precautions (see Table 5.4-6). Secondly, the detected dengue virus RNA could for the most part belong to non-functional dengue virus particles (Choy et al. 2013). This second point would also be the case for studies that used qRT-PCR, as well as those that used RT-PCR

A significant number of studies calculated MIRs or MLEs by combining data from all dengue virus serotypes (see Table 4.5) and given that different serotypes of dengue virus may be horizontally or vertically transmitted at different rates, as well as being present in different ratios within the mosquito population. This could lead to an inaccurate estimation in the rate of vertical transmission of dengue virus serotypes. The data on dengue virus infection rates in mosquitoes used for calculating the MIR or MLE varied greatly. Many studies calculated MIRs or MLEs for specific months in specific areas, whereas others calculated these statistics for combined data. This makes comparing surveys of dengue virus infection rates difficult. The ecology of different areas varies, thereby making the standardization by a specific area for a specific length of time not necessarily relevant. However, if future studies attempted to standardize sampling methodologies it would nevertheless be easier to compare infection rates among studies.

4.5 Overview

Despite a lack of testing of the relative sensitivity of screening methods, the ease of carrying out the more modern techniques has led to a growth in the literature on various aspects of vertical transmission of dengue viruses. In Table 4.1-7 I summarise these studies. In the text, I discuss the mechanistic basis of vertical transmission (see section 4.6), transgenerational vertical transmission (see section 5.7), vertical transmission to desiccated and diapausing eggs (see section 4.8), field studies that

compare *Ae. aegypti* and *Ae. albopictus* (see section 4.9), comparison of vertical transmission in larvae and adults (see section 4.10), field studies comparing vertical and horizontal transmission (see section 4.11), seasonality in vertical transmission (see section 4.12), dengue fever epidemic prediction (see section 4.13), field studies that failed to find evidence of vertical transmission (see section 4.14), a recent laboratory experiment in relation to vertical transmission and subsequent horizontal transmission of dengue virus between larval mosquitoes (see section 4.15) and mathematical models (see section 4.16). I finish with a discussion of the current state of knowledge of the role of vertical transmission in dengue virus ecology (see section 4.17) and an overall summary (see section 4.18).

Table 4.1: Summary of laboratory studies on vertical transmission of dengue viruses in common vectors.

Source	Host Species	Infection route of parent generation	Serotype of dengue	Sample Size (individual specimens)*	Infection rate*	Type of infection rate	Origin of dengue serotype	Methodology of Screening	Use of Controls in screening
(Jousset 1981)	*Ae. aegypti*	Intrathoracic inoculation	DENV-2	1494-1903	0.52-2/1000	MIR	New Guinea C strain of DENV-2, passed 25 times through mice	Amplification in mice, followed by complement fixation test.	Not mentioned
(Rosen et al. 1983)	*Ae. albopictus*	Intrathoracic inoculation	DENV-1	293-7522	1.7-14/1000	MIR	Many virus strains had been used. A few had been passed through monkeys; all had been passed through mosquitoes.	Amplification in *Tx. amboinensis* , followed by Direct Fluorescent Antibody Technique (DFAT)	Not mentioned
			DENV-2	2193-4080	0.91-2.5/1000	MIR			
			DENV-3	1280-4420	0.23-0.78/1000	MIR			
			DENV-4	194-1070	0.22-5.2/1000	MIR			
	Ae. aegypti		DENV-1	1543	0.65/1000	MIR			
	Ae. albopictus	Oral inoculation	DENV-1	790-1197	1.2-13/1000	MIR			
(Ramalingam et al. 1986)	*Ae. aegypti*	Oral inoculation	Unknown serotypes	5320	None occurred	NA	Mosquitoes where fed on suspected human DF/DHF cases in Malaysia.	DFAT. Confirmation by amplification in *Ae. pseudoscutellaris* cell line or *Toxorhynchites* mosquitoes, followed by DFAT	Not mentioned
(Rosen 1987a)	*Ae. albopictus*	Intrathoracic inoculation	DENV-1	1570-2661	0.38-5.1/1000	MIR	Fiji, 1975. Method of passage unknown	Amplification in *Tx. amboinensis*, followed by Fluorescent Antibody Technique (FAT).	Not mentioned

*Where multiple infection rates were taken for the same type of host species, at the same stage of life-cycle, the range of sample sizes and infection rates are listed.

Table 4.1 continued.

Source	Host Species	Infection route of parent generation	Serotype of dengue	Sample Size (individual specimens)*	Infection rate*	Type of infection rate	Origin of dengue serotype	Methodology of Screening	Use of Controls in screening
(Rosen 1988)	*Ae. albopictus*	Oral inoculation	DENV-1	96-4336	2.3-21/1000	MIR	Fiji	Amplification in *Tx. amboinensis* , followed by IFAT	Not mentioned
(Mitchell & Miller 1990)	*Ae. albopictus*	Parenteral inoculation	DENV-1 DENV-4	84-626 1022	1.6-12/1000 0.98/1000	MIR MIR	Puerto Rico (1985), passed once through *Tx. amboinensis*.	Amplification in *Ae. albopictus* or *Tx. amboinensis*, followed by DFAT	Not mentioned
(Bosio et al. 1992)	Various strains of *Ae. albopictus* *Ae. aegypti*	Oral inoculation	DENV-1 DENV-1	391-797 756	11.1-41.2% 0.13-2.9% 3% 0.13%	VTR FIR VTR FIR	Jamaica. Passed once through a mosquito and C6/36 *Ae. albopictus* cells.	IFAT	Not mentioned
(Joshi et al. 1996)	*Ae. aegypti*	Intrathoracic inoculation	DENV-3	17	88%	VIR	Unknown	IFAT	Not mentioned
(Lee et al. 1997)	*Ae. aegypti*	Oral inoculation	DENV-1-4	390	15/1000	MIR	Human serum, passed once through C6/36 *Ae. albopictus* cells.	Peroxidase-antiperoxidase staining (PAP) and RT-PCR	Not mentioned
(Gokhale et al. 2001)	*Ae. albopictus*	Intrathoracic inoculation	DENV-2	1965 1046 1083	1.5/1000 5.7/1000 6.5/1000	MIR MIR MIR	Jammu, India. Passed 8 times through mice.	ELISA and further confirmation through amplification in a mosquito, followed by IFAT	Not mentioned

*Where multiple infection rates were taken for the same type of host species, at the same stage of life-cycle, the range of sample sizes and infection rates are listed.

Table 4.1: Continued

Source	Host Species	Infection route of parent generation	Serotype of dengue	Sample Size (individual specimens)*	Infection rate*	Type of infection rate	Origin of dengue serotype	Methodology of Screening	Use of Controls in screening
(Mourya et al. 2001)	*Ae. aegypti* *Ae. aegypti*	Oral inoculation Intrathoracic inoculation	DENV-2	250-750	2.2-5/1000 7.1-63%	MIR Pools of infected larvae (of unknown size)	Jammu, India. Passed 8 times through mice.	ELISA	Not mentioned
(de Castro et al. 2004)	4th instar larvae *Ae. albopictus*	Oral inoculation	DENV-2	284	56/1000	MIR	Rio de Janeiro (1998). Passed an unspecified number of times through C6/36 *Ae. albopictus* cells.	IFAT and confirmation with RT-PCR	Positive and negative for RT-PCR
	Adult female *Ae. albopictus*			92	98/1000	MIR			
	4th instar larvae *Ae. aegypti*			521	17/1000	MIR			
	Adult female *Ae. aegypti*			59	51/1000	MIR			
(Guo et al. 2007)	Non-diapausing *Ae. albopictus* eggs	Oral inoculation	DENV-2	NA	3/5 pools	NA	New Guinea, passed at last once through a mouse.	RT-PCR and Southern-blot	Positive controls
	Diapausing *Ae. albopictus* eggs			NA	2/5 pools	NA			
(Buckner et al. 2013)	*Ae. aegypti* *Ae. albopictus*	Oral inoculation	DENV-1	36 18	8% 11%	VTR VTR	Florida (2010). Passed 3 times through kidney cells.	qRT-PCR	Positive and negative

*Where multiple infection rates were taken for the same type of host species, at the same stage of life-cycle, the range of sample sizes and infection rates are listed.

Table 4.2: Summary information of laboratory studies on vertical transmission of dengue viruses in uncommon dengue vectors.

Source	Host Species	Infection route of parent generation	Serotype of dengue	Sample Size (individual specimens)*	Infection rate*	Type of infection rate	Origin of dengue serotype	Methodology of Screening	Use of Controls in screening
(Gubler et al. 1985)	*Ae. mediovittatus* (Coquillett)	Parenteral Inoculation	DENV-1	384	2.6 /1000	MIR	Puerto Rican strain, passed at least once through a mosquito species.	Amplification in a mosquito, followed by DFAT	Not mentioned
(Freier & Rosen 1987)	Various mosquitoes of the *Ae. scultellaris* (Walker) group	Intrathoracic inoculation	DENV-1 DENV-2 DENV-3 DENV-4	50-1724 90-1500 341-2031 169-2958	0-1.7% 0-1.4% 0-1.9% 0-0.6%	FIR FIR FIR FIR	DENV-1 Fiji (1975), DENV-2 Thailand (1974), DENV-3 Burma (1976) and DENV-4 Indonesia (1973). All were passed through *Tx. amboinensis*	Amplification in *Tx. amboinensis*, followed by Indirect fluorescent antibody test (IFAT).	Not mentioned
(Freier & Rosen 1988)	*Ae. mediovittatus*	Intrathoracic inoculation	DENV-1 DENV-2 DENV-3 DENV-4	6-19 Families º 7-15 Families º NA 12 Families º	26.7-94.7% 23.1-75% 12.5% 66.7%	VTR VTR VTR VTR	Many virus strains had been used. All had been passed through *Tx. amboinensis* at least once.	Amplification in *Tx. amboinensis* , followed by IFAT	Not mentioned
			DENV-1 DENV-2 DENV-3 DENV-4	130-720 114-601 257-510 310-506	0.7-18.8% 0.7-20.3% 0.7-3.1% 0.3-10.5%	FIR FIR FIR FIR	Fiji (1975). Passed 3 times through *Tx. amboinensis*.		
(de Souza & Freier 1991)	*Haemagogus equinus* (Theobald)	Intrathoracic inoculation	DENV-1	989 1500	2/1000 1.3/1000	MFIR		IFAT	Not mentioned

*Where multiple infection rates were taken for the same type of host species, at the same stage of life-cycle, the range of sample sizes and infection rates are listed.

º Offspring of a single female.

Table 4.3: Summary information of field surveys studying vertical transmission of dengue viruses in mosquitoes that list infection rates for individual dengue virus serotypes.

Source	Location	Host Species #	Serotype	Sample Size (individual specimens)	Infection rate	Type of infection rate	Methodology of Screening	Use of Controls in screening
(Khin & Than 1983)	Rangoon, Myanmar	*Ae. aegypti* larvae	DENV-2	6200	0.48/1000	MIR	Amplification in *Tx. amboinensis* followed by Direct Fluorescent Antibody Technique (DFAT).	Positive and negative controls
		Larval male *Ae. aegypti* (RAL)	DENV-2	7730	0.26/1000	MIR		
(Hull et al. 1984)	Trinidad	*Ae. aegypti* eggs (RAL)	DENV-4	10957	0.091/1000	MIR	Amplification in *Ae. psedoscutellaris* cell lines, followed by Indirect fluorescent antibody test (IFAT).	Not mentioned
(Serufo et al. 1993)	Campos Altos City, Brazil	Larval *Ae. albopictus*	DENV-1	1128	1.77/1000	MIR	Amplification in *Ae. albopictus* C6/C36 cell lines, followed by Fluorescent Antibody Technique (FAT). ELISA followed by RT-PCR was used to identify serotyping.	Not mentioned
(Joshi et al. 1996)	Jalore, India	Larval *Ae. aegypti* (RAL)	DENV-3	388	5.7%	VIR	IFAT	Not mentioned
(Thenmozhi et al. 2000)	Tamil Nadu, India	Adult male *Ae. aegypti*	DENV-2	3701	0.54/1000	MIR	ELISA. Serotype confirmation through amplification in *Tx. splendens*, followed by IFAT.	Not mentioned
			DENV-3	3701	0.27/1000	MIR		
		Male larvae *Ae. aegypti* (RAL)	Unknown	3583	0.28/1000	MIR		
		Female larvae *Ae. aegypti* (RAL)	Not found		NA	NA		

Specimens caught in the field and raised to adulthood in the laboratory are listed with the acronym (RAL).

Table 4.3: Continued

Source	Location	Host Species #	Serotype	Sample Size (individual specimens)	Infection rate	Type of infection rate	Methodology of Screening	Use of Controls in screening
(Günther et al. 2007)	Oaxaca, Mexico	Larval *Ae. aegypti* Larval *Ae. aegypti* (RAL)	Not found DENV-4 DENV-2&3	280 and 340 400 460	NA 5/1000 4.3/1000	NA MIR MIR	RT-PCR. Confirmation not mentioned.	Use of negative controls for all but positive only mentioned in a figure
(Akbar et al. 2008)	Bandung, Indonesia	Larval *Ae. aegypti* (RAL) Larval *Ae. aegypti*	DENV-2 Not found	653	1.5/1000 NA	MIR NA	RT-PCR. Confirmation not mentioned.	Not mentioned
(Cecílio et al. 2009)	Minas Gerais, Brazil	Immature *Aedes* species raised to 4th instar larvae in the lab Immature *Ae. albopictus* raised to 4th instar larvae in the lab Female larval *Ae. albopictus* (RAL) Male larval *Ae. albopictus* (RAL)	Combined DENV-1-4 DENV-1 DENV-2 DENV-2	3482 1241 1241 7 11	22/1000 1.6/1000 28/1000 4/7 individuals 4/11 individuals	MIR MIR MIR VIR VIR	RT-PCR. Confirmation not mentioned.	Negative and positive control
(Le Goff et al. 2011)	Santa Cruz, Bolivia	Larvae and pupae *Ae. aegypti* (RAL)	DENV-1 DENV-3 DENV-1 DENV-3	1383 1383 1383 1383	3.6/1000 6.5/1000 0.37% 0.68%	MIR MIR TIR TIR	RT-PCR. Confirmation not mentioned.	Negative controls
(Martins et al. 2012)	Fortaleza, Ceara, Brazil	Female larvae and pupae *Ae. aegypti* (RAL) Female larvae and pupae *Ae. albopictus* (RAL)	DENV-2 DENV-2 DENV-3	2005 212 212	0.5/1000 4.7/1000 9.4/1000	MIR MIR MIR	Amplification in *Ae. albopictus* C6/C36 cell lines, followed by IFAT. Further confirmation with RT-PCR followed by nucleotide sequencing.	Negative control for IFAT. Negative and positive control for RT-PCR

Specimens caught in the field and raised to adulthood in the laboratory are listed with the acronym (RAL).

Table 4.4a: Summary information of field surveys that failed to find evidence of vertical transmission of dengue viruses in mosquitoes.

Source	Location	Host Species#	Serotype	Sample Size (individual specimens)	Infection rate	Type of infection rate	Methodology of Screening	Use of Controls in screening
(Ramalingam et al. 1986)	Kelang, Malaysia	Immature female *Ae. aegypti* (RAL) Immature male *Ae. aegypti* (RAL) Immature female *Ae. albopictus* (RAL) Immature male *Ae. albopictus* (RAL)	Not found Not found Not found Not found	20 34 215 221	NA NA NA NA	NA NA NA NA	Amplification in *Tx. splendens* or *Ae. aegypti*, followed by Direct fluorescent antibody test (DFAT).	Not mentioned
(Hutamai et al. 2007)	Northern Thailand	Larval *Ae. aegypti* (RAL). Larval *Ae. albopictus* (RAL).	Not found Not found	9825 150	NA NA	NA NA	RNA extraction and the molecular technique NASBA	Positive and negative controls
(Zeidler et al. 2008)	Roraima, Brazil	*Ae. aegypti* eggs raised to 4th instar larvae	Not found	Larva resulting from 1422 eggs	NA	NA	RT-PCR. Confirmation not mentioned.	Positive controls

Specimens caught in the field and raised to adulthood in the laboratory are listed with the acronym (RAL).

Table 4.4b: Summary information of field surveys that failed to find evidence of vertical transmission of dengue viruses in mosquitoes, but did find evidence of horizontal transmission of dengue viruses.

Source	Location	Host Species #	Serotype	Sample Size (individual specimens)	Infection rate	Type of infection rate	Methodology of Screening	Use of Controls in screening
(Watts et al. 1985)	Bangkok, Thailand	Adult female *Ae. aegypti* in Bangkok	DENV-2	268	5.2%	IR	DFAT. Confirmation by amplification in *Ae. pseudoscutellaris* cell line or *Tosorhynshites* mosquitoes, followed by DFAT	Not mentioned
		Adult male *Ae. aegypti* in Bangkok	Not found	85	NA	NA		
		Larvae *Ae. aegypti* in Bangkok	Not Found	5839	NA	NA		
		Pupae *Ae. aegypti* in Bangkok	Not Found	39	NA	NA		
		Immature female *Ae. aegypti* in Ban Yang (RAL)	Not found	505	NA	NA		
		Immature male *Ae. aegypti* in Ban Yang (RAL)	Not found	187	NA	NA		
		Immature female *Ae. albopictus* in Ban Yang (RAL)	Not found	1740	NA	NA		
		Immature male *Ae. albopictus* in Ban Yang (RAL)	Not found	1459	NA	NA		
(Ilkal et al. 1991)	Maharashtra, India	Adult Female *Ae. aegypti*	DENV-2	375	2.1%	IR	IFAT. Confirmation by amplification in *Ae. aegypti,* followed by IFAT. Serotype identification through amplification in *Ae. albopictus* cell lines or mice, followed by complement fixation.	Not mentioned
			DENV-3	375	0.8%	IR		
			Unknown	375	4.8%	IR		
		Adult Male *Ae. aegypti*	Not Found	64	NA	NA		
		Larval Male *Ae. aegypti* (RAL)	Not Found	281	NA	NA		
		Larval Female *Ae. aegypti* (RAL)	Not Found	323	NA	NA		

Specimens caught in the field and raised to adulthood in the laboratory are listed with the acronym (RAL).

Table 4.4b: Continued

Source	Location	Host Species	Serotype	Sample Size (individual specimens)*	Infection rate*	Type of infection rate	Methodology of Screening	Use of Controls in screening
(Chow et al. 1998)	Singapore	Adult Female *Ae. aegypti*	DENV-1	409	44/1000	MIR	RT-PCR. Confirmation of DENV-1 and DENV-2 by nucleotide sequencing.	Not mentioned
			DENV-2	409	4.9/1000	MIR		
			DENV-3	409	2.5/1000	MIR		
		Larval *Aedes*	Not Found	53 pools of 1-10 individuals	NA	NA		
		Adult Female *Ae. albopictus*	DENV-1	784	10/1000	MIR		
			DENV-2	784	6.4/1000	MIR		
			DENV-4	784	1.3/1000	MIR		
(Romero-Vivas et al. 1998)	Puerto Triunfo Columbia	Adult Female *Ae. aegypti*	DENV-1	130-670	0.15-0.77%	IR	FAT	Positive controls
			DENV-2	359-670	0.27-1.9%	IR		
		Adult Male *Ae. aegypti*	Not Found	1522	NA	NA		
(Pinheiro et al. 2005)	Manaus, Amazonas, Brazil	Adult Female *Ae. aegypti*	DENV-3	374	37/1000	MIR	RT-PCR. And further confirmation by amplification in C6/C36 cells, followed by IFAT and nucleotide sequencing.	Negative controls for IFAT
		Adult Male *Ae. aegypti*	Not Found	300	NA	NA		
		Immature *Ae. aegypti*	Not Found	1142	NA	NA		
(Chen et al. 2010)	Southern Taiwan	Adult female *Ae. aegypti* pooled across all sample	Combined	12372	0.97/1000	MLE	qRT-PCR. Confirmation by nucleotide sequencing.	Not mentioned
			DENV-1-4	12372	0.97/1000	MIR		
		Adult female *Ae. aegypti* by month	DENV-1-4	939-1996	0.5-2.2/1000	MLE		
		Adult female *Ae. aegypti* by month and city level	DENV-1-4	75-1364	0.73-12.6 /1000	MLE		
		Adult male *Ae. aegypti*	Not found	49759	NA	NA		
		Adult female *Ae. albopictus*	Not found	57,319	NA	NA		
		Adult male *Ae. albopictus*	Not found	21,996	NA	NA		

*Where multiple infection rates were taken for the same type of host species, at the same stage of life-cycle, the range of sample sizes and infection rates are listed.

Table 4.5: Summary information of field surveys studying vertical transmission of dengue viruses in mosquitoes that list infection rates for dengue virus serotypes combined.

Source	Location	Host Species #	Sample Size (individual specimens)*	Infection rate *	Type of infection rate	Methodology of Screening	Use of Controls in screening
(Ahmad et al. 1997)	Malaysia	Larval Ae. aegypti (RAL)	14605	0.14/1000	MIR	Amplification in Ae. albopictus C6/C36 cell lines, followed by PAP. Further confirmation with RT-PCR	Positive and negative controls for PAP staining.
		Larval Ae. albopictus (RAL)	47804	0.15/1000	MIR		
(Kow et al. 2001)	Singapore	Adult male *Ae. aegypti*	600	1.33%	VIR	RT-PCR. Confirmation by nucleotide sequencing.	Negative controls
		Adult male *Ae. albopictus*	837	2.15%	VIR		
(Lee & Rohani 2005)	Kuala Lumpur, Malaysia	Immature *Ae. aegypti* (raised to 3rd instar larvae within the lab).	19434	5.77-40/1000	MIR	Amplification in C6/C36 cells, followed by PAP.	Not mentioned
		Immature *Ae. albopictus* (raised to 3rd instar larvae within the lab).	3759	2.4-14/1000	MIR		
(Joshi et al. 2006)	Johpur City, India	Tree-hole found larvae *Ae. albopictus* (RAL)	67	9%	VIR	IFAT	Not mentioned
		Tree-hole found larvae *Ae. aegypti* (RAL)	23	Not found	NA		
		Tree-hole found larvae *Ae. vittatus* (Bigot) (RAL)	4	Not found	NA		
(Rohani et al. 2007)	Malaysia	Larval *Ae. aegypti* (via PAP staining)	40-510	3.9-31/1000	MIR	Amplification in C6/C36 cells followed by PAP staining and RT-PCR	Positive and negative controls for RT-PCR
		For All Larval *Ae. aegypti* (via PAP staining)	2250	16/1000	MIR		
		Larval *Ae. aegypti* (via RT-PCR)	20-290	6.9-50/1000	MIR		
		For All Larval *Ae. aegypti* (via RT-PCR)	990	12/1000	MIR		
		Larval *Ae. albopictus* (via PAP staining)	10-340	2.9-100/1000	MIR		
		For All Larval *Ae. albopictus* (via PAP staining)	2130	17/1000	MIR		
		Larval *Ae. albopictus* (via RT-PCR)	220-290	4.5-10/1000	MIR		
		For All Larval *Ae. albopictus* (via RT-PCR)	780	7.7/1000	MIR		

Specimens caught in the field and raised to adulthood in the laboratory are listed with the acronym (RAL).

*Where multiple infection rates were taken for the same type of host species, at the same stage of life-cycle, the range of sample sizes and infection rates are listed.

Table 4.5: Continued.

Source	Location	Host Species #	Sample Size (individual specimens)*	Infection rate *	Type of infection rate	Methodology of Screening	Use of Controls in screening
(Arunachalam et al. 2008)	Chennai, Tamil Nadu, India	Adult male *Ae. aegypti*	5408	2.7-28/1000	MIR	ELISA. Serotype confirmation through amplification in *Tx. splendens*, followed by IFAT.	Not mentioned
(Angel et al. 2008)	Rajasthan, India	Larval *Ae. aegypti* (RAL) Jodhpur Strain Larval *Ae. aegypti* (RAL) Kota Strain Larval *Ae. vittatus* (RAL) Jodhpur Strain Larval *Ae. vittatus* (RAL) Jaipur Strain Larval *Ae. albopictus* (RAL) Jodhpur Strain	37 35 61 11 39	160/1000 29/1000 16/1000 180/1000 26/1000	VIR VIR VIR VIR VIR	IFAT, further confirmed by pooling positives, amplifying in mice and retesting by IFAT.	Not mentioned
(Angel & Joshi 2008)	Rajasthan, India	Larval *Ae. aegypti* (RAL) Larval *Ae. albopictus* (RAL) Larval *Ae. vittatus* (RAL)	977 251 383	11.2% 15.9% 8.4%	VIR VIR VIR	IFAT, further confirmed by pooling positives, amplifying in mice and retesting by IFAT.	Positive and negative controls
(Thongrungkiat et al. 2011)	Bangkok, Thailand	Larval *Ae. aegypti* dark form strain (RAL) Larval *Ae. aegypti* pale form strain (RAL)	15179 278	15.6/1000 12.9/1000	MIR MIR	RT-PCR. Confirmation not mentioned.	Positive and negative control in example figure.

Specimens caught in the field and raised to adulthood in the laboratory are listed with the acronym (RAL).
*Where multiple infection rates were taken for the same type of host species, at the same stage of life-cycle, the range of sample sizes and infection rates are listed.

Table 4.6: Summary information of field survey that searched for vertical and horizontal transmission of dengue viruses in mosquitoes.

Source	Location	Host Species #	Serotype	Sample Size (individual specimens)	Infection rate	Type of infection rate	Methodology of Screening	Use of Controls in screening
(Ibáñez-Bernal et al. 1997)	Reynosa, Mexico	Adult female *Ae. aegypti*	Not found	1051	NA	NA	Amplification in (*Ae. albopictus)* C6/C36 cells or VERO (green-monkey kidney) cells, followed by haemagglutination, IFAT and confirmation by RT-PCR.	Negative controls
		Adult male *Ae. aegypti*	Not found	1600	NA	NA		
		Adult female *Ae. albopictus*	Not found	2339	NA	NA		
		Adult male *Ae. albopictus*	DENV-2	647	1.5/1000	MIR		
			DENV-3	647	1.5/1000	MIR		
(Fouque et al. 2004)	French Guiana	Eggs *Ae. aegypti* (RAL).	DENV-4	3435	0.58/1000	MIR	Amplification in AP61 cell culture, followed by IFAT.	Not mentioned
		Larval *Ae. aegypti* (RAL).	DENV-4	4078	0.25/1000	MIR		
		Adult Male *Ae. aegypti*	Not found	502	NA	NA		
		Adult Female *Ae. aegypti*	Not found	251	NA	NA		
(Thavara et al. 2006)	Southern Thailand	Adult Female *Ae. aegypti*	Combined DEN1-4	145	15%	IR	RT-PCR. Confirmation not mentioned.	Positive and negative controls
		Adult Male *Ae. aegypti*	Combined DEN1-4	324	16%	VIR		
(Thenmozhi et al. 2007)	Kerala, Southern India	Female larval *Ae. albopictus* (RAL).	DENV-1	1485	0.67/1000	MIR	ELISA. Serotype confirmation through amplification in *Tx. splendens*, followed by IFAT.	Positive and negative controls used for ELISA
		Adult female *Ae. albopictus*	Unknown serotype	1445	0.69/1000	MIR		
		Adult male *Ae. albopictus*	Unknown serotypes	1817	2.2/1000	MIR		
(Das Bina et al. 2008)	Jaipur and Delhi, India	Adult male *Ae. aegypti*	Not found	3	NA	NA	ELISA	Positive and negative controls
		Adult Female *Ae. aegypti*	Not found	3	NA	NA		
		Larval *Ae. aegypti* (RAL)	Combined DENV-1-4	63	48/1000	MIR		

Specimens caught in the field and raised to adulthood in the laboratory are listed with the acronym (RAL).

Table 4.6: Continued

Source	Location	Host Species #	Serotype	Sample Size (individual specimens)*	Infection rate *	Type of infection rate	Methodology of Screening	Use of Controls in screening
(Guedes et al. 2010)	Recife, Brazil	*Ae. aegypti* eggs (RAL)	DENV-1	2972	1/1000	MIR	RT-PCR. Confirmation not mentioned.	Not mentioned
			DENV-2	2972	3.4/1000	MIR		
			DENV-3	2972	1.3/1000	MIR		
		Adult Female *Ae. aegypti*	DENV-1	301	17/1000	MIR		
			DENV-2	301	6.6/1000	MIR		
			DENV-3	301	6.6/1000	MIR		
(Vilela et al. 2010)	Minas Gerais, Brazil	Female adult *Ae. aegypti*	DENV-3	137	21.9/1000	MIR	RT-PCR. Confirmation by nucleotide sequencing.	Not mentioned
		Male adult *Ae. aegypti*	DENV-3	100	10/1000	MIR		
		Ae. aegypti eggs raised to larvae for identification	DENV-3	5573	0.18/1000	MIR		
(de Figueiredo et al. 2010)	Various regions of Brazil	Female adult *Haemagogus leucocelaenus* (Dyar & Shannon)	DENV-1	170	5.8/1000	MIR	RT-PCR. DENV-3 confirmed by nucleotide sequencing.	Positive and negative control in example figure.
		Female adult *Ae. aegypti*	DENV-1	43	23/1000	MIR		
		Female adult *Ae. aegypti*	DENV-2	403	2.5/1000	MIR		
		Larval *Ae. albopictus*	DENV-3	542	5.5/1000	MIR		
		Female adult *Ae. albopictus*	Not found	49	NA	NA		
		Male adult *Ae. albopictus*	Not found	39	NA	NA		
		Male adult *Ae. aegypti*	Not found	31	NA	NA		

Specimens caught in the field and raised to adulthood in the laboratory are listed with the acronym (RAL).

*Where multiple infection rates were taken for the same type of host species, at the same stage of life-cycle, the range of sample sizes and infection rates are listed.

Table 4.6: Continued

Source	Location	Host Species #	Serotype	Sample Size (individual specimens)*	Infection rate *	Type of infection rate	Methodology of Screening	Use of Controls in screening
(Mulyatno et al. 2012)	Surabaya, Indonesia	Female adult *Ae. aegypti*	DENV-1	65-94	23-32/1000	MIR	Amplification in C6/C36 cells followed by RT-PCR. Confirmation not mentioned.	Not mentioned
			DENV-2	127	16/1000	MIR		
		Male adult *Ae. aegypti*	DENV-1	62-78	16-26/1000	MIR		
		Female larval *Ae. aegypti* (RAL)	DENV-1	250-453	8-2.1/1000	MIR		
		Male larval *Ae. aegypti* (RAL)	DENV-1	350-582	5.7-8.6/1000	MIR		
		Larval *Ae. aegypti*	DENV-1	550	5.5/1000	MIR		
(Das et al. 2013)	Orissa state, India	Adult Female *Ae. albopictus*	DENV-2	112	8.92/1000	MLE	RT-PCR. 4 DENV-2 pools and 1 DENV-3 pool confirmed by nucleotide sequencing.	Positive controls (negative control in an example figure.)
		Pupae *Ae. albopictus* (RAL)	DENV-2	687	6.09 /1000	MLE		
		Pupae *Ae. aegypti* (RAL)	DENV-2	381	2.63/1000	MLE		
		Adult Female *Ae. albopictus*	DENV-2	112	8.9/1000	MIR		
		Pupae *Ae. albopictus* (RAL)	DENV-2	687	5.8/1000	MIR		
			DENV-3	687	1.5/1000	MIR		
		Pupae *Ae. aegypti* (RAL)	DENV-2	381	2.6/1000	MIR		
		Pupae *Ae. albopictus* (RAL) from control site	DENV-2	440	2.27/1000	MLE		
(Martínez et al. 2014)	Acapulco, Mexico	Male larvae *Ae. aegypti* (RAL)	DENV-1	Unclear	1.4/1000	MLE	RT-PCR. Confirmation not mentioned.	Not mentioned
		Female larvae *Ae. aegypti* (RAL)	Not found	Unclear	NA	NA		
		Adult male *Ae. aegypti*	DENV-1	Unclear	6.18/1000	MLE		
		Adult female *Ae. aegypti*	Not found	Unclear	NA	NA		

Specimens caught in the field and raised to adulthood in the laboratory are listed with the acronym (RAL).

*Where multiple infection rates were taken for the same type of host species, at the same stage of life-cycle, the range of sample sizes and infection rates are listed.

Table 4.6: Continued

Source	Location	Host Species #	Serotype	Sample Size (individual specimens)	Infection rate	Type of infection rate	Methodology of Screening	Use of Controls in screening
(Sanchez-Rodríguez et al. 2014)	Monterrey, Mexico	Larvae *Ae. aegypti* (RAL)	Not found	833	NA	NA	RT-PCR. Confirmation not mentioned.	Positive and negative controls
		Larvae *Ae. albopictus* (RAL)	Unknown	1836	0.544/1000	MIR		
		Adult male *Ae. aegypti*	Not found	81	NA	NA		
		Adult female *Ae. aegypti*	Not found	67	NA	NA		
		Adult male *Ae. albopictus*	Not found	151	NA	NA		
		Adult female *Ae. albopictus*	Not found	405	NA	NA		
(Yang et al. 2014)	Shenzhen, China	Larvae *Ae. aegypti* (RAL)	DENV-1	9000	0.3/1000	MIR	RT-PCR. Unclear if nucleotide sequence confirmation was used mosquito samples.	Not mentioned for entomology screenings
		Adult male *Ae. aegypti*	Not found	Not mentioned	NA	NA		
		Adult female *Ae. aegypti*	Not found	Not mentioned	NA	NA		
(Espinosa et al. 2014)	Puerto Iguazú, Misiones, Argentina	Adult male *Ae. aegypti*	DENV-3	Not mentioned	1 positive pool	NA	RT-PCR Confirmation by nucleotide sequencing.	Not mentioned
		Adult female *Ae. aegypti*	Not found	Not mentioned	NA	NA		

Specimens caught in the field and raised to adulthood in the laboratory are listed with the acronym (RAL).

Table 4.7: Summary information of laboratory studies on the vertical transmission of dengue viruses through several generations of mosquitoes.

Source	Host Species	Infection route of F0 Generation	Serotype of dengue	Sample Size (Generation followed by individuals tested)	Infection rate	Type of infection rate	Origin of dengue serotype	Methodology of Screening	Use of Controls in screening
(Shroyer 1990)	*Ae. albopictus* F0 to F3	Intrathoracic inoculation	DENV-1	F0 279, F1 475	0.7%	VTR	Fiji. Passed once through *Ae. albopictus* and twice in *Tx. amboinensis*.	Individual samples directly analyzed by IFAT. Followed by double checking of negatives, by amplification in *Tx. amboinensis*, followed by IFAT.	Not mentioned
				F1 2, F2 104	100%	VTR			
			DENV-1	F0 279, F1 475	0.4%	FIR			
				F1 2, F2 24 and 80	2.5 and 4.2%	FIR			
				F2 2, F3 11 and 59	0 and 3.4%	FIR			
(Joshi & Sharma 2001)	*Ae. aegypti* F0 to F7	Intrathoracic inoculation	DENV-3	F0 16, F1 50	52%	VIR	Other than the serial number for the National Institute of Virology, Pune, India, where the virus is stored information is scant (633978).	IFAT	Not mentioned
				F1 Unclear, F2 142	55.6%	VIR			
				F2 Unclear, F3 431	55.6%	VIR			
				F3 Unclear, F4 37	67.5%	VIR			
				F4 Unclear, F5 65	30.7%	VIR			
				F5 Unclear, F6 26	23.0%	VIR			
				F6 Unclear, F7 180	15.5%	VIR			
(Joshi et al. 2002)	*Ae. aegypti* F0 to F7	Intrathoracic inoculation	DENV-3	F0 Unclear probably 200, F1 123	2.8%	VIR	Thailand (1963). Passed through mice 21 times.	Amplification in a mosquito, followed by IFAT	Not mentioned
				F1 200, F2 158	8.6%	VIR			
				F2 200, F3 258	13.0%	VIR			
				F3 200, F4 122	11.7%	VIR			
				F4 200, F5 154	11.6%	VIR			
				F5 200, F6 145	11.7%	VIR			
				F6 200, F7 157	12.6%	VIR			

Table 4.7: Continued

Source	Host Species	Infection route of F0 Generation	Serotype of dengue	Sample Size (Generation followed by individuals tested)	Infection rate	Type of infection rate	Origin of dengue serotype	Methodology of Screening	Use of Controls in screening
(Wasinpiyamongkol et al. 2003)	Pale *Ae. aegypti* F0 to F1	Oral inoculation	DENV-2	F0 41, F1 36 from a vertically transmitting family, 11, 10 and 5 from non- vertically transmitting families	2.7%	FIR	Thailand passed a number of times through Toxorhynchites mosquitoes.	IFAT	Not mentioned
	Dark *Ae. aegypti* F0 F3			F0 63, F1 54 from a vertically transmitting family, 17 and 5 from non- vertically transmitting families	3.7%	FIR			
				F1 2, F2 59 from a vertically transmitting family, 17 from a non- vertically transmitting family	3.3%	FIR			
				F2 2, F3 70 from a vertically transmitting family	1.4%	FIR			
(Rohani et al. 2008)	*Ae. aegypti* F0 to F5	Oral inoculation	DENV-2	F0 100-150, F1 200	45/1000	MIR	Malaysia (2004). Passed an unspecified number of times through C6/36 *Ae. albopictus* cells.	Amplification in cell culture, followed by PAP.	Positive and negative control
				F1 approximately 250, F2 200	45/1000	MIR			
				F2 approximately 250, F3 200	35/1000	MIR			
				F3 approximately 250, F4 200	35/1000	MIR			
				F4 approximately 250, F5 200	30/1000	MIR			

4.6 The mechanistic basis of vertical transmission

Rosen (1987a) suggests that the reason for the low rate of vertical transmission of dengue viruses may be due to its mechanical basis. Infection of *Aedes* would have to take place after the development of the egg via the micropyle during fertilisation at the time of oviposition (transmission occurring from either the father or mother), due to the fact that the eggs begin to develop as soon as the blood meal is taken and once developed the eggs are surrounded by a thick chorion. This chorion would present a barrier for dengue viruses reaching the eggs.

Many field studies in this review use the detection of dengue viruses in male mosquitoes to infer that vertical transmission has taken place because male mosquitoes do not feed on blood (Service 2012). In order to discount the possibility that male mosquitoes have not acquired dengue viruses through sexual transmission, Rosen in 1987(b) determined that whilst male *Ae. albopictus* could sexually infect female *Ae. albopictus*, females could not infect males. However this is the only direct research on sexual transmission of dengue viruses in mosquitoes. Also worth noting is that this study found that some of the offspring of female *Ae. albopictus* infected from males, were dengue virus positive and suggested that the incubation rate for vertical transmission via this route was quicker than through the oral or intrathoracic inoculation. Rosen (1987b) suggested that these findings support his previous suggestion (Rosen 1987a) that vertical transmission occurs during fertilisation at the time of oviposition.

Immunoflourscent techniques have been used to stain the organs of orally and parenterally DENV-1 infected *Ae. albopictus* and *Ae. aegypti* (Chen et al. 1993). DENV-1 infections were found in the female ovarioles, oviducts and accessory glands. The male testes, vas deferens, seminal vesicles and accessory glands were infected but

the spermathecae were not, possibly due to a chitinous barrier. This work supports the previous finding that whilst male *Ae. albopictus* could sexually infect female *Ae. albopictus*, females could not infect males sexually (Rosen 1987b). Chen et al. (1993) also suggest that the mechanism of vertical infection is during fertilisation at the time of oviposition (Rosen 1987a), however their report suggests that further study would be needed.

Tu et al. (1998) used IFAT and electron-microscopy to study the reproductive system of DENV-2 intrathoracically infected male *Ae. aegypti*. As with previous studies (Chen et al. 1993) most of the tissue of male reproductive system was found to be infected but the actual germ cells (i.e. spermatogonia, spermatocytes, spermatid and spermatozoa) were not infected. This work further supports the findings of Rosen (1987b) that infected males have the ability to sexually transmit dengue viruses and also transmit the virus to their offspring at fertilisation (Rosen 1987a; Rosen 1987b).

In a survey of ovarian proteins and dengue virus infections of *Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus* (caught in the field as larvae and raised to adulthood in the lab) Angel et al. (2008) suggested a negative association between dengue virus infections and a 200kDa protein (see Table 4.5). If associations exist between ovarian proteins and dengue virus infections this could shed light on the mechanism of vertical transmission and any genetic associations with vertical transmission within the vector. However Angel et al. (2008) provided no statistical evidence for this association.

Using RT-PCR and Western blot screening for dengue virus E protein, Zhang et al. (2010) detected no DENV-2 replication in the ovaries of intrathoracic infected *Ae. albopictus*. This may well add to Rosen (1987a) suggestion that vertical transmission occurs during fertilisation at the time of oviposition. Zhang et al. (2010) suggest that the lack of viral replication in the ovaries may explain why vertical transmission occurs at

such a low rate and that further study on the mechanism of vertical transmission may shed light on this. As such there is relatively little knowledge of the mechanistic basis of vertical transmission of dengue viruses, although the research available is reasonably consistent.

4.7 Transgenerational vertical transmission of dengue virus

Shroyer (1990) found that DENV-1 virus could be transmitted vertically across 3 generations, by selecting the eggs of those females that had been infected through vertical transmission. The VTR increased dramatically from the F_1 to the F_2 generation from 0.7% to 100% (see Table 5.7), suggesting that there was heritable variation in VTR for dengue viruses within either the mosquito host or the dengue virus. The number of offspring that had acquired DENV-1 from their mother ranged from 0-4.2% FIR. Bosio et al. (1992) in a lab study using DENV-1 infected several strains of *Ae. albopictus* and measured the variation in their FIR and VTR. Bosio et al. (1992) found that the variation in FIR was insignificant between strains of *Ae. albopictus*. However Bosio et al. (1992) found a greater source of variation in FIR in the different families, within the strains (≈95.2%) (see Table 4.1). The study by Bosio et al. (1992) finding high variation in FIR between different families and the findings of Shroyer (1990) selection experiment suggest that higher rates of FIR and VTR could be selected for.

Joshi et al. (2002) demonstrated using a lab strain of *Ae. aegypti*, that DENV-3 viruses could persist through vertical transmission for 7 generations (see Table 5.7). The vertically infected lines had an increased larval mortality rate compared to control lines, but no statistical analysis was carried out (Joshi et al. 2002). In what seems to have been a preliminary study to Joshi et al. 2002, the progeny of *Ae. aegypti* infected with DENV-3, were measured for success in reaching adulthood, for seven generations (Joshi & Sharma 2001) (see Table 5.7). It is unclear whether each of the seven

generations was intrathoracically infected with DENV-3, or whether the eggs of vertically infected adults were selected for use in the proceeding generation. Joshi & Sharma (2001) state there was a higher failure rate in reaching adulthood for the virus infected line compared to the control line (however no statistical analyses was presented). Wasinpiyamongkol et al. (2003) was similarly able to demonstrate using *Ae. aegypti* that DENV-2 viruses could persist through vertical transmission for 3 generations of mosquitoes (see Table 5.7). The experimental procedure of selecting eggs from mothers that were found to be positive for DENV-2, was similar to Joshi et al. (2002) but unlike Joshi et al. (2002) no comparisons were made with a control group.

Rohani et al. (2008) using a laboratory strain of *Ae. aegypti* and DENV-2 further showed that dengue viruses can persist through several generation of *Ae. aegypti* via vertical transmission (see Table 5.7). The experimental set up was similar to that of Joshi et al. (2002). It is worth noting that the decrease in MIR found with subsequent generations led to DENV-2 not persisting past the 5^{th} generation. Rohani et al. (2008) report a low hatching rate of 50% within this study but as no experimental control line was used during their experiment, the effect of dengue viruses upon *Ae. aegypti* egg hatching rate cannot be inferred.

Taken together these studies suggest that there may be heritability in vertical transmission and that the virus has the potential to persist through multiple generations, via vertical transmission. Furthermore some studies suggest that such heritability in vertical transmission of dengue viruses could lead to its selection. However it remains unclear with many studies whether heritability of this trait is within the host or the virus and it is unknown how important is such selection in nature.

4.8 Lab studies on vertical transmission to desiccated and diapausing eggs

As stated in the introduction to this chapter (section 4.1) the eggs of both *Ae. aegypti* and *Ae. albopictus* can withstand desiccation but only certain strains of *Ae. albopictus* eggs undergo diapause (M. Service 2012b; WHO 2015d). The vertical transmission of dengue virus to either diapausing or desiccated mosquito eggs could provide a reservoir of dengue virus capable of persisting through seasons of low adult vector abundance. In a lab experiment using *Ae. albopictus*, Gokhale et al. (2001) found that the larvae and adult offspring of *Ae. albopictus*, intrathoracically infected with DENV-2, had higher rates of DENV-2 infection if the eggs from which they had emerged were desiccated for 1-2 months (see Table 4.1). Whilst this is one of the few studies to look at the role of diapausing or desiccated eggs on vertical transmission of dengue viruses, Gokhale et al. (2001) do not present the statistics used in their findings and the pools used to analyse their samples were of different sizes leading to a possible bias. Mourya et al. (2001) similarly found that the larvae, adult male and female offspring of female adult *Ae. aegypti* intrathoracically infected with DENV-2, had higher rates of DENV-2 infection if the eggs from which they had emerged were desiccated for 1-2 months (see Table 4.1). However again no statistic comparison is made and the pool sizes of samples used in the analysis are not discussed.

In one of the few studies to look at dengue virus replication in *Aedes* eggs, Guo et al. (2007) found that DENV-2 was replicating in non-diapausing *Ae. albopictus* but not in diapausing *Ae. albopictus* (see Table 4.1). Guo et al. (2007) suggests that dengue viruses could be surviving in diapausing eggs in a quiescent state. This would seem to suggest that leaving dengue virus infected *Aedes* eggs in a diapausing state for a long period of time should have no positive effect on whether the mosquito still has the dengue virus infection later in life. If dengue viruses were replicating during diapause

you would expect a higher viral load at the start of the mosquito's life cycle and therefore a decreased chance of the mosquito clearing the infection as it progresses through the life-cycle. Unusually Guo et al. (2007) only used eggs from the second and third gonotrophic cycle and no reason is given as to why eggs from the first gonotrophic cycle were not used. Guo et al. (2007) found no significant difference between the survivorship of DENV-2 exposed and non-DENV-2 exposed eggs but did find significantly higher hatching rates in infected non-diapausing eggs compared to infected diapausing eggs. However, Guo et al. (2007) did not make a comparison between infected and non-infected diapausing eggs. If such a comparison was made it would be possible to determine if dengue virus infection has a detrimental effect on *Aedes* eggs surviving through diapause.

Questions remain on whether infection in diapausing or desiccated eggs is detrimental to the pharate embryo (Adams and Boots 2010). The work of Joshi and Sharma (2001) and Joshi et al. (2002) suggests that vertically acquired dengue virus could have a detrimental effect on a mosquito's mortality rate and fecundity due to lost resources and damage through the viral infection. Some laboratory studies have demonstrated that horizontally acquired dengue virus can also have a negative effect on *Ae. aegypti* longevity and fecundity (Maciel-de-Freitas et al. 2011; Sylvestre et al. 2013) but others have found no effect on *Ae. aegypti* mortality rate (Carrington et al. 2015). As such not only is there a need to assess the efficiency of dengue virus transmission to eggs but any effects on the fitness of vertically infected mosquitoes, including mosquitoes that survive diapause or being desiccated as eggs.

4.9 Field and lab studies comparing vertical transmission in *Ae. albopictus* and *Ae. aegypti*

Malaysian strains of *Ae. albopictus* and *Ae. aegypti*, were fed a blood meal containing either one or all four of dengue virus serotypes (Lee et al. 1997). It is unclear if all four dengue virus serotypes were in the infectious blood meal or in separate blood meals given to separate groups of mosquitoes. Pools of fourth instar *Ae. aegypti* from the resulting second batch of eggs were found to be positive for dengue viruses (see Table 4.1) but no dengue viruses were detected in the larvae from the first and second batch of *Ae. albopictus* or the first batch *Ae. aegypti*. This suggests that *Ae. aegypti* has a higher rate of vertical transmission.

Countering this, in comparing a strain of DENV-2 infected *Ae. albopictus* (derived from specimens collected in Rio de Janeiro and kept in a lab for a year) with a well-established lab colony of DENV-2 infected *Ae. aegypti*, de Castro et al. (2004) suggest that *Ae. albopictus* have a higher vertical transmission rate (see Table 4.1), implying that *Ae. albopictus* could be playing a role as reservoir for dengue virus through vertical transmission (de Castro et al. 2004). However, statistical analysis comparing the larval infection rates of *Ae. albopictus* and *Ae. aegypti* was not presented and de Castro et al. (2004) only give the percentage of pools positive for DENV-2.

Studies from the field would possibly suggest that *Ae. albopictus* may well have a higher rate of vertical transmission. In areas known for dengue fever in humans within Singapore, Kow et al. (2001) used RT-PCR to individually screen field caught adult male *Ae. aegypti* and *Ae. albopictus*. Kow et al. (2001) report relatively high rates of infection with dengue virus, which was higher in *Ae. albopictus* (2.15% VIR, as opposed to 1.33%), however no statistical analysis was presented (see Table 4.5) .

Furthermore a survey of immature *Aedes* species in Jodhpur, Rajasthan, India (Joshi et al. 2006), found that *Ae. aegypti* and *Ae. vittatus* in tree-holes, domestic and peri-domestic water containers, were negative for dengue viruses, while some *Ae. albopictus* were found to be positive (Joshi et al. 2006) (see Table 4.5). Finally Das et al. (2013) following the first outbreak of dengue fever in 2010 in Orissa state, India, conducted a screening of adult, larval and pupae *Ae. aegypti* and *Ae. ablopictus*, in areas known for dengue virus infection (see Table 5.6). Das et al. (2013) found a higher MLE for immature *Ae. albopictus* than in immature *Ae. aegypti*.

Overall then, there is some evidence of higher vertical transmission in *Ae. albopictus* but the data is rather limited. It should be noted, however that a meta-analysis by Lambrechts et al. (2010) suggests that *Ae. albopictus* may not be an important vector of dengue virus.

4.10 Field work comparing vertical transmission in adult or late stage larvae

Günther et al. (2007) in a RT-PCR field survey of larval *Ae. aegypti* in two locations of Oaxaca, Mexico found dengue viruses in the samples, in larvae raised to adulthood in the lab, but none in samples that were analysed as larvae (see Table 4.3). They suggested that vertically transmitted dengue virus is more likely to be detected in adults. Unfortunately the sample size of samples analysed as adults from the two locations was much higher than the sample size for those analysed as larva.

In a survey of dengue virus infections in *Aedes* larvae in the Pampulha region of Brazil, eggs of *Ae. albopictus* were collected using ovitraps in areas known for dengue fever (Cecílio et al. 2009) (see Table 4.3). These eggs were raised to 4^{th} instar within the lab and then screened for dengue virus infection, in pools of 50. Upon confirmation of dengue virus infection in larvae in certain areas Cecílio et al. (2009) made another collection of eggs from those areas. These eggs were then raised to adulthood within the

lab and the resulting adult *Ae. albopictus* were then screened for Dengue viruses individually (4/7 females and 4/11 males were found positive for DENV-2). Cecílio et al. (2009) give no reason as to why this second collection of eggs, raising to them to adults and individual screening took place. If it was to compare the ability to detect vertical transmission in adult *Aedes* to that of larvae, then there are several issues with this approach. First the sample size from the eggs that were raised to adults was much lower than those analysed as larvae. Secondly the result is biased towards the eggs that were raised to adults (through the individual screening and selection of collections based upon areas known for vertical transmission of dengue virus). Thirdly if there was a time difference between the two sets of egg collection then there may have been a difference in the proportion eggs infected with dengue virus.

In one season of the study conducted by Mulyatno et al. (2012) *Ae. aegypti* larvae were collected and either screened for dengue viruses as larvae or raised to adulthood in the laboratory and then screened as adults (see Table 5.6). Other than stating the DENV-1 MIRs for these samples, no comparisons are made between these groups (this may be because the sample size is too small but this is not stated).

Overall the faults with these three studies and the lack of data in this area mean that it is still inconclusive as to whether vertically acquired dengue virus infection is more readily detected in certain stages of the *Aedes*' life cycle. Further work on this area would not only shed light on the best morphological stage for detecting vertically acquired dengue virus, but also determine if vertically acquired dengue virus can be cleared by the mosquitoes during their lifecycle. Within the laboratory Nelms et al. (2013) measured significantly higher rates of vertically transmitted West Nile Virus when screening first instar larvae of *Culex pipiens* complex than adult *C. pipiens*, suggesting that West Nile virus infection was lost in development from larvae to adult.

4.11 Field based studies comparing horizontal transmission and vertical transmission of dengue virus

The possession of data from the field on dengue viruses in adult female mosquitoes and either the rate of infection in adult male mosquitoes or immature mosquitoes, could allow vertical transmission rates to be inferred statistically. However, the studies that survey such subgroups of *Aedes* populations have run into a number of problems that have prevented this analysis.

Some field surveys fail to find evidence of vertical transmission. For instance, Ilkal et al. (1991) in a field study in Maharashta, India, found dengue virus antigens in wild caught female *Ae. aegypti* but none within wild caught adult male *Ae. aegypti* and *Ae. aegypti* larvae that were caught as larvae, which were then reared to adulthood in the lab (see Table 5.4a). Similarly in 16 sites known for dengue virus transmission across Singapore Chow et al. (1998) found dengue virus infection in female adult *Ae. aegypti* and *Ae. albopictus* but not in the larvae (see Table 5.4b). A virological screening of adult *Ae. aegypti* in Puerto Triunfo, Colombia, found DENV-1 and DENV-2 in females but not adult males (Romero-Vivas et al. 1998) (see Table 5.4b). Likewise, in the city of Manaus (Amazonas, Brazil) a survey of dengue virus infections of *Ae. aegypti* found virus in adult females but not in the 1142 larvae and pupae analysed (Pinheiro et al. 2005) (see Table 5.4b). Pinheiro et al. (2005) suggest that the low sample size could be the reason for not detecting dengue viruses in immature *Ae. aegypti*. In an extensive survey of *Ae. aegypti* and *Ae. albopictus* adults conducted in southern Taiwan from 2004-2007, Chen et al. (2010) found a few pooled samples of *Ae. aegypti* females, positive for one of the dengue virus serotypes (see Table 5.4b) but none of the male *Ae. aegypti*, male *Ae. albopictus* or female *Ae. albopictus* turned out to be infected with any dengue viruses.

Other studies found evidence of vertical transmission, but found no sign of dengue virus infections in adult females. Ibáñez-Bernal et al. (1997) were the first to detect dengue virus infection in wild adult male *Ae. albopictus* but detected no dengue virus infections in the adult females (see Table 5.6). A survey of dengue virus infections in *Ae. aegypti* in French Guiana found DENV-4 in eggs and larvae (Fouque et al. 2004) (see Table 5.6) yet no dengue viruses were isolated from adult *Ae. aegypti*, this may not be surprising considering that the adult sample size was 753, compared to 3435 eggs and 4078 larvae. In May 2007, within the urban area of Jaipur and Delhi, India Das Bina et al. (2008) despite extremely low sample sizes found 3 pools of *Ae. aegypti* that had been field caught as larvae positive for dengue virus but found no virus in female or male adults (see Table 5.6).

Thavara et al. (2006) found dengue viruses in adult male and female *Ae. aegypti* that were collected in the rainy season in villages in southern Thailand, which had experienced recent DHF cases (see Table 5.6). As Thavara et al. (2006) states that serotypes DENV-2, DENV-3 and DENV-4 were found but neglects to mention the proportions or numbers of each serotype. It is therefore not possible to calculate an estimate of vertical infection rate for each of the dengue virus serotypes.

In a survey of adult and larvae (which were raised to adulthood in the lab) *Ae. albopictus* in Kerala, southern India, Thenmozhi et al. (2007) found low MIRs for dengue virus in both the adult female mosquitoes, adult males and larvae (see Table 5.6). Other than identifying the one pool of female larvae as positive for DENV-2 Thenmozhi et al. (2007) were unable to identify the serotypes of dengue virus found. This and the low number of samples positive for dengue viruses means calculating an estimate for vertical transmission is not possible.

Vilela et al. (2010) and Guedes et al. (2010) both screened adult female *Ae. aegypti* for dengue viruses and eggs caught from ovitraps in Minas Gerais, Brazil and Racife, Brazil respectively (see Table 5.6). Guedes et al. (2010) calculate MIR for combined dengue virus serotypes, eventhough the number of pools positive for dengue virus is listed for individual serotypes (see Table 5.6). Neither Vilela et al. (2010) or Guedes et al. (2010) can easily be used to crudely infer vertical transmission rate, as either the adult caught *Ae. aegypti* or the larvae/adult raised in the lab from field caught eggs, have small sample sizes (see Table 5.6).

In a survey of dengue virus infections of the genera *Psoropora, Haemagogus* and *Aedes* from across Northeast, Southeast, and South Brazil, de Figueiredo et al. (2010) found 3 pools of *Ae. albopictus* larvae positive for DENV-3 (542 individuals) from the city of Santos but did not find any DENV-3 in any of the 88 adults (see Table 5.6). The low sample size of adult *Ae. albopictus* from Santos and lack of data on where in Santos the samples were collected means a statistical inference of the rate of vertical transmission cannot be made.

Mulyatno et al. (2012) conducted several surveys of dengue virus infections in adult and larval *Ae. aegypti* in Surabaya, Indonesia (see Table 5.6). Other than stating the findings that there was a generally higher MIR for dengue virus infections in wild caught females (horizontal transmission) than in specimens caught as larvae, Mulyatno et al. (2012) do not attempt to quantify the rate of vertical transmission (this may be because the sample size is too small but this is not stated). Das et al. (2013) found dengue virus infection in both female adult *Ae. albopictus* and pupal *Ae. albopictus*. However very few adult female *Aedes* were caught, making a comparison of horizontal and vertical infection rates difficult (see Table 5.6). As such there is still a lack of good

data comparing adult and larval infection in the field, which could be used to infer the vertical transmission rate of dengue virus.

4.12 Seasonality

Several studies suggest a peak in vertical transmission of dengue virus in certain seasons. In a survey of adult male *Ae. aegypti* in Chennai, India conducted from March 2003-December 2004, Arunachalam et al. (2008), suggested a peak in MIRs in June and July of 2003 (see Table 4.5). Arunachalam et al. (2008) conclude that vertical transmission helps in the maintenance of dengue viruses through the dry season (when adult vector numbers would be low). However, Arunachalam et al. (2008) provide no statistical evidence for this spike in MIRs and a similar rise in MIRs did not occur the following year. Similarly Angel & Joshi (2008) found a higher rate of dengue virus infection in the larvae of *Ae. albopictus* in winter. Suggesting that *Ae. albopictus* acts as a reservoir for dengue viruses through the winter, when incidents of dengue fever in humans is low (see Table 4.5) (although again no statistical analyses was presented).

Mulyatno et al. (2012) conducted several surveys of dengue virus infections in adult and larval *Ae. aegypti* in Surabaya, Indonesia (see Table 5.6) and suggested that there are higher rates of vertical transmission in the rainy season but do not present a statistical analyses. Overall then the picture is far from clear and without statistical analysis it is unclear whether there is significant seasonality in the vertical transmission of dengue virus.

4.13 Predicting dengue outbreaks

Chow et al. (1998) found dengue viruses in female adult *Aedes* species 6 weeks before the 1995 and 1996 outbreaks of dengue fever (see Table 5.4b). They suggest that monitoring of adult female *Aedes* mosquitoes for dengue virus serotypes could be used

to predict when outbreaks of human dengue fever would occur. A similar suggestion but using the monitoring of *Aedes* larvae would later be made by Lee & Rohani (2005).

It is interesting to note that previously, Joshi et al. (1996) in a survey of dengue virus infections in *Ae. aegypti* larvae, in Jalore, India, suggests a peak in infected *Ae. aegypti* larvae coincided with an outbreak of dengue fever but provided no statistical analyses backing this (see Table 4.1).

Lee & Rohani (2005) based upon a study of dengue viruses in *Ae. aegypti* and *Ae. albopictus* larval caught as eggs in ovitraps in Kuala Lumpa (August 1996-December 1997) (see Table 4.5), suggest that since they detect dengue virus in larval *Ae. aegypti* and *Ae. albopictus* 7-41 days before dengue fever cases in humans, ongoing surveillance of immature *Ae. aegypti* and *Ae. albopictus* could be used as an early warning system for dengue fever cases occurring. However, between 7 and 41 days is a large amount of variation and a statistical model would be needed to test this assertion.

In a monthly survey of dengue virus infections in larvae of two strains of *Ae. aegypti* (see Table 4.5), Thongrungkiat et al. (2011) suggests there was a peak in dengue virus infections four months before cases of dengue fever. They suggest that the monitoring of *Aedes* larvae for dengue viruses could therefore be used as an early warning system for dengue fever outbreaks. However whilst Thongrungkiat et al. (2011) do use a statistical analyses to rule out a correlation between dengue virus infections of larvae with either rainfall or human cases, they do not provide any statistical evidence for a significant peak in dengue virus infections in mosquito larvae four months before cases of dengue fever.

Martins et al. (2012) conducted a quarterly survey of female immature *Ae. aegypti* and *Ae. albopictus* in Fortaleza, Brazil (March 2007-July2009) (see Table 4.3). After finding evidence of vertical transmission, before the largest epidemic of dengue

fever seen in Fortaleza (2008), they suggest that the monitoring of immature *Aedes* could be used as an early warning system for detecting dengue fever epidemics. However the three pools of immature mosquitoes positive for dengue viruses were collected in May 2007, July 2007 and January 2008 and no statistical evidence for an association were provided.

When considering the costs in resources and man power of such an early warning system, the monitoring of *Aedes* larvae for dengue virus would be unfeasible. As suggested by Zeidler et al. (2008) after finding no signs of vertical transmission of dengue virus. This becomes more apparent when you consider the sampling effort undertaken in finding samples of immature *Aedes* specimens infected with dengue virus through vertical transmission (running from the 100s to the 10000s of samples) (see Table 4.3-6), the low rates of vertical transmission seen in the field (see Table 4.3-6) and the field based studies that found no evidence of vertical transmission (see Table 5.4a-b and section 4.14 below).

4.14 Field based studies that found no evidence of vertical transmission

Several field studies that I have already discussed found no evidence for vertical transmission of dengue viruses (Watts et al. 1985; Ramalingam et al. 1986; Ilkal et al. 1991; Romero-Vivas et al. 1998; Chow et al. 1998; Pinheiro et al. 2005; Chen et al. 2010) (see Table 4a-b). There are a number of other studies that have also failed to find evidence for vertical transmission of dengue viruses. A survey across 17 locations in the Chiang Mai and Lumpang provinces of Thailand detected no dengue viruses in field caught *Ae. aegypti* (9825) and *Ae. albopictus* (150) larvae (Hutamai et al. 2007) (see Table 5.4a).

Within areas of high dengue fever incidence, in the city Boa Vista, Brazil, Zeidler et al. (2008) used ovitraps to collect *Ae. aegypti* mosquitoes and then raise them

within the lab to third or fourth instar larvae (see Table 5.4a). Using a RT-PCR technique, Zeidler et al. (2008) were unable to detect dengue viruses in the 1,172 larvae screened. Zeidler et al. (2008) use their findings and those from studies that either find low or none existent dengue virus infection rates in immature and male *Aedes* species, to suggest that the movement of people may play a greater role in the epidemiology of dengue virus than vertical transmission. A similar argument would be made by Chen et al. (2010), who pointed out that results of their study and other studies suggest that the dengue virus is not endemic to Taiwan but it is constantly being reintroduced by travel from other countries. This may explain Chen et al. (2010) finding no dengue virus in either *Ae. aegypti* and *Ae. albopictus* adult males. Given the likelihood of a reporting bias against studies that do not find vertical transmission, this large body of studies may indicate that vertical transmission is often absent or very low in many areas endemic for dengue virus, even in areas with high incidences of dengue fever.

4.15 Vertical transmission followed by horizontal transmission between larval mosquitoes, via cannibalism of dead or living infected larvae.

Bara et al. (2013) measured the susceptibility *Ae. aegypti* and *Ae. albopictus* 2nd instar larvae to DENV-2, DENV-3 and DENV-4 infections via horizontal transmission. Bara et al. (2013) tested horizontal transmission of dengue viruses occurring through either the larval growth environment or dengue virus infected tissue culture with viral supernatant. The mosquitos were then screened for dengue virus infection as pupae or occasionally as late instar larvae in pools. Bara et al. (2013) found that both species were susceptible to the three serotypes but there was significant variation in the infection rates and viral titres between the serotypes. Bara et al. (2013) suggest, given the low viral titres needed for horizontal transmission of dengue viruses, it is possible for larvae to become infected via ingestion of dead infected larvae, which have acquired

dengue viruses through vertical transmission. Bara et al. (2013) go on to suggest that future research on the viral titer of larvae infected through vertical transmission, in natural populations, is needed to verify this. It should be noted, that if it is possible for larvae to become infected via ingestion of dead infected larvae, that have acquired dengue viruses through vertical transmission, then it is also possible for larvae to become infected via ingestion of dead female adult mosquitoes that have acquired dengue virus through blood feeding.

Given the low viral titers needed for horizontal transmission in these experiments, it would seem possible for larvae to become infected via cannibalism of not only dead but also living vertically dengue virus infected larvae. Whilst this could contribute to the persistence of dengue virus, it should be noted that in order for the oral transmission of dengue virus in *Aedes* larvae to occur, the already rare event of vertical transmission of dengue virus must have occurred. Bara et al. (2013) work may also suggest that the rates of vertical transmission in the wild could be an overestimate.

4.16 Theoretical models of the role of vertical transmission in the persistence of dengue viruses

Although field and laboratory studies have shown that vertical transmission of dengue virus occurred, mathematical models can provide insight as to whether vertical transmission is important to the epidemiology of the dengue virus and point towards needed empirical work. The first model to examine this was by Esteva & Vargas (2000) who modelled the effect of both partial blood feeding and vertical transmission on dengue virus epidemiology. They suggested that vertical transmission favoured an endemic level of dengue virus and that vertical transmission of dengue viruses could be important in areas of low human density. Subsequently, Coutinho et al. (2006) developed models that suggested that vertical transmission could aid dengue viruses in

surviving through seasons of low adult vector populations, although they did not persist in the long term and Coutinho et al. (2006) used a high FIR of 50%, as stated in section 4.3 FIRs for *Ae. albopictus* and *Ae. aegypti* when not selected for are less than 5% (see Table 4.1).

An extensive modelling treatment of the question of the impact of vertical transmission on dengue virus epidemiology was given by Adams & Boots (2010). They examined the impact on endemic and epidemic dynamics of different levels of vertical transmission and focus on the persistence of the virus using stochastic models. Overall they conclude that the levels of vertical transmission generally seen suggest that it is not likely to be important to either epidemic dynamics or the persistence of the virus. Only the exceptional high rates of dengue virus infections in larvae seen by Angel & Joshi (2008), in Rajasthan, would affect the epidemic persistence of dengue virus in these models (see Table 4.5). The models do however highlight the importance of conducting further research into vertical transmission of dengue viruses to diapausing or desiccated mosquito eggs as a possible role in the persistence of dengue viruses (Adams & Boots 2010).

Similarly Charron et al. (2013) used mathematical models to test various methods of persistence of several dipteran born viral infections through unfavourable seasons. Charron et al. (2013) finds that vertical transmission to a diapausing insect vector could lead to persistence over many years, especially when the vector numbers in unfavourable seasons and vertical transmission rate was high. Thus Charron et al. (2013) suggest that vertical transmission to diapausing eggs, could not always guarantee persistence through unfavourable seasons. However Charron et al. (2013) cite values for parameters from a review conducted in 1987 when more up to date studies were available.

Based on the findings of Bara et al. (2013) (see section 4.15), Tennakone (2014) modelled of vertical transmission of dengue viruses followed by horizontal transmission between larvae via cannibalism. Tennakone (2014) found that these two processes in combination could cause persistence of dengue virus without human transmission. However, Tennakone's (2014) model is rather limited, a more in depth model including diapause, seasonality and a range of parameter values is needed to explore how these processes combined could relate to persistence.

Amaku et al. (2014) included vertical transmission at a VIR of 0.1 when assessing different dengue vector control strategies. Amaku et al. (2014) found that killing adult mosquitoes followed by reducing the mosquito biting rate were the most effective ways of controlling dengue and that larvaciding followed by reducing larval carrying capacity were the least effective control strategies. This would suggest that vertical transmission had little effect on dengue virus epidemiology. As a whole the mathematical modelling suggests that only high levels of vertical transmission are likely to significantly impact on the epidemiology of dengue virus but its most important role may be in diapause or combined with horizontal transmission between larvae via cannibalism.

4.17 The role of vertical transmission in persistence of disease

From looking at the levels of vertical transmission that are most often seen in the laboratory and the field, as well as the trends from mathematical models, it is unlikely that vertical transmission of dengue viruses within *Aedes* species plays an important role in the epidemiological persistence of dengue viruses. As suggested by Adams & Boots (2010) only one study (Angel & Joshi 2008) suggests a high enough vertical transmission rate to have a positive effect on the epidemiological persistence of dengue

viruses. It therefore seems likely that dengue viruses persist due to asymptomatic human infections and spatial reintroductions.

Many studies suggest that the vertical transmission of dengue virus in mosquitoes is acting as a reservoir, when asymptomatic dengue virus infections of humans are more likely to be acting as a reservoir for dengue virus. The outbreak of dengue fever in the Florida Keys 2009 is a case in point. Using DENV-1 isolated in the 2010 outbreak of dengue fever in Key West, Florida, Buckner et al. (2013) orally infected *Ae. aegypti* and *Ae. albopictus* (see Table 4.1). If the adult mosquitoes had undergone full DENV-1 dissemination, their late instar larvae were then screened for DENV-1. Buckner et al. (2013) suggests from the high VIR rates found and the findings of Muñoz-jordán et al. (2013), genetic sequencing showing that the 2010 DENV-1 outbreak in Key West was just a continuation of the 2009 outbreak and that DENV-1 had persisted via vertical transmission through the interepidemic period. Another possible explanation for the continuation of the outbreak from 2009 to 2010 could be that the majority of DENV-1 infection in humans were asymptomatic and acted as a reservoir of infection. Evidence for this comes from the fact that 27 cases of dengue fever were reported in Key West in 2009 (Trout et al. 2010), yet a seroprevelance survey of dengue virus antibodies conducted in September 2009 suggested that approximately 600-1000 residents of Old Town, Key West (population 19,846) were infected during the 2009 outbreak (Radke et al. 2012). Das et al. (2013) following the first outbreak of dengue fever in 2010 in Orissa state, India, conducted a screening of adult, larval and pupae *Ae. aegypti* and *Ae. ablopictus* in areas known for dengue fever and areas 1.5 km away not known for dengue fever (as control sites). Das et al. (2013) find an MLE of 2.27 for DENV-2 in *Ae. albopictus* pupae, in the control sites (see Table 5.6). On similar note, Yang et al. (2014) after finding evidence of vertical transmission

of DENV-1 (see Table 5.6) suggested that the importation of vertically infected diapausing eggs had been responsible for an outbreak of dengue fever in Shenzhen, China. The introduction of dengue to the area by asymptomatically infected humans who were missed by the health services may also have been responsible. Further demonstrating that asymptomatic dengue virus infection in humans may well be acting as a more likely reservoir for dengue virus than the vertical transmission of dengue viruses in mosquitoes.

A review by Grange et al. (2014) of asymptomatic infections found that 20-97% of dengue virus infections were asymptomatic, the mean percentage of infections that were asymptomatic in cohort studies was 76% and for index cluster studies it was 37% (Grange et al. 2014). This under reporting of dengue virus infections may not just be due to asymptomatic infection but also misdiagnosis, as many of the symptoms of dengue fever, are similar to other viral illnesses (WHO 2015c). The suggested peak in dengue virus infections in larvae and adult male *Aedes* occurring before a peak in dengue fever cases may reflect an under reporting of dengue fever cases in humans. Perception of the disease increases as a dengue fever outbreak progresses; this may well lead to greater reporting of dengue fever, in the latter stages of an outbreak.

Dengue viruses will also go extinct in certain areas and then be reseeded from other areas where dengue viruses still persist by human movement. From a statistical analysis of DHF cases in Thailand, Cummings et al. (2004) found that DHF cases seem to move in a wave emanating from Bangkok at a speed 148 km per month. Furthermore Gubler (2004) suggested that Cummings et al. (2004) work is evidence to support the hypothesis that dengue viruses are maintained in large urban centres and move out to smaller communities after periodic extinction. A metapopulation model by Adams and Kapan (2009) showed how patches of large vector populations can act as hubs and

reservoirs of dengue virus. Increased movement of viremic humans among these patches increases the influence of the large vector population patches in establishing new foci of transmission.

Stoddard et al. (2014) found that low-level transmission of dengue virus occurred throughout the year when evidenced by the occurrence of clinical illness in the small city of Iquitos, Peru. Combined with the review by Grange et al. (2014), this would suggest that dengue virus persists though seasons of low vector capacity in large enough towns or cities (Gubler 2004) at a low level, with most human dengue virus infections being unreported due to either a mild range or complete lack of symptoms. Furthermore, Stoddard et al. (2013) and Reiner et al. (2014) demonstrated that in the light of *Ae. aegypti's* spatial heterogeneity and lack of dispersal (Getis et al. 2003; Rodhain & Rosen 1997; Harrington et al. 2005; Maciel-de-Freitas et al. 2010), that human movement was responsible for the spread of dengue virus. Dengue virus infected humans who are asymptomatic are likely to be more mobile than those who are symptomatic. As such the movement of asymptomatic dengue virus infections may reseed dengue virus to an area after its extinction at small spatial scales, leading to dengue virus persistence at the larger spatial scale.

4.18 Summary

Further research into vertical transmission of dengue viruses may be logistically difficult due to its low rate and sampling effort attested to in Table 4.1-7. That being said there are a few areas where research is lacking. Before such research takes place there needs to be an assessment of the different screening techniques for detecting vertically acquired dengue virus taking into account accuracy, resource costs and labour. Specifically there is a lack of work on whether vertically acquired dengue virus infection is more readily detected in certain stages of the *Aedes*' life cycle. Further work

on this would not only shed light on the best morphological stage for detecting vertically acquired dengue virus but also determine if vertically acquired dengue virus can be cleared by the mosquitoes during their lifecycle. On a similar note there has been relatively little work on the mechanistic basis of vertical transmission of dengue viruses since the 1990s. Field work aiming to quantify the rate of vertical transmission from the proportion of dengue virus infected adult female mosquitoes and larval or adult male mosquitoes has so far either lacked a large enough sample size or adequate statistical analyses. There is some evidence of a higher rate of vertical transmission of dengue viruses in *Ae. albopictus* than *Ae. aegypt* but the data is rather limited. However, the work by Lambrechts et al. (2010) suggests that *Ae. albopictus* may not be an important vector of dengue virus. Within many of the laboratory studies on transgenerational vertical transmission of dengue virus it was unclear if heritability for vertical transmission of dengue virus is within the mosquito host or the virus. It also remains unknown how important selection for such a trait is in nature. Studies that suggest a seasonal peak in vertical transmission of dengue virus have lacked systematic statistical modelling. Research into detecting dengue virus infections in larval *Aedes* species as a dengue fever epidemic warning system is not a priority as such a system would be economically and logistically unfeasible. The possibility of vertical transmission of dengue virus to larvae, leading to the horizontal transmission of dengue virus between larvae via cannibalism of larvae needs to be further explored as a possible contributing factor in the persistence of dengue virus. Furthermore, there is a lack of work on vertical transmission of dengue virus into diapausing or desiccated eggs and the impact of dengue virus infection on survivorship. Data on this area is lacking despite the fact that if vertical transmission is likely to have any effect on the maintenance of dengue virus then it will be through diapausing or desiccated eggs.

Overall, however, it must be stated that the low rates of vertical transmission seen (typically less than 10/1000 MIR or MLE) in the field, the sheer sampling effort in obtaining such results (running from 100s to 10000s of samples) and the many field studies, which found no evidence of vertical transmission of dengue virus would point to the vertical transmission of dengue virus being of little importance to the epidemiology and persistence of dengue virus. A combination of asymptomatic dengue virus infection in humans and movement of dengue virus infected humans may well be more important.

Chapter 5: Frequency dependent models of asymptomatic dengue infections: their relation to epidemic success, persistence and population at risk of developing dengue haemorrhagic fever

Abstract

Recent estimates suggest that there are 390 million dengue virus infections a year, but only 96 million episodes of dengue illness. Furthermore, a recent review found that 20-97% of dengue infections are asymptomatic. In order to further the understanding of the role of asymptomatic dengue virus infections on dengue's epidemic success, persistence and the population at risk of developing dengue haemorrhagic fever, I adapted two frequency dependent SIR type dengue models that include an asymptomatic class (SAIR models). In the first model upon infection individuals either become symptomatic or asymptomatic. In the second model infected individuals first become asymptomatic and then progressed to either being symptomatic or immune. For both models the level and duration of transmission from the asymptomatic class was varied over a wide range due to the lack of data. Whilst the inclusion of asymptomatic infections did not lead to the dengue virus becoming endemic, asymptomatic infections could lead to dengue virus persisting for several years in lower transmission settings. Furthermore, it was found that a larger proportion of the population could also be left at risk of DHF, than suggested by symptomatic dengue infections.

5.1 Introduction

Recent work by Bhatt et al. (2013) estimates that there are 390 million dengue infections a year, but only 96 million episodes of dengue illness. Whilst suggesting that this RNA positive flavivirus is the most important arboviral pathogen on the planet

(Guzman et al. 2010; Rodenhuis-Zybert et al. 2010), this raises the question of how important unreported infections are to the epidemiology of dengue viruses. Primary infection of a dengue virus can lead to asymptomatic infection through to dengue fever (DF). After a suggested 1-3 year period after primary infection by one of the serotypes of dengue virus (DENV-1, DENV-2, DENV-3 and DENV-4), heterotypic immunity to the other dengue serotypes wanes (Guzman et al. 2010; Rodenhuis-Zybert et al. 2010; Reich et al. 2013). At this point subsequent infection with another dengue serotype can lead to dengue haemorrhagic fever (DHF) (Guzman et al. 2010; Rodenhuis-Zybert et al. 2010; Andraud et al. 2012; WHO 2015b). DHF can cause dengue shock syndrome (DSS), both DHF and DSS are life threatening. Grouped together as severe dengue, DHF and DSS have been estimated to cause 500,000 cases of illness, mostly in children and have a mortality rate of 2.5% (WHO 2015b). Therefore unreported dengue infections may not only affect dengue's epidemiology in terms of dengue's persistence and spread but also in determining the proportion of the population left immune to a dengue serotype after an epidemic, that would thereby be at risk of developing DHF in an epidemic of a differing dengue serotype.

Since the 1980s there has been a growth in studies that attempt to quantify the proportion of dengue infections that are asymptomatic (Grange et al. 2014). A recent review by Grange et al. (2014) found that 20-97% of dengue infections were inapparant. In many studies the term apparent dengue infection is used to describe a dengue infection sufficiently severe to alter a person's regular schedule causing absenteeism from school or work. Whereas an inapparent dengue infection is one that does not cause such disruption to a person's routine. Therefore an inapparent dengue infection ranges from asymptomatic to mildly symptomatic infection.

Considering that asymptomatic dengue viral infections seem so common, the question remains as to what extent an asymptomatic dengue infection is transmissible to a biting mosquito and thereby playing a role in the persistence of dengue viruses. Not a single study has directly researched transmission from asymptomatic dengue viral infections to biting mosquitoes. However, a key to such transmission is to determine the level of viremia in asymptomatic dengue infections. Two studies using RT-PCR have demonstrated that asymptomatic dengue infections can produce detectable levels of viremia in the blood (Beckett et al. 2005; Reyes et al. 2010). However this method of detection does not quantify the level of viremia (Carrington & Simmons 2014). Duong et al. (2011) found a lower but not significantly different level of viremia in asymptomatic dengue infections compared to symptomatic infections using real-time RT-PCR, although Duong et al. (2011) sample size of asymptomatic infections is low (13 compared to 176 symptomatic dengue infections). Nguyet et al. (2013) found that whilst ambulatory mildly symptomatic dengue infections had a lower viremia than dengue infections that lead to hospitalisation, many still had levels of viremia greater than the relevant MID_{50} for the infecting dengue serotype (Nguyet et al. 2013). MID_{50} (Mosquito Infective Dose 50%) being the viremia level corresponding to a 50% probability of human-mosquito transmission (Nguyet et al. 2013). If Nguyet et al. (2013) work is any indication then many asymptomatic dengue infections may well be infectious to a biting mosquito but fewer asymptomatic dengue infections could be above the MID_{50} when compared to symptomatic dengue infection. Therefore, many asymptomatic dengue infections may be less infectious when compared to symptomatic dengue infection. Countering this asymptomatic dengue infected humans are probably more ambulatory than symptomatic dengue infected humans. Considering that the *Aedes* species that act as vectors of dengue virus bite during the day and early evening

(M. Service 2012b), this could lead to asymptomatic dengue infected humans having greater contact with these biting mosquitoes, thereby having a higher transmission rate.

Given that asymptomatic infections are so common and have the potential to transmit dengue viruses, it is important to model their role in the epidemiology of dengue. To this end, I adapted two SIR type dengue models that include an asymptomatic class (SAIR models) based on the flu models by Robinson & Stilianakis (2013). The first (Model A) assumes that on infection individuals either become symptomatic or asymptomatic, while in the second (Model B) individuals first become asymptomatic and then progress to either being symptomatic or immune. Both of these models are reasonable assumptions of how asymptomatic infections may work and it is important that in the absence of clear data on how the process works that the implications of both frameworks are examined. The assumption of transmission from the asymptomatic class was varied over a wide range due to the lack of data concerning asymptomatic viremia and the possibility of asymptomatic dengue infected humans having a greater ambulatory nature leading to greater contact with biting mosquitoes. The duration of the asymptomatic class's transmissibility was varied as to my knowledge there is no work on the duration of viremia in asymptomatic infections. The models were carried out in low, medium and high dengue transmission settings.

5.2 Methods

5.2.1 Description of Model A under frequency dependent transmission

I developed a frequency dependent transmission version of the 2nd framework described in Robinson & Stilianakis (2013) (here referred to as Model A), as described by Equation 5.1-4 below and the flow diagram in Figure 6.1. Table 6.1 contains a list of all Model A's parameter and variables. I assumed frequency dependent transmission as

many other models of dengue transmission do (Johansson et al. 2011; Andraud et al. 2012), justifying this simplification of vector transmission into an aggregated mean vector mediated transmission rate (Johansson et al. 2011), as the vector population is considered to be dense and timescale of transmission is sufficiently short (Andraud et al. 2012).

$$\frac{\Delta S}{\Delta t} = \mu N - \frac{S(\beta_A A + \beta_I I)}{N} - \mu S$$ **Equation 5.1**

$$\frac{\Delta A}{\Delta t} = (1-p)\frac{S(\beta_A A + \beta_I I)}{N} - \gamma_A A - \mu A$$ **Equation 5.2**

$$\frac{\Delta I}{\Delta t} = p\frac{S(\beta_A A + \beta_I I)}{N} - \gamma_I I - \mu I$$ **Equation 5.3**

$$\frac{\Delta R}{\Delta t} = \gamma_I I + \gamma_A A - \mu R$$ **Equation 5.4**

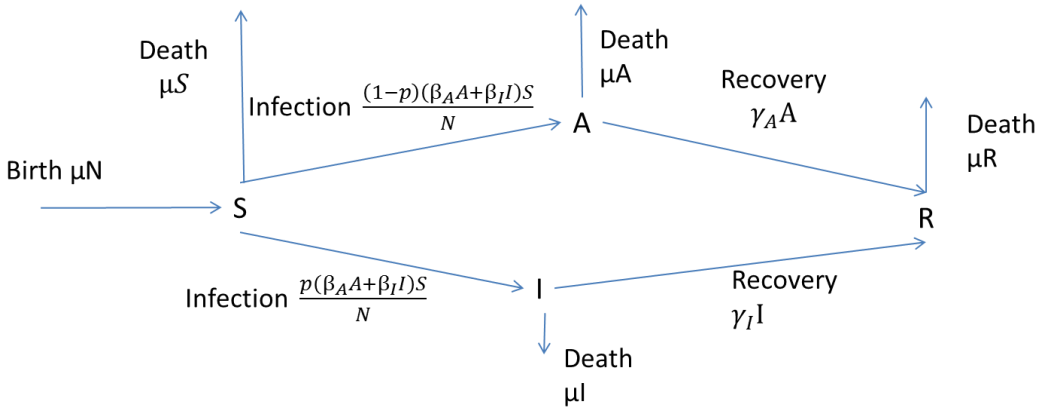


Figure 5.1: Flow diagram representing Model A

Table 5.1 Variables at starting value and parameters used in Model A

Symbol	Name	Value
μ	Birth and death rate (per day)	$1/(60*365)$
c	Infectivity of asymptomatic infections compared to symptomatic infections	0 to 2
β_I	Symptomatic transmission rate (per day)	$200/365$ to $400/365$
β_A	Asymptomatic transmission rate (per day)	$c*\beta_I$
φ	Days symptomatic	8
γ_I	Symptomatic recovery rate (per day)	φ^{-1}
d	Asymptomatic recovery rate compared to symptomatic recovery rate	0.05 to 2
γ_A	Asymptomatic recovery rate (per day)	$(d\varphi)^{-1}$
p	Probability of being symptomatic	0 to 1
S	Susceptible population	10^6-1
A	Asymptomatic population	0 to 1
I	Symptomatic population	0 to 1
R	Recovered population	0
N	Total population	10^6

The total human population (N) is divided into susceptible (S), asymptomatically infected (A), symptomatically infected (I) and recovered (R) classes. Every class of human experiences loss due to a constant death rate μ, however the human population remains constant as humans are born into the susceptible class at a birth rate of μN. Susceptible humans become infected at a frequency dependent transmission term that is the sum of transmission from asymptomatically and symptomatically infected humans, given by $S(\beta_A A+ \beta_I I)/N$. Upon infection humans either become asymptomatic at a rate of $(1-p)S(\beta_A A+ \beta_I I)/N$ or symptomatically infected at a rate of $pS(\beta_A A+ \beta_I I)/N$. Asymptomatically infected people gain complete lifelong immunity at a rate of γ_A and symptomatically infected people gain complete lifelong immunity at a rate of γ_I, in both cases moving to the recovered class (R).

The formula for models A's basic reproduction number (R_0) is listed with equations below (Robinson & Stilianakis 2013).

$$R_0 = (1-p)\left(\frac{\beta_A}{\gamma_A+\mu}\right) + p\frac{\beta_I}{\gamma_I+\mu} \qquad \textbf{Equation 5.5}$$

5.2.2 Description of Model B under frequency dependent transmission

A frequency dependent version of the 1st framework described in Robinson & Stilianakis (2013) (here to referred to as Model B), where all infections start as asymptomatic, is expressed in Equation 5.6-9 and the flow diagram in Figure 6.2. Table 6.2 contains a list of all the parameters and variables used in Model B.

$$\frac{\Delta S}{\Delta t} = \mu N - \frac{S(\beta_A A + \beta_I I)}{N} - \mu S \qquad \textbf{Equation 5.6}$$

$$\frac{\Delta A}{\Delta t} = \frac{S(\beta_A A + \beta_I I)}{N} - \delta A - \gamma_A A - \mu A \qquad \textbf{Equation 5.7}$$

$$\frac{\Delta I}{\Delta t} = \delta A - \gamma_I I - \mu I \qquad \textbf{Equation 5.8}$$

$$\frac{\Delta R}{\Delta t} = \gamma_I I + \gamma_A A - \mu R \qquad \textbf{Equation 5.9}$$

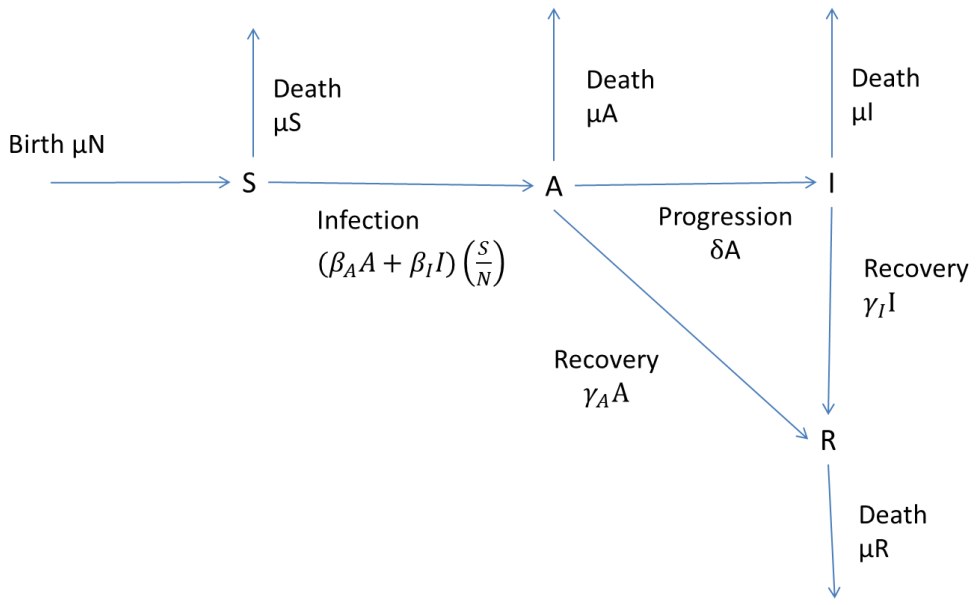


Figure 5.2: Flow diagram representing Model B

Table 5.2: Variables at starting value and parameters used in Model B

Symbol	Name	Value
μ	Birth and death rate (per day)	$1/(60x365)$
c	Infectivity of asymptomatic infections compared to symptomatic infections	0 to 2
β_I	Symptomatic transmission rate (per day)	$200/365$ to $400/365$
β_A	Asymptomatic transmission rate (per day)	$c\beta_I$
φ	Days symptomatic	8
γ_I	Symptomatic recovery rate (per day)	φ^{-1}
d	Asymptomatic recovery rate compared to symptomatic recovery rate	0.05 to 2
γ_A	Asymptomatic recovery rate (per day)	$(d\varphi)^{-1}$
p	Probability of being symptomatic	0 to 0.95
δ	Progression rate from asymptomatic to symptomatic (per day)	$P(\gamma_A+\mu)/(1-p)$
S	Susceptible population	10^6-1
A	Asymptomatic population	0 to 1
I	Symptomatic population	0 to 1
R	Recovered population	0
N	Total population	10^6

Again the total human population (N) is divided into susceptible (S), asymptomatically infected (A), symptomatically infected (I) and recovered (R) classes. Every class of human experiences loss due to a constant death rate μ, however the human population remains constant as humans are born into the susceptible class at a birth rate of μN. Susceptible humans become infected at a frequency dependent transmission term that is the sum of transmission from asymptomatically and symptomatically infected humans, given by $S(\beta_A A+ \beta_I I)/N$, upon this infection susceptible humans move to the asymptomatic class. Asymptomatically infected people gain complete lifelong immunity at a rate of γ_A, die μ or progress to symptomatic infection δ. This means that a proportion p, which is equal to $\delta/(\delta+\gamma_A+\mu)$, of asymptomatic infection will become symptomatically infected. Symptomatically

infected people gain immunity at a rate of γ_I. Once immunity is gained in the symptomatic or asymptomatic class humans move to the recovered class.

Formula for the basic reproduction number (R_0) is listed with equations below (Robinson & Stilianakis 2013).

$$R_0 = \frac{\beta_A}{\gamma_A + \delta + \mu} + \frac{\delta}{\gamma_A + \delta + \mu}\left(\frac{\beta_I}{\gamma_I + \mu}\right) \qquad \textbf{Equation 5.10}$$

5.2.3 Analyses of Model A and Model B

Model A and Model B were coded in Matlab. The parameter space was then explored with ode45, under the 'RelTol', 10^{-6} setting, using Dengue parameters sourced as mid-range values from the parameters listed in Andraud et al. (2012) literature review of Dengue transmission models (unless otherwise stated) (see Table 6.1-2).

In principle asymptomatic individuals could be transmitting dengue at a lower rate due to a lower viremia. However asymptomatic individuals could also be transmitting dengue at a higher rate due to the higher exposure of infected individuals to mosquito bites through their lack of illness causing greater mobility. For these reasons parameter c of both models, the coefficient relating β_A to β_I (β_A=c β_I), was varied 0-2. For each of these settings of c, the proportion of infection leading to symptoms (p) was varied from 0-1 in Model A. In Model B however for each of these settings of c parameter p (proportion of infection leading to symptoms) was varied from 0-0.95 due to the fact that altering p alters δ as $\delta=p(\gamma_A+\mu)/(1-p)$ and at p=1 $\delta=\infty$. Parameter d, the coefficient relating γ_A and γ_I ($\gamma_A=1/d\varphi$, $\gamma_I=1/\varphi$) was kept at 1. There is no information on the immune recovery rate for asymptomatically dengue infected humans. I therefore varied parameter d from 0.05-2, as at d=0 γ_A would equal infinity ($\gamma_A=1/d\varphi$, $\gamma_I=1/\varphi$). For each of these settings of d parameter p (proportion of infection leading to symptoms) was varied from 0-1 in Model A, while in Model B for each of these settings of d

parameter p was varied from 0-0.95 due to the fact that altering p alters δ as $\delta=p(\gamma_A+\mu)/(1-p)$ and at p=1 $\delta=\infty$.

The range in parameter space for the probability of a dengue virus infection leading symptoms (p) could be considered a large sample. Therefore it was decided to highlight a conservative sample of parameter space for this parameter through labelling certain axis values regarding this parameter in green on figures that display model outputs with respect to changes in this parameter. The lower limit to this conservative parameter space p=0.2 is based on the rounded down mean possibility of symptomatic dengue virus infection from cohort studies in Grange et al. (2014). The upper limit to this conservative parameter space of p=0.7 is based on the rounded up mean possibility of symptomatic dengue virus infection from index cluster studies in Grange et al. (2014). The non-rounded mean symptomatic dengue virus infection rate (p) in cohort studies was 24% whereas in index cluster studies it was 63% (Grange et al. 2014). Cohort studies quantify the ratio of asymptomatic to symptomatic dengue infections by following a cohort of people and use case reporting, absenteeism and/or symptom questionnaires, combined with blood screening for dengue antibodies at regular intervals (Endy 2002a; Endy 2002b; Arguello et al. 2015). Index cluster studies sample people surrounding an index case of dengue illness. Sampled individuals' symptoms are quantified through symptom questionnaires or clinical diagnosis and their blood is screened for dengue antibodies (Singh et al. 2000; Beckett et al. 2005; Reyes et al. 2010; Wang et al. 2015).

Likewise, the range in parameter space for the level and duration of transmission from asymptomatic dengue virus infections (c and d, respectively) could be considered large samples. Therefore it was decided to highlight conservative samples of parameter space for these parameters through labelling certain axis values regarding these

parameters in green for figures that display model outputs with respect to changes in these parameters. The lower limit of these conservative parameter spaces is 0.5 and the upper limit is 1.5. Note that at the time of submission of this thesis there was no data regarding the level or duration of transmission from asymptomatic dengue virus infected humans available, which could be used to base a more conservative region of parameter space for both of these parameters. For the exploration of the parameter space surrounding the asymptomatic class's transmission rate β_A and recovery rate γ_A, Model A and B were run with 999,999 susceptible humans and the arrival of a single symptomatically infected individual. At a very low rate of transmission or duration of transmission for the asymptomatic class (c and d, respectively) an epidemic may start with the arrival of a symptomatic dengue infected human but not with the arrival of an asymptomatic dengue infected human. For this reason I then reran the same simulation with 999,999 susceptible humans and the arrival of an asymptomatically infected individual. The exploration of the two models' sets of parameter space was conducted at the low-transmission setting of β_I=200/365, mid-level transmission setting of β_I=300/365 and high-level transmission setting of β_I=400/365, (this is the range of transmission setting cited in the review by Andraud et al. (2012)).

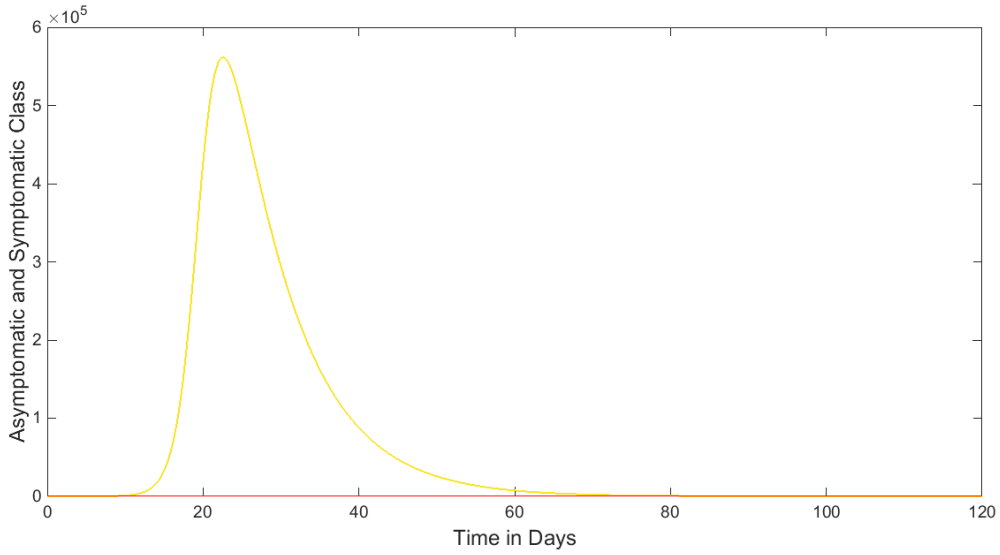
5.3 Results

5.3.1 Epidemic dynamics

When no infections lead to symptoms (p=0), the level and duration of transmission in both the symptomatic and asymptomatic class is the same (c=1 and d=1 respectively) models A and B both become a simple frequency dependent SIR model, with the asymptomatic class taking the place of the symptomatic class. This demonstrates a typical SIR epidemic as the disease dramatically spreads through the

population and declines as the pool of susceptible humans is used up (see Figure 5.3). As the proportion of infections leading to symptoms (p) increases in both models the symptomatic curve takes the place of the asymptomatic curve, however in Model B the symptomatic curve has a larger spread and lower peak for mid-levels of infections leading to symptoms (p) (see Figure 5.4-5). At a different set of parameter Robinson & Stilianakis (2013) observed that in Model B the symptomatic curve had a higher peak, than Model A, which aids in demonstrating the two different models can produce a different peak incidence of asymptomatic and symptomatic infections. The epidemic patterns seen across Model A and B's parameter space follows this pattern, except in differing heights and spreads in the symptomatic and asymptomatic curves. However epidemics with a basic reproduction number R_0 of less than 1 or started with the arrival of an asymptomatic individual when there is no transmission from that class (c=0) in Model A, fail to spread through the population (see Figure 5.6-14).

A)



B)

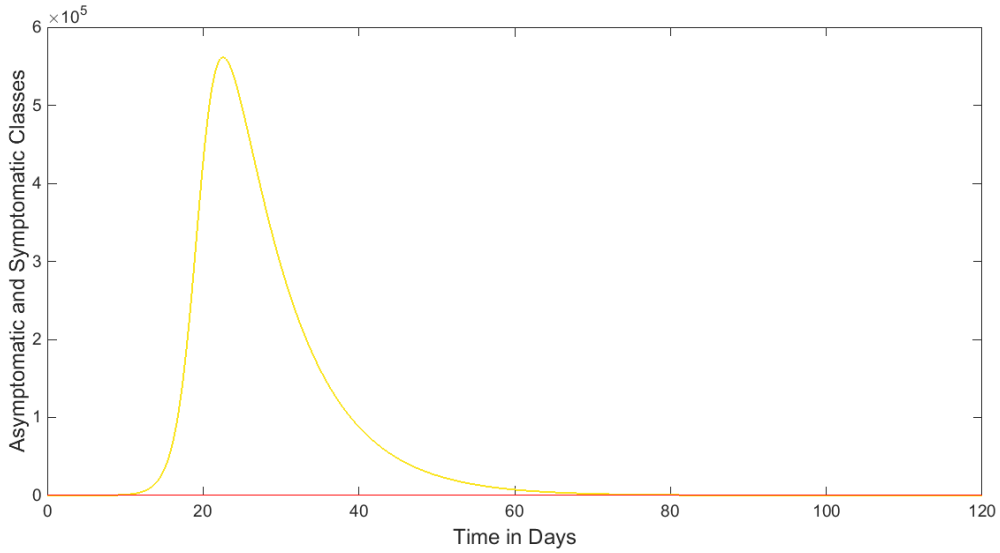
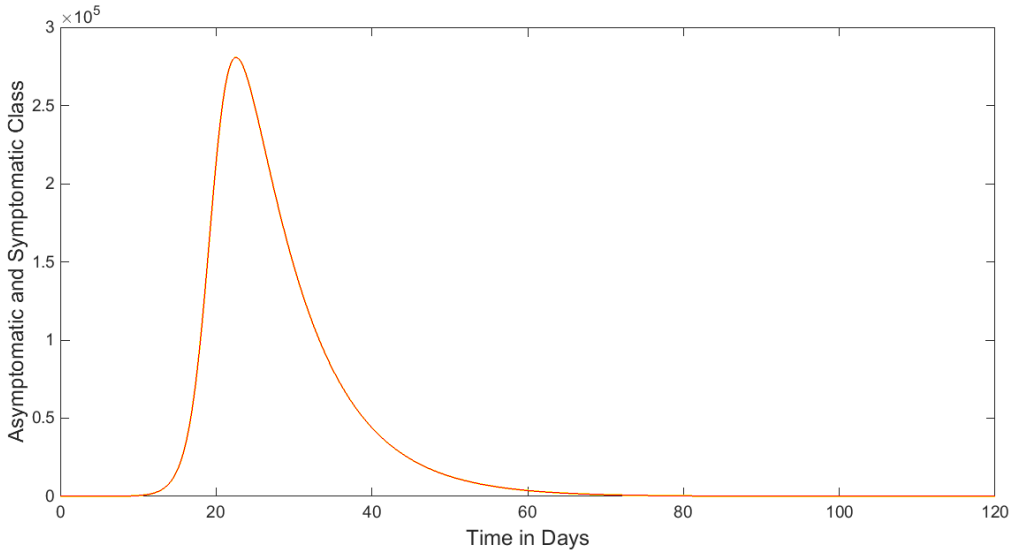


Figure 5.3 Infected human population after the arrival of symptomatically infected human at mid-level transmission (β_I = 300/365), with no symptomatic infections (p=0), the duration of an asymptomatic and symptomatic infection being the same (d=1) and the transmission from an asymptomatic and symptomatic infection being the same (c=1). Symptomatically infected humans in red and asymptomatically infected humans in yellow. A) Model A. B) Model B

A)



B)

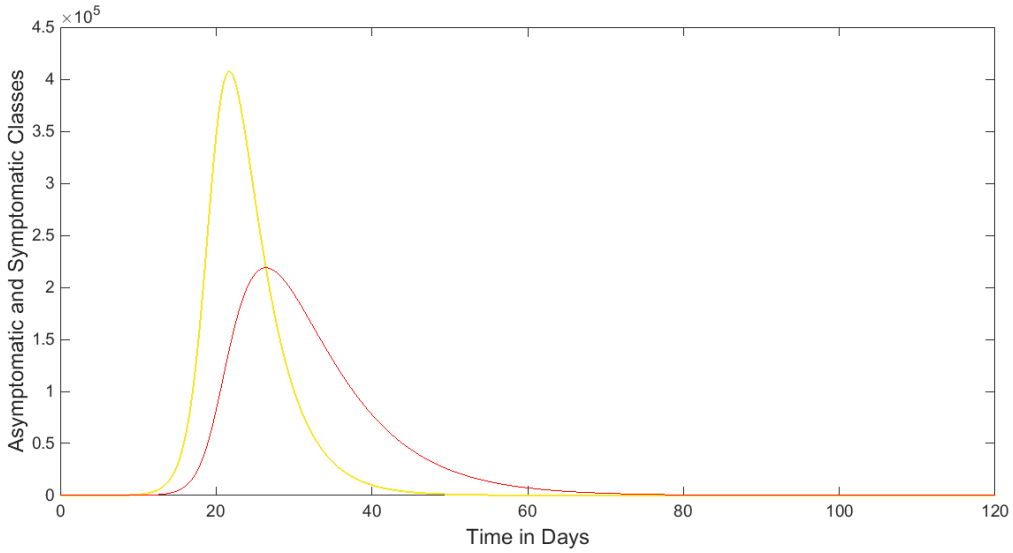
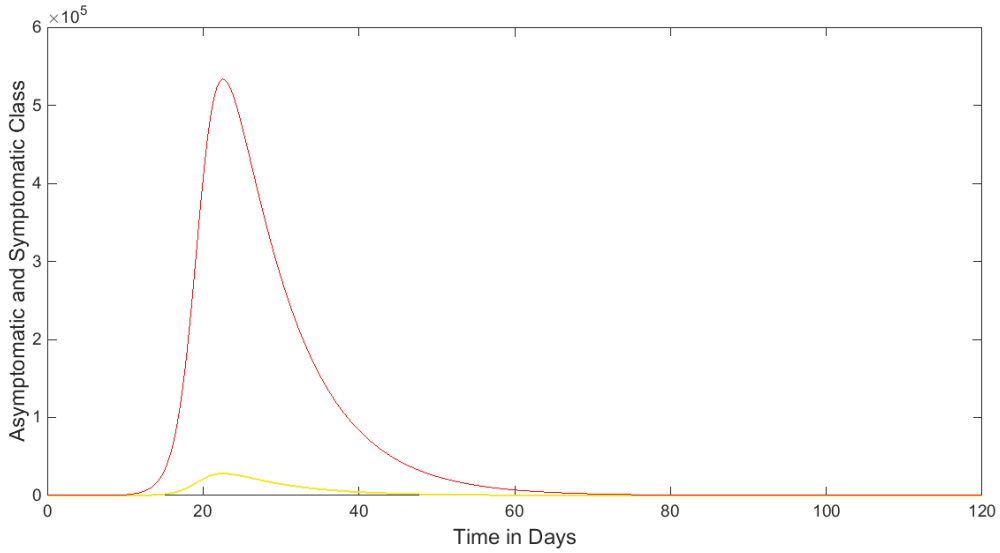


Figure 5.4: Infected human population after the arrival of symptomatically infected human at mid-level transmission (β_I = 300/365), with 50% of infections being symptomatic (p=0.5), the duration of an asymptomatic and symptomatic infection being the same (d=1) and the transmission from an asymptomatic and symptomatic infection being the same (c=1). Symptomatically infected humans in red and asymptomatically infected humans in yellow. A) Model A. B) Model B

A)



B)

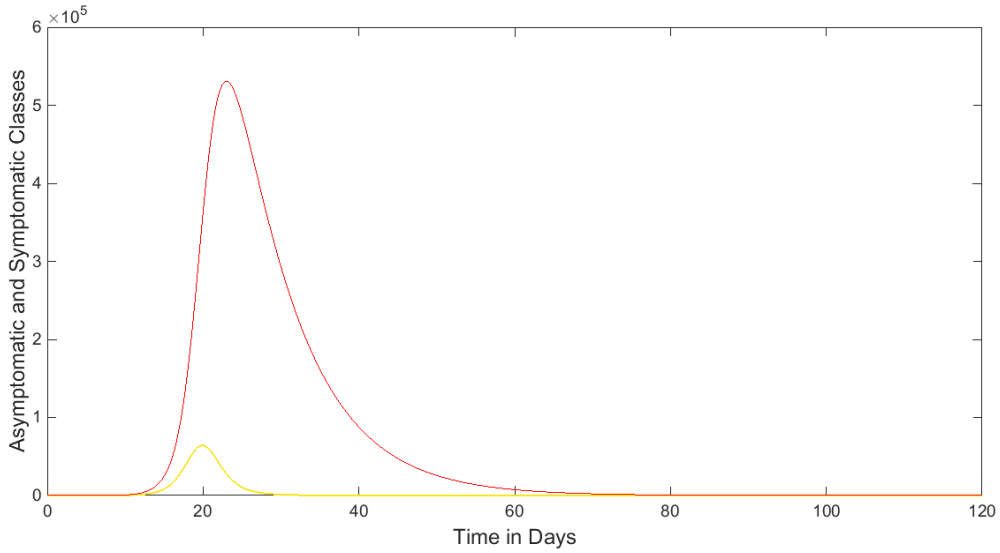


Figure 5.5: Infected human population after the arrival of symptomatically infected at mid-level transmission (β_I = 300/365), with 95% of infections being symptomatic (p=0.95), the duration of an asymptomatic and symptomatic infection being the same (d=1) and the transmission from an asymptomatic and symptomatic infection being the same (c=1). Symptomatically infected humans in red and asymptomatically infected humans in yellow. A) Model A. B) Model B

5.3.1 Basic reproduction number R_0

Both models A and B produce the same basic reproduction number R_0 for the same transmission settings (β_I), proportion of infections leading to symptoms (p), level and duration of transmission in the asymptomatic class (c and d respectively) (see Figure 5.6-8). As a note of intuitive sense for all three transmission settings (β_I) when the level and duration of transmission is the same in both the asymptomatic and symptomatic class (c=1 and d=1, respectively), the R_0 value is the same no matter the proportion of infections leading to symptoms (p) (see Figure 5.8-8). Furthermore this happens to be the same R_0 value for Model A when all infections are symptomatic (p=1) no matter the level and duration of transmission in the asymptomatic class (c and d, respectively) (see Figure 5.6-8). Beyond this R_0 increases with a lower proportion of infections leading to symptoms (p) combined with a high level or duration of transmission in the asymptomatic class (c and d respectively) but decreases with a low proportion of infections leading to symptoms (p) combined with a low level or duration of transmission in the asymptomatic class (c and d respectively) (see Figure 5.6-8). This pattern in the value of R_0 is similar across all three transmission setting (β_I) but the overall values of R_0 increases as β_I increases from 200/365 to 400/365.

At the low transmission setting (β_I=200/365) the areas of parameter space that lead to an R_0 below 1 and therefore produce no epidemic, occur below a threshold. This threshold forms a line on the figures depicting the epidemic effects of varying levels of transmission in the asymptomatic class (c) combined with varying the proportion of infections leading to symptoms (p), from c=0.2 and p=0 to c=0 and p=0.2 (see Figure 5.6 A-B, Figure 5.9 and Figure 5.15). For figures depicting the epidemic effects of varying durations of transmission in the asymptomatic class (d) combined with varying the proportion of infections leading to symptoms (p), this line occurs from d=0.2 and

p=0 to d=0.05 and p=0.15 (see Figure 5.6 C-D, Figure 5.10 and Figure 5.16). The area of this parameter space decreases at the medium transmission setting (β_I=300/365) as this threshold moves to the bottom left hand corner. For figures depicting epidemic effects of varying levels of transmission in the asymptomatic class (c) combined with varying the proportion of infections leading to symptoms (p), the line now occurs from c=0.15 and p=0 to c=0 and p=0.15 (see Figure 5.7 A-B, Figure 5.11 and Figure 5.17). For figures depicting varying durations of transmission in the asymptomatic class (d) combined with varying the proportion of infections leading to symptoms (p), this line occurs from d=0.15 and p=0 to d=0.05 and p=0.1 (see Figure 5.7 C-D, Figure 5.12 and Figure 5.18). This area of parameter space decreases even further in the high transmission setting (β_I=400/365) as this threshold moves further to the bottom left hand corner for figures depicting epidemic effects of varying levels of transmission in the asymptomatic class (c) combined with varying the proportion of infections leading to symptoms (p), the line now occurring from c=0.1 and p=0 to c=0 and p=0.1 (see Figure 5.8 A-B, Figure 5.13 and Figure 5.19). For figures depicting varying durations of transmission in the asymptomatic class (c) combined with varying the proportion of infections leading to symptoms (p), this line occurs from d=0.1 and p=0 to d=0.05 and p=0.05 (see Figure 5.8 C-D, Figure 5.14 and Figure 5.20).

The more conservative samples of parameter space for the proportion of infections leading to symptoms (p), the level and duration of transmission in the asymptomatic class (c and d respectively) do not lead to the lower values of R_0 seen in the bottom left corner of the sub-figures of Figure 5.6-8. Likewise they do not lead to the higher values of R_0 seen in the top left corner of the sub-figures of Figure 5.6-8. This means that the areas of parameter space that lead to an R_0 below 1 (see Figure 5.6-

8) and therefore lead to no epidemic spreading through the population, are not included in the more conservative sample of parameter space (see Figure 5.9-14).

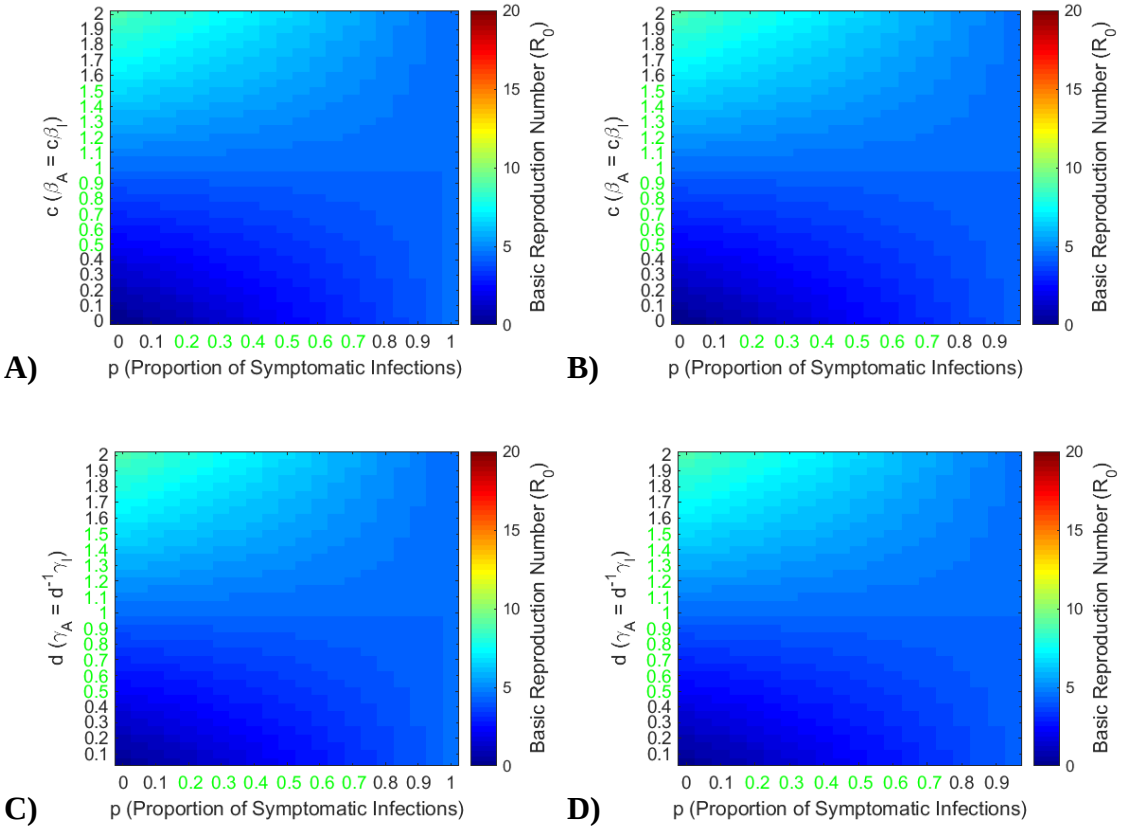


Figure 5.6: Basic reproduction number (R_0) at low-level transmission (β_I=200/365). A) Model A at different proportions of infections developing symptoms (p) and different levels of transmission in asymptomatic infections (c), when the duration of asymptomatic and symptomatic infections is the same (d=1). B) Model B at different proportions of infections developing symptoms (p) and different levels of transmission in asymptomatic infections (c), when the durations of asymptomatic and symptomatic infections is the same (d=1). C) Model A at different proportions of infections developing symptoms (p) and different durations of asymptomatic infections (d), when transmission from the asymptomatic and symptomatic infections is the same (c=1). D) Model B at different proportions of infections developing symptoms (p) and different durations of asymptomatic infection (d), when transmission from the asymptomatic and symptomatic infections is the same (c=1). For all sub-figures the more conservative parameter space is displayed in green.

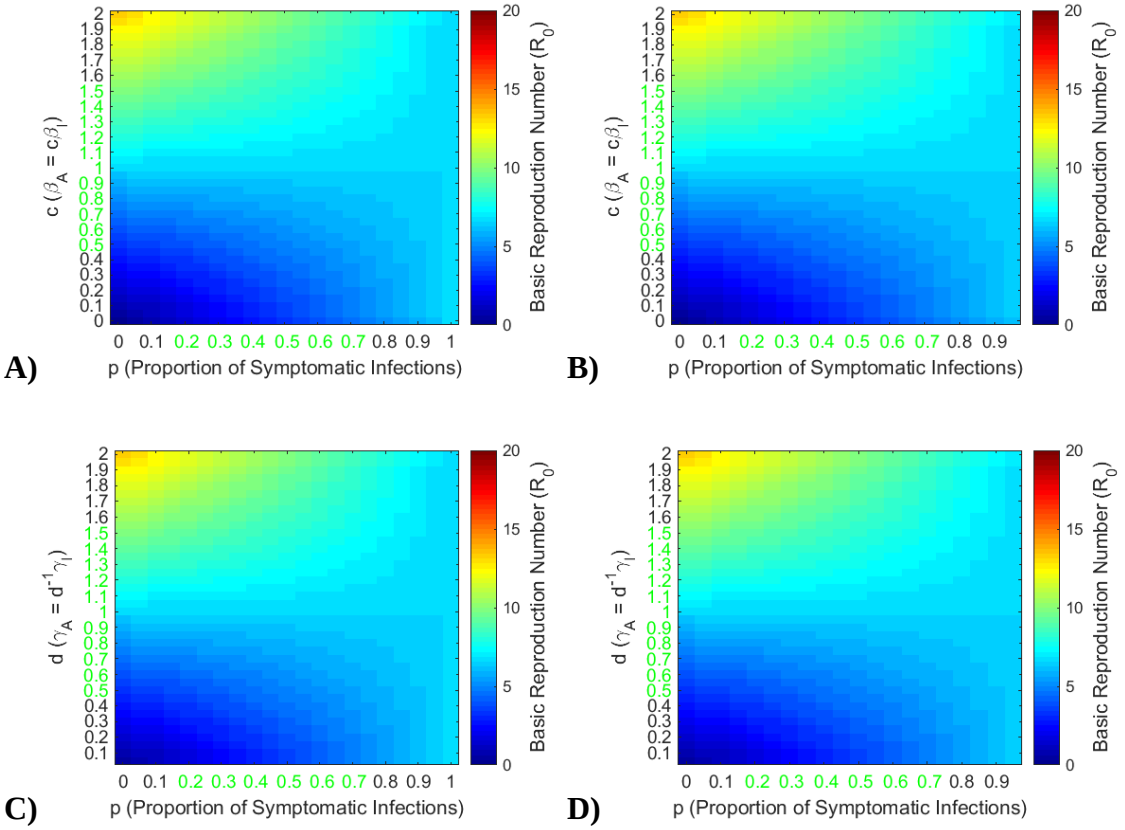


Figure 5.7: Basic reproduction number (R_0) at mid-level transmission (β_I=300/365). A) Model A at different proportions of infections developing symptoms (p) and different levels of transmission in asymptomatic infections (c), when the duration of asymptomatic and symptomatic infections is the same (d=1). B) Model B at different proportions of infections developing symptoms (p) and different levels of transmission in asymptomatic infections (c), when the duration of asymptomatic and symptomatic infections is the same (d=1). C) Model A at different proportions of infections developing symptoms (p) and different durations of asymptomatic infections (d), when transmission from the asymptomatic and symptomatic infections is the same (c=1). D) Model B at different proportions of infections developing symptoms (p) and different durations of asymptomatic infection (d), when transmission from the asymptomatic and symptomatic infections is the same (c=1). For all sub-figures the more conservative parameter space is displayed in green.

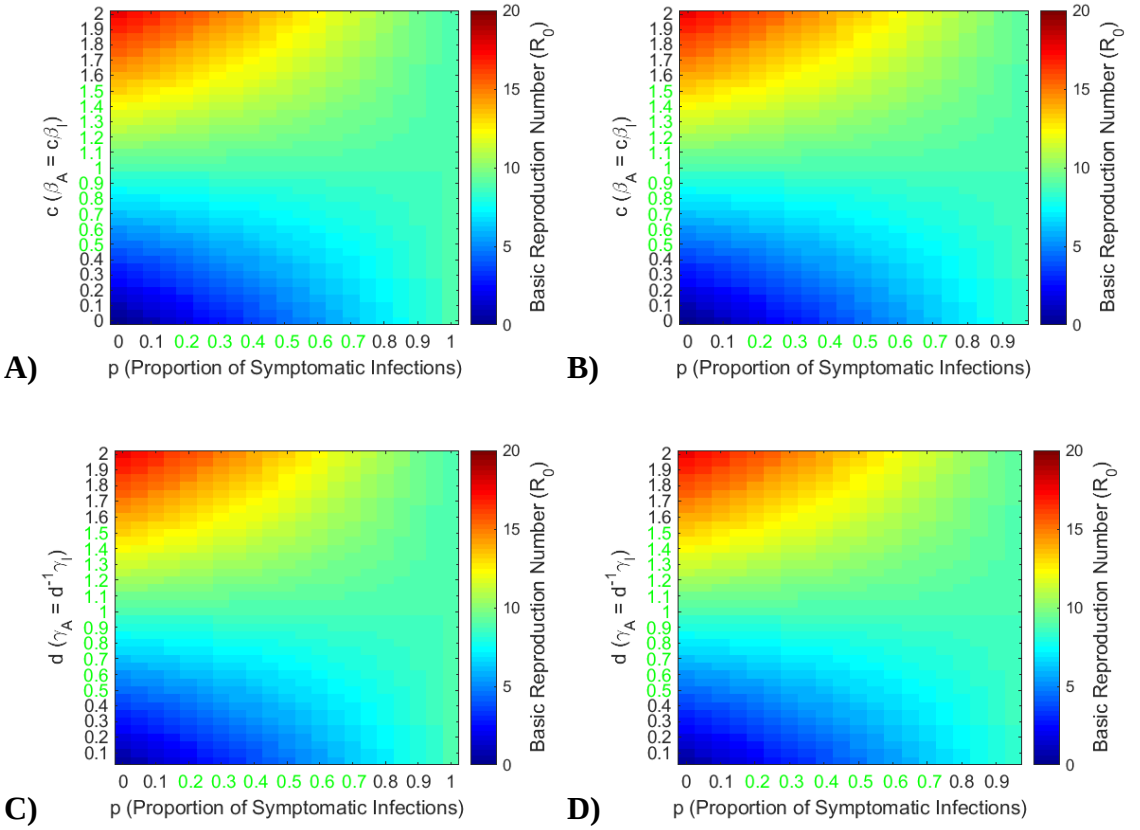


Figure 5.8: Basic reproduction number (R_0) at high level transmission (β_I=400/365). A) Model A at different proportions of infections developing symptoms (p) and different levels of transmission in asymptomatic infections (c), when the duration of asymptomatic and symptomatic infections is the same (d=1). B) Model B at different proportions of infections developing symptoms (p) and different levels of transmission in asymptomatic infections (c), when the durations of asymptomatic and symptomatic infections is the same (d=1). C) Model A at different proportions of infections developing symptoms (p) and different durations of asymptomatic infection (d), when transmission from the asymptomatic and symptomatic infections is the same (c=1). D) Model B at different proportions of infections developing symptoms (p) and different durations of asymptomatic infection (d), when transmission from the asymptomatic and symptomatic infections is the same (c=1). For all sub-figures the more conservative parameter space is displayed in green.

5.3.2 Epidemic persistence

For the vast majority of epidemics asymptomatic and symptomatic dengue infections die out after three months to a year, regardless of the level and duration of transmission in the asymptomatic class (c and d respectively), the proportion of infections leading to symptoms (p), transmissions setting (β_I) or model (see Figure 5.9-14). Epidemics that last more than a year occur, for a lower proportion of infections leading to symptoms (p) combined with low levels or durations of transmission in the asymptomatic class in proportion to the symptomatic class (c and d respectively). The lower the transmission settings (β_I) the longer the epidemics that last for greater than a year last. Epidemics that last more than 5 years and in some cases longer than 10 years occur at the lowest transmission setting (β_I=200/365) (see Figure 5.9-10). For the medium transmission setting (β_I=300/365) the longer lasting epidemics range from 1.5-3 years (see Figure 5.11-12). For the high transmission setting (β_I=400/365) the longer lasting epidemics range from just over a year to 3 years (see Figure 5.13-14). Across all three transmission settings (β_I) these longer lasting epidemics also coincide with the R_0 being only slightly over 1, or put another way, for the parameter values just above the threshold lines for R_0 values less than 1 described in section 5.3.1 above (compare Figure 5.9-14 with Figure 5.6-8). This suggests that whilst at these parameter settings dengue can spread through the population, R_0 being greater than 1, dengue spreads slowly, due to a low force of infection. It should be noted that the more conservative samples of parameter space for the proportion of infections leading to symptoms (p), the level and duration of transmission in the asymptomatic class (c and d respectively), did not produce the longer lasting epidemics (see Figure 5.9-14). This was in a similar fashion to the more conservative samples of parameter space not producing the lower values of R_0 (compare Figure 5.6-8 with Figure 5.9-14).

This epidemic pattern is broadly similar between models A and B, whether an epidemic is started by the arrival of an asymptomatic individual or symptomatic individual. However in Model B and an epidemic started by the arrival of an asymptomatic individual lengthen the course of the longer lasting epidemics. Another exception is that an epidemic fails to even start with the arrival of an asymptomatic individual when there is no transmission from that class (c=0) in Model A (see Sub-Figure A of Figure 5.9, Figure 5.11 and Figure 5.13).

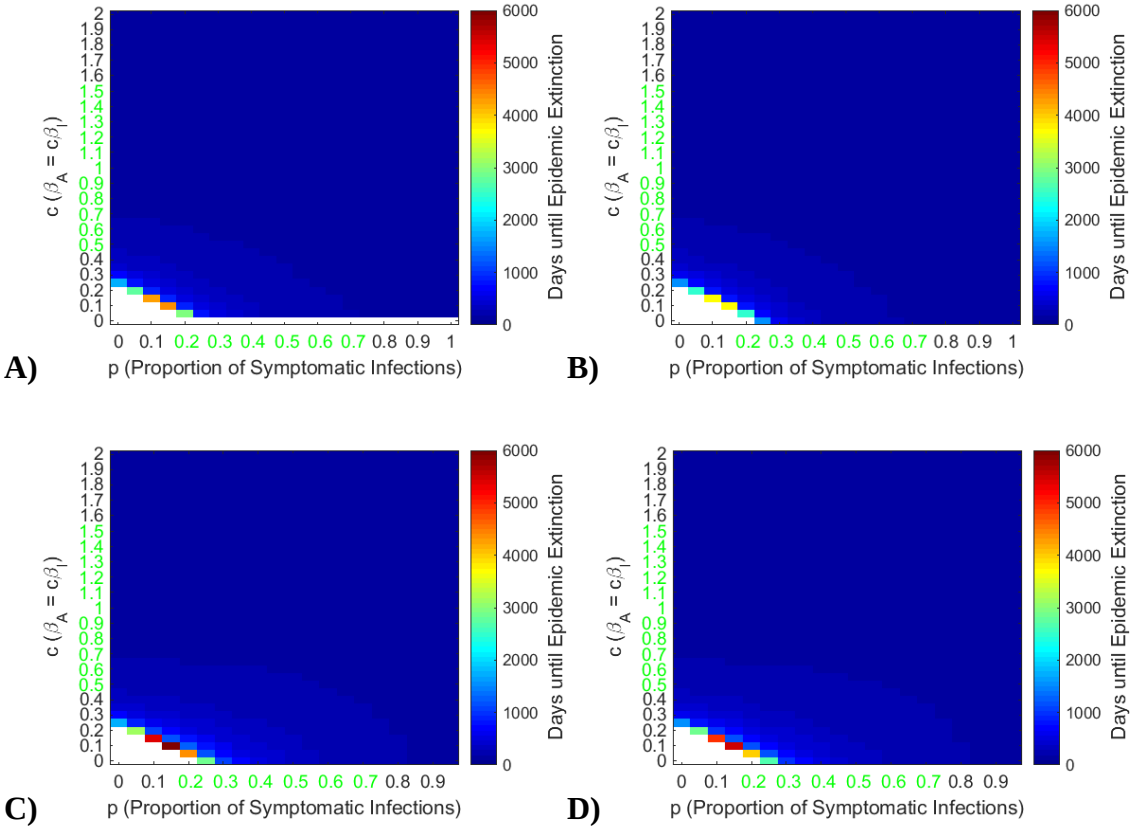


Figure 5.9: Days until epidemic extinction against different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under low-level transmission (β_I=200/365). Days until epidemic extinction is defined as the first occurrence of the asymptomatic and symptomatic classes being less than 1 after the peak total of symptomatic and asymptomatic infections. Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. Note the colour scale represents 0-6000 days for subfigures A-D. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

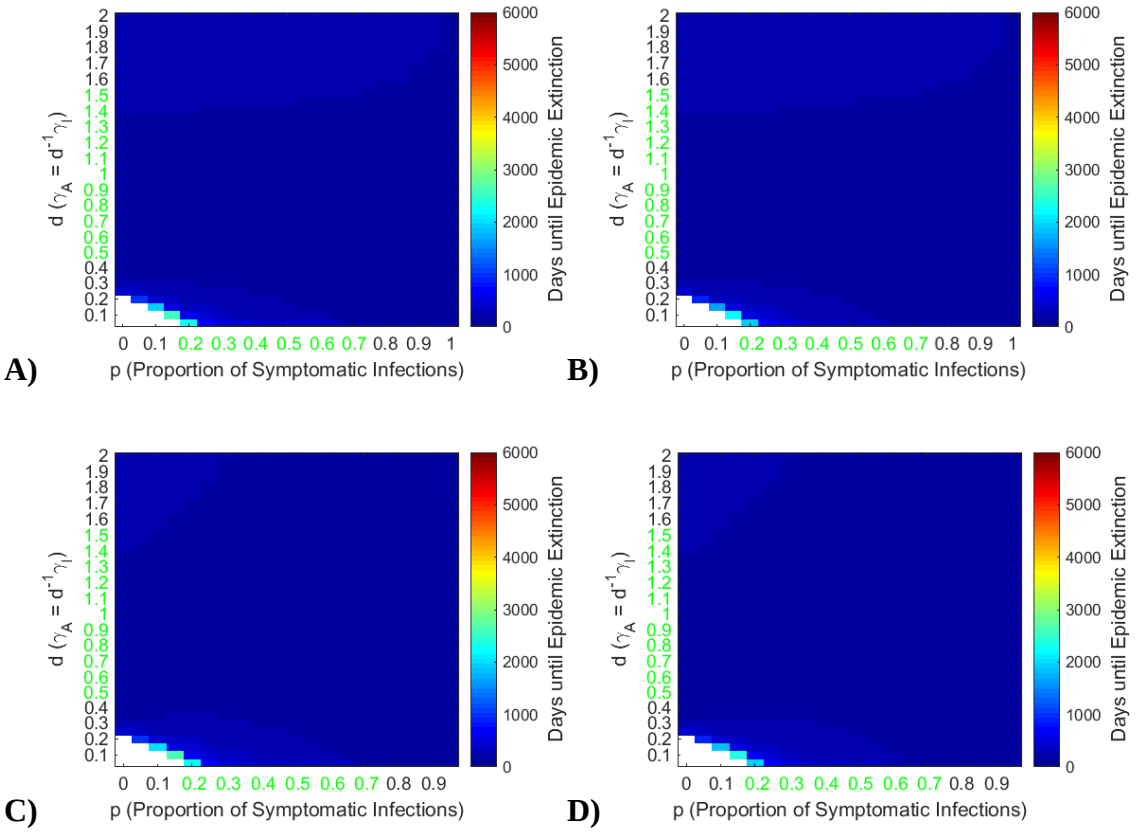


Figure 5.10: Days until epidemic extinction against different durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under low-level transmission (β_I=200/365). Days until epidemic extinction is defined as the first occurrence of the asymptomatic and symptomatic classes being less than 1 after the peak total of symptomatic and asymptomatic infections. Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. Note the colour scale represents 0-6000 days for subfigures A-D. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

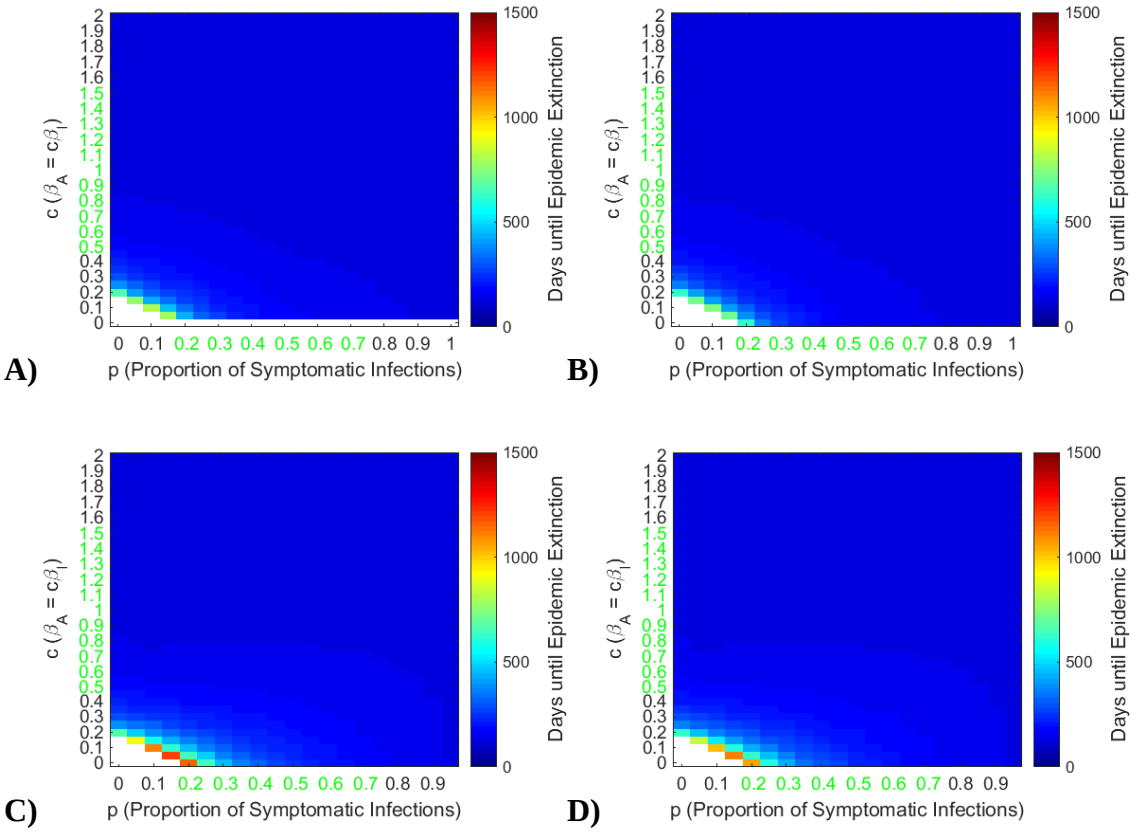


Figure 5.11: Days until epidemic extinction against different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under mid-level transmission (β_I=300/365). Days until epidemic extinction is defined as the first occurrence of the asymptomatic and symptomatic classes being less than 1 after the peak total of symptomatic and asymptomatic infections. Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. Note the colour scale represents 0-1500 days for subfigures A-D. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

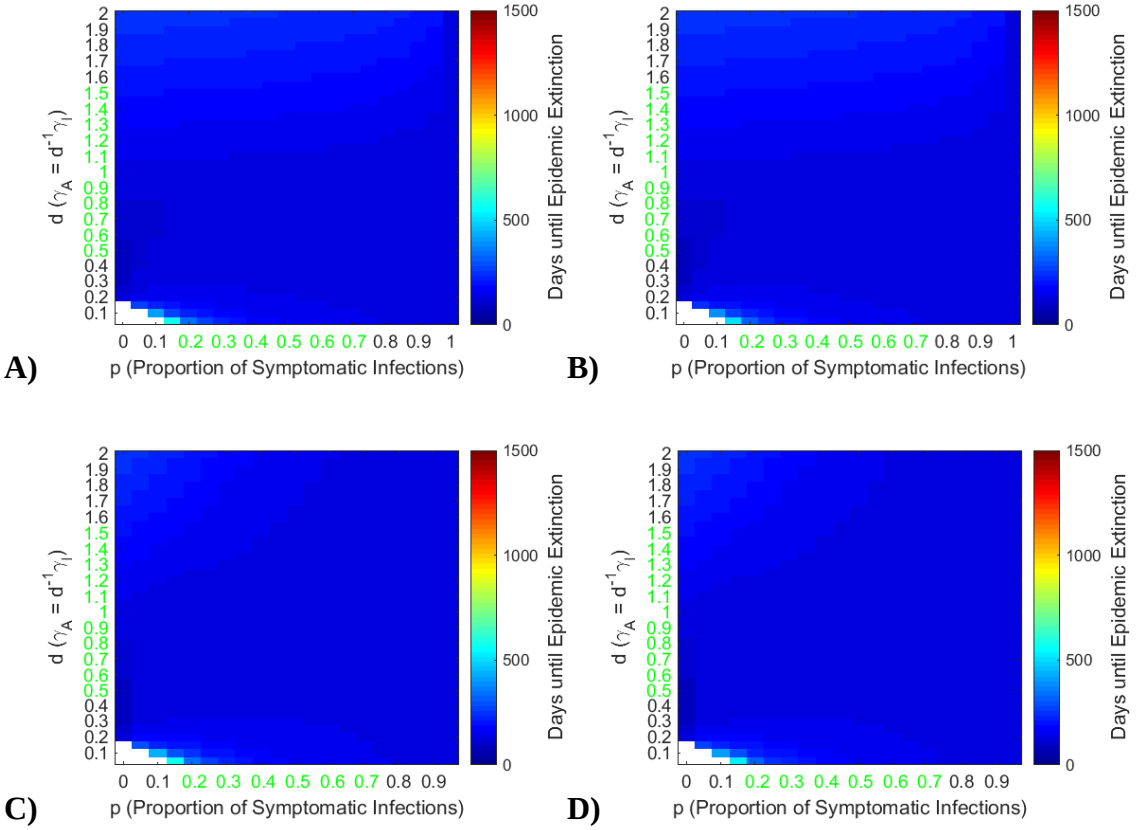


Figure 5.12: Days until epidemic extinction against different durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under mid-level transmission (β_I=300/365). Days until epidemic extinction is defined as the first occurrence of the asymptomatic and symptomatic classes being less than 1 after the peak total of symptomatic and asymptomatic infections. Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. Note the colour scale represents 0-1500 days for subfigures A-D. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

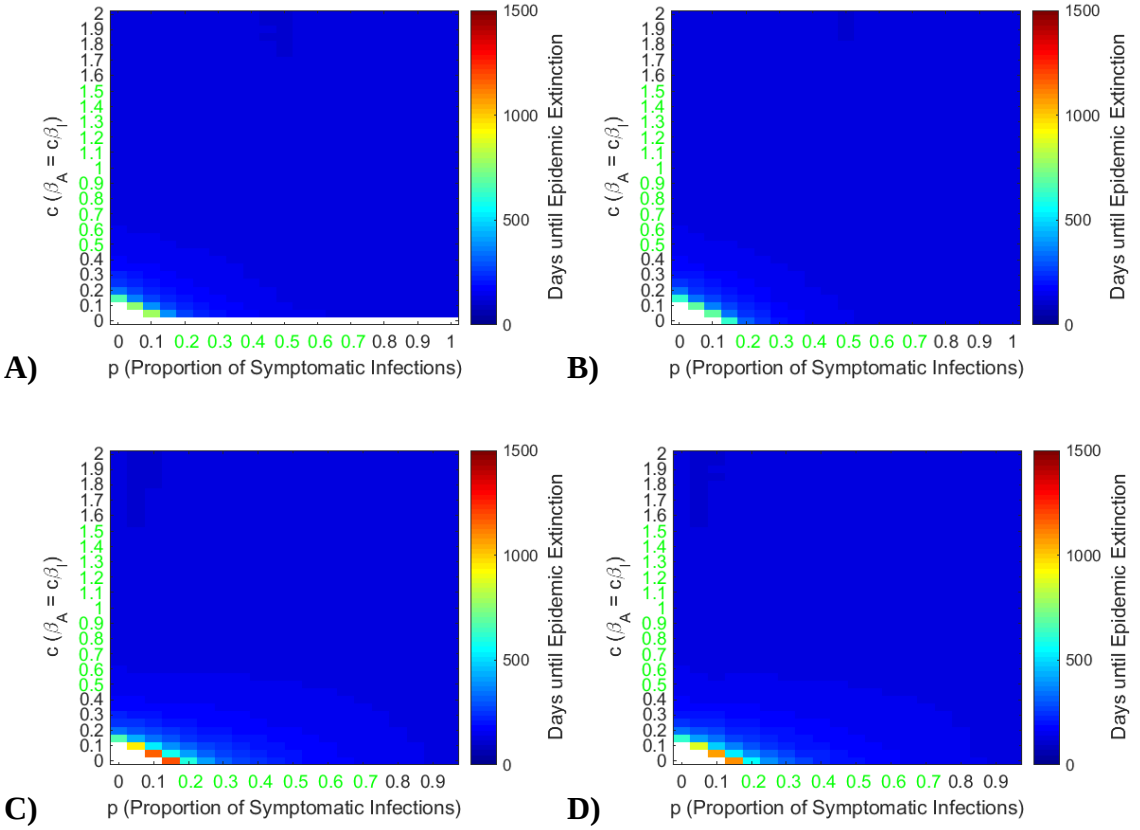


Figure 5.13: Days until epidemic extinction against different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under high-level transmission (β_I=400/365). Days until epidemic extinction is defined as the first occurrence of the asymptomatic and symptomatic classes being less than 1 after the peak total of symptomatic and asymptomatic infections. Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. Note the colour scale represents 0-1500 days for subfigures A-D. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

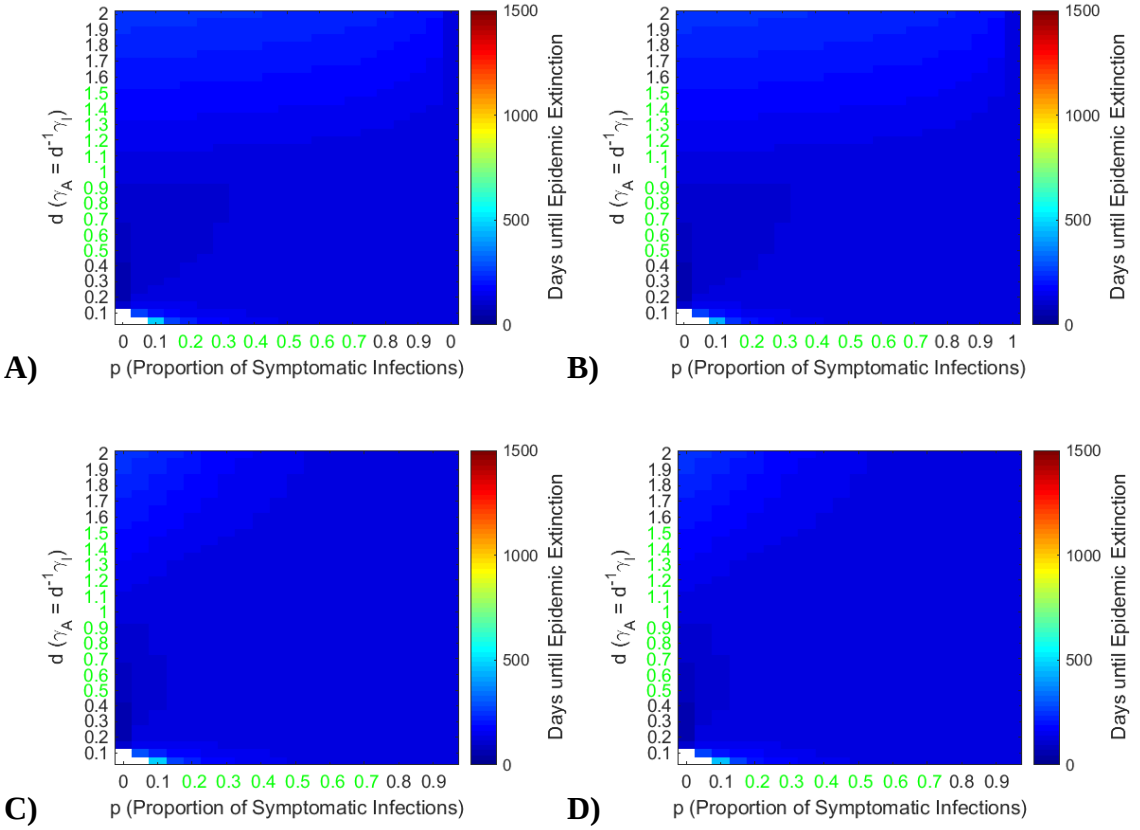


Figure 5.14: Days until epidemic extinction against different durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under high-level transmission (β_I=400/365). Days until epidemic extinction is defined as the first occurrence of the asymptomatic and symptomatic classes being less than 1 after the peak total of symptomatic and asymptomatic infections. Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. Note the colour scale represents 0-1500 days for subfigures A-D. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

5.3.3 Percentage of the population resistant at the end of an epidemic

Both models A and B produce an extremely similar percentage of population resistant to dengue by the end of the epidemic. At each of the transmission settings (β_I), proportion of infections leading to symptoms (p), level and duration of transmission in the asymptomatic class in proportion to the symptomatic class (c and d respectively), regardless if an outbreak is started by the arrival of a symptomatic or asymptomatic individual (see Figure 5.15-20).

In the lower transmission setting of β_I=200/365, levels of transmission in the asymptomatic class lower than the symptomatic class (c<1) combined with not all infections becoming symptomatic (p<1), leads to the percentage of population resistant to dengue dropping from near 100% to less than 10 % with falling levels of transmission in the asymptomatic class (c) and decreasing proportions of infections leading to symptoms (p) (see Figure 5.15). This is also true for the duration of transmission in the asymptomatic class (d) (see Figure 5.16). Meaning that at the low level transmission setting (β_I=200/365) the more conservative samples of parameter space for the proportion of infections leading to symptoms (p), the level and duration of transmission in the asymptomatic class (c and d respectively), produce epidemics where above 85% of the population is resistant to dengue virus (see Figure 5.15-16).

In the medium transmission setting of β_I=300/365 for levels of transmission in the asymptomatic class (c) less than 0.7 combined with a proportion of infections leading to symptoms (p) less than 0.7, the percentages of population resistant to dengue drops from near 100% to less than 40 % with a decreasing level of transmission in the asymptomatic class (c) and proportion of infections leading to symptoms (p) (see Figure 5.17). This is also true for the duration of transmission in the asymptomatic class in proportion to the symptomatic class (d) (see Figure 5.18). Meaning that at the mid-level

transmission setting (β_I=300/365) the more conservative samples of parameter space for the proportion of infections leading to symptoms (p), the level and duration of transmission in the asymptomatic class (c and d respectively), produce epidemics where above 90% of the population is resistant to dengue virus (see Figure 5.17-18).

In the higher transmission setting (β_I=400/365) for levels of transmission in the asymptomatic class less than half that of the symptomatic class ($c<0.5$) combined with less than half the of infections becoming symptomatic ($p<0.5$), the percentages of population resistant to dengue drops from near 100% to less than 40 % with decreasing levels of transmission in the asymptomatic class (c) and proportions of infections leading to symptoms (p) (see Figure 5.19). This is also true for the duration of transmission in the asymptomatic class (d) (see Figure 5.20). Meaning that at the high-level transmission setting of β_I=400/365 the more conservative samples of parameter space for the proportion of infections leading to symptoms (p), the level and duration of transmission in the asymptomatic class (c and d respectively), produce epidemics where above 95% of the population is resistant to dengue virus (see Figure 5.19-20).

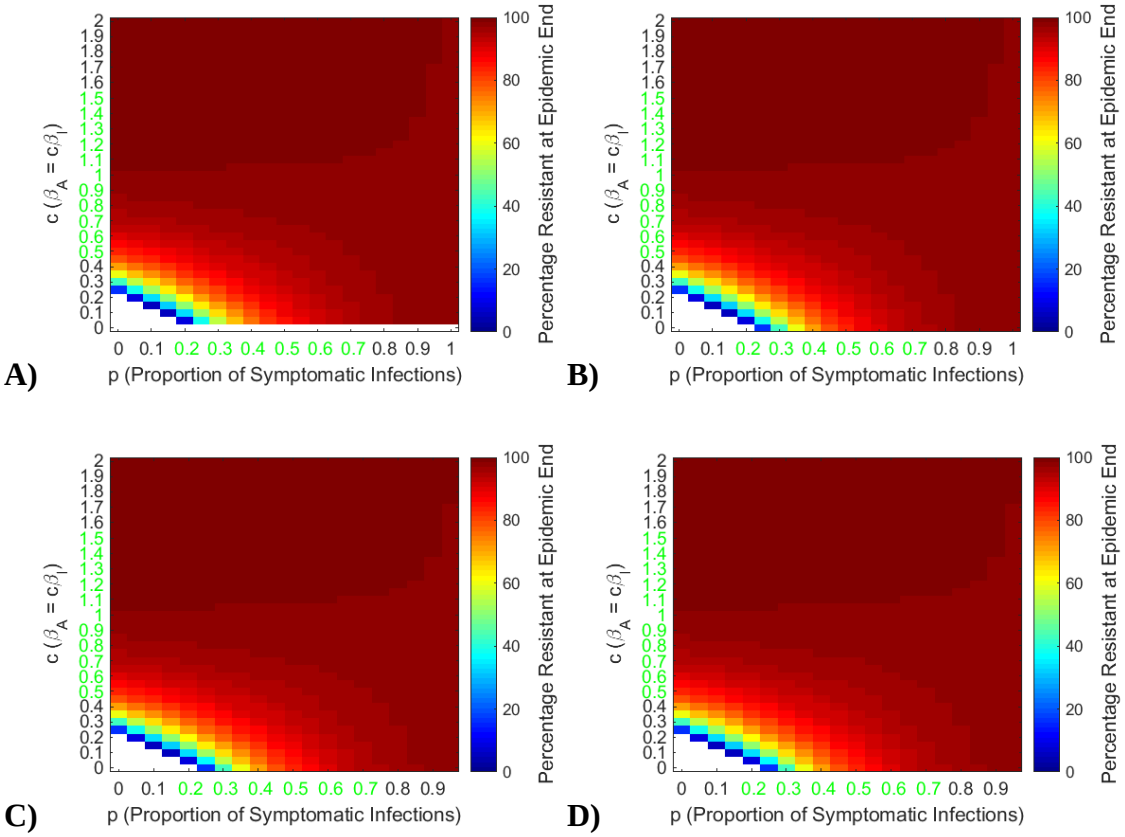


Figure 5.15: Percentage of the population resistant at the end of the epidemic against different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under low-level transmission (β_I=200/365). Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

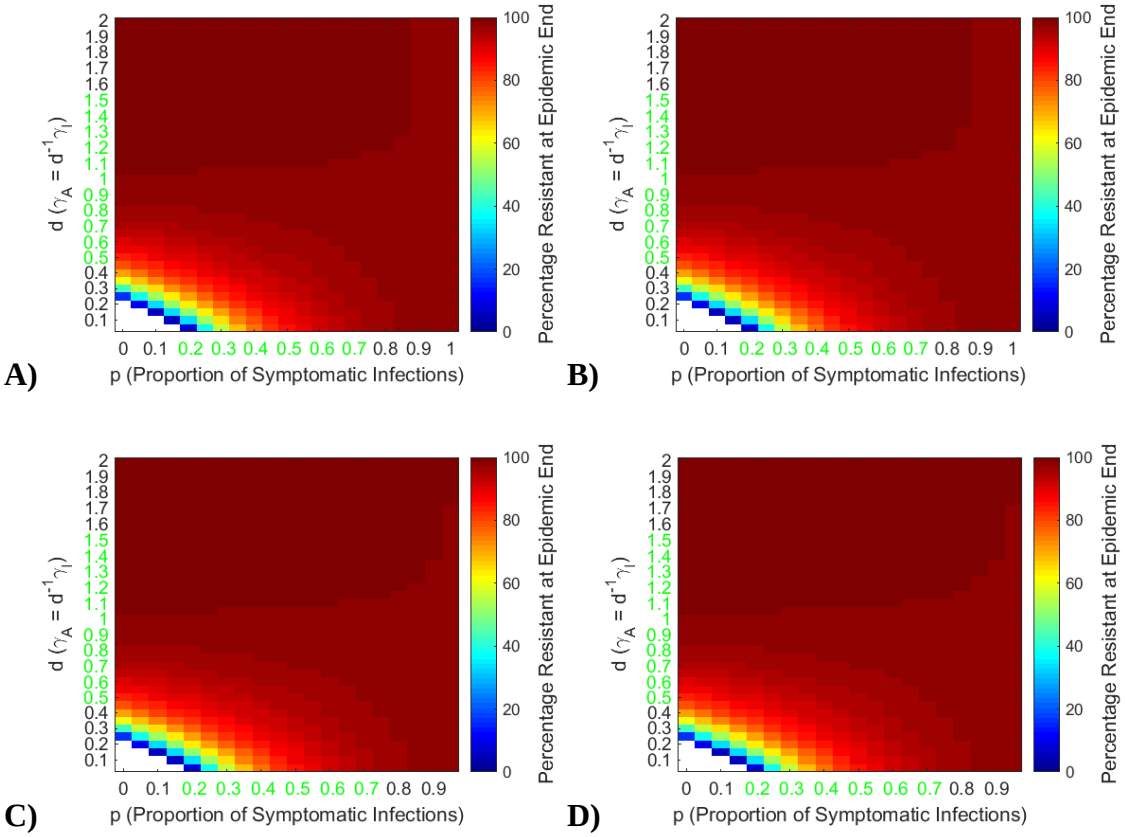


Figure 5.16: Percentage of the population resistant at the end of the epidemic, against different durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under low-level transmission (β_I=200/365). Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

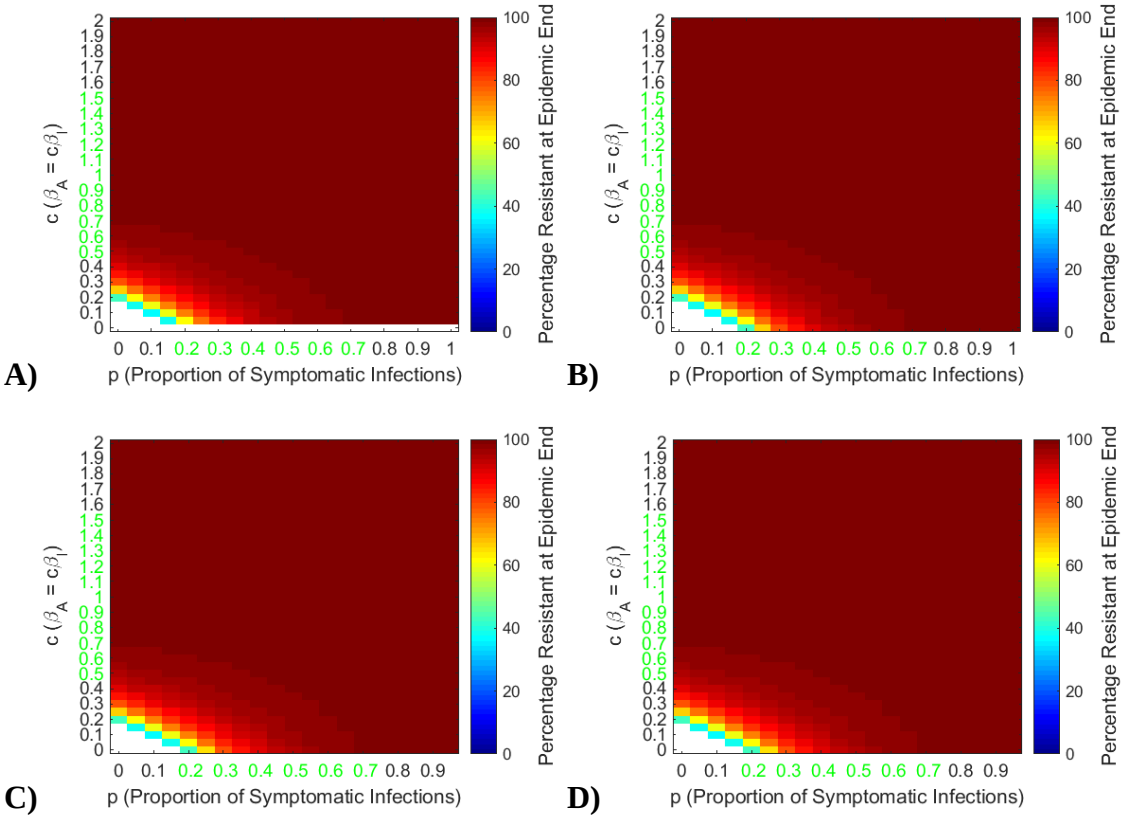


Figure 5.17: Percentage of the population resistant at the end of the epidemic, against different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under mid-level transmission (β_I=300/365). Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

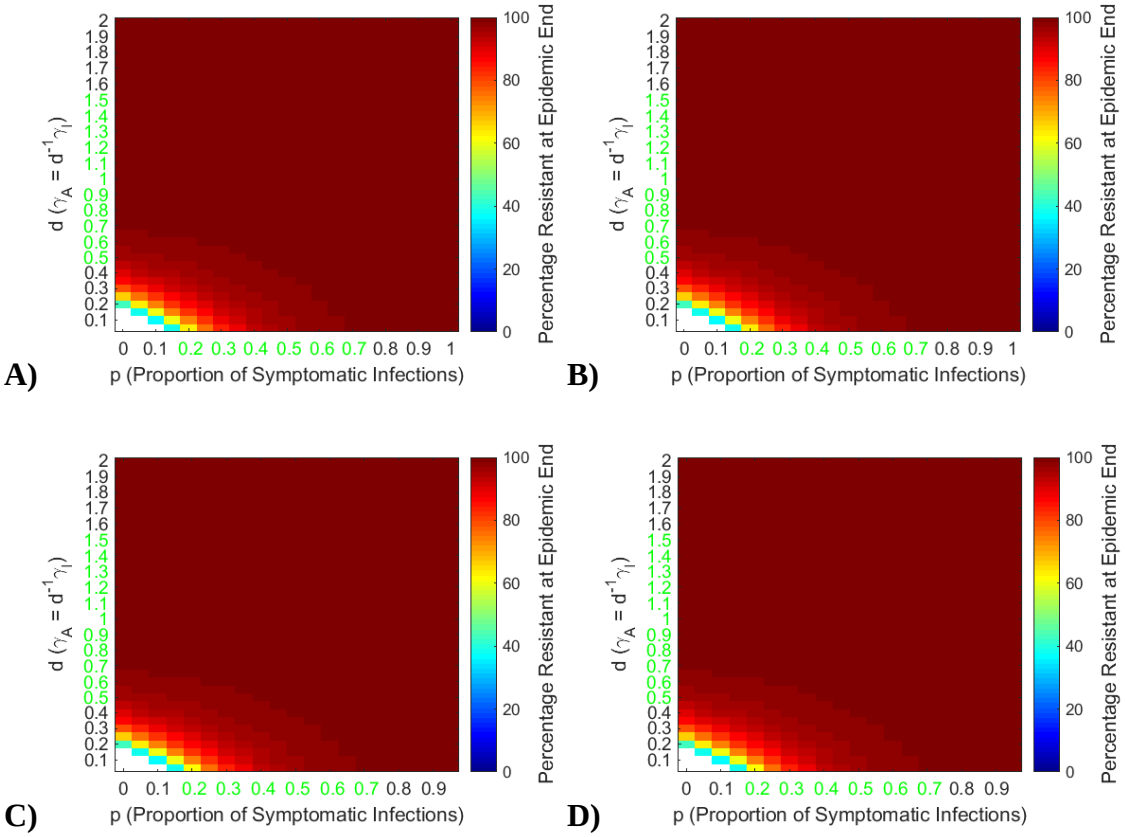


Figure 5.18: Percentage of the population resistant at the end of the epidemic, against different durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under mid-level transmission (β_I=300/365). Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

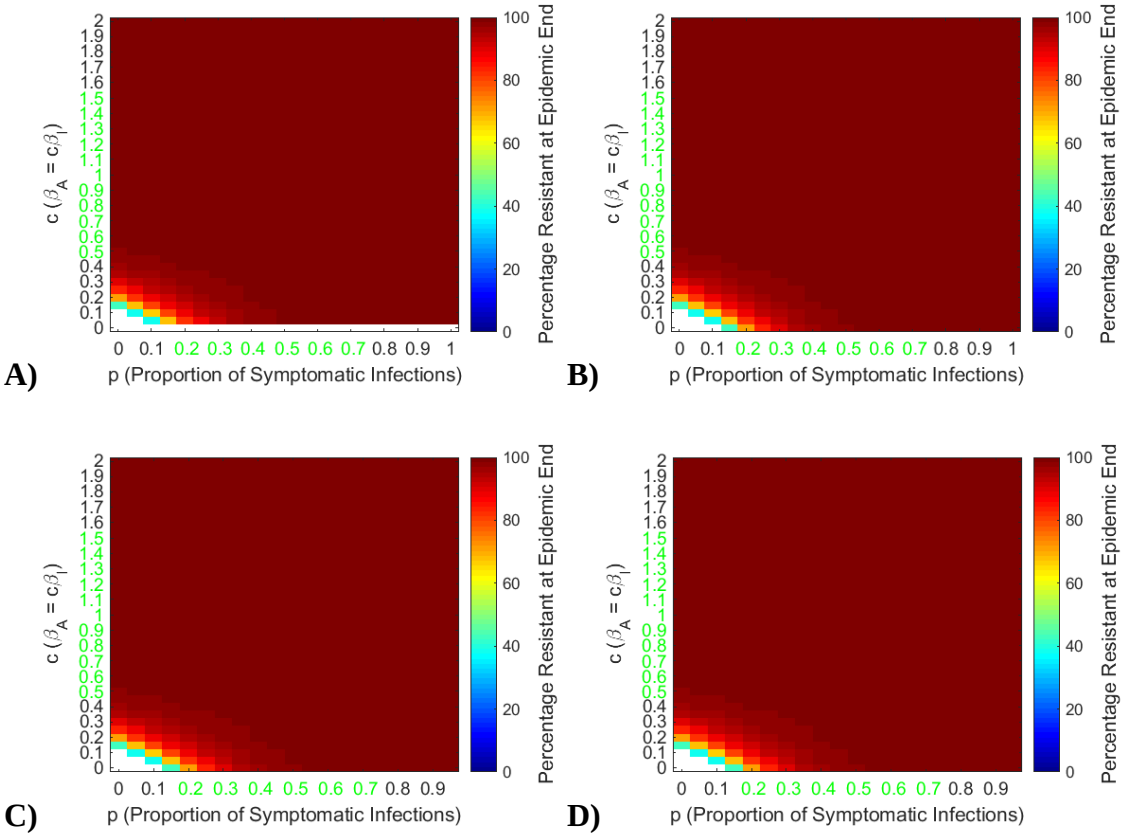


Figure 5.19: Percentage of the population resistant at the end of the epidemic, against different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under high-level transmission (β_I=400/365). Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

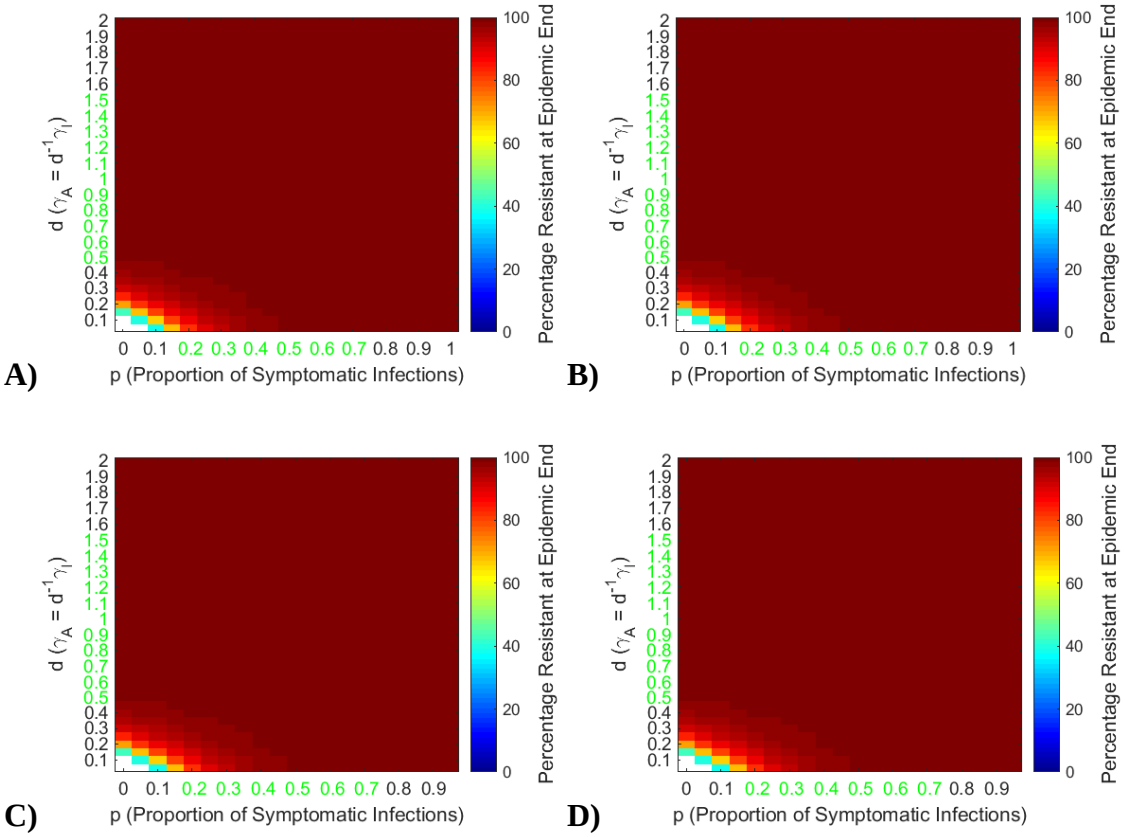


Figure 5.20: Percentage of the population resistant at the end of the epidemic, against durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under high-level transmission (β_I=400/365). Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

5.3.4 Biological unrealistic time lengths for progression from an asymptomatic infection to a symptomatic infection (δ^{-1}) within Model B

Results from Model B should be interpreted with a cautionary note in terms of what is biologically realistic. Recalling Equation 5.7 and Equation 5.8, δ represents the

rate at which an infected human develops symptoms and moves from the asymptomatic class to the symptomatic class (Robinson & Stilianakis 2013). Therefore δ^{-1} represents the number of days an individual would spend asymptomatically infected if they do not die (μ) or their immune system fails to clear the infection (γ_A) (Robinson & Stilianakis 2013). Considering that $p=\delta/(\delta+\gamma_A+\mu)$ and $\gamma_A=1/d\varphi$, where φ is the number of days until immunity clears infection in a symptomatic infection and d represents how long immunity clears infection in an asymptomatic infection in proportion to a symptomatic infection, Equation 5.11 can be derived.

$$\delta^{-1} = \frac{1-p}{p((d\varphi)^{-1}+\mu)} \qquad \textbf{Equation 5.11}$$

Equation 5.11 demonstrates that the number of days spent with an asymptomatic infection before progression to a symptomatic infection (δ^{-1}) can be affected by both the proportion of infections that are symptomatic (p) and the time until immunity clears all dengue virus in an asymptomatic infection in proportion to a symptomatic infection (d). As demonstrated by Figure 5.21 this means that certain combinations of these two proportions can lead to the period of time spent from becoming asymptomatic infected until progressing to a symptomatic infection (δ^{-1}), being biologically unfeasibly long, depending if longer than a month, two weeks or a week is seen as being biologically unfeasible (See Figure 5.21 B, C and D respectively).

This not only affects the biological realism of the results obtain from using Model B when altering the time until immunity clears all dengue virus in an asymptomatic infection (d), but the biological realism of the results when varying the transmission from asymptomatic infection (c), along with the proportion of infections that lead to development of symptoms (p). This is because whilst acquisition of immunity was kept equal between asymptomatic and symptomatic infection (i.e. d was kept at 1), the proportion of infections leading to symptomatic infection was altered (p).

Therefore if longer than a month is considered a biologically unlikely amount of time spent from becoming asymptomatic infected until progressing to a symptomatic infection (δ^{-1}), then 0-0.2 are unrealistic values for the proportion of infections that lead to development of symptoms (p), when the symptomatic and the asymptomatic infections develop immunity at the same rate (d=1) (see Figure 5.21B). Likewise if longer than two weeks is considered a biologically unlikely amount of time spent from becoming asymptomatic infected until progressing to a symptomatic infection (δ^{-1}), then 0-0.35 are unrealistic values for the proportion of infections that lead to development of symptoms (p), when the symptomatic and the asymptomatic infections develop immunity at the same rate (d=1) (see Figure 5.21C). If longer than a week is considered a biologically unlikely amount of time spent from becoming asymptomatic infected until progressing to a symptomatic infection (δ^{-1}), then 0-0.5 are unrealistic values for the proportion of infections that lead to development of symptoms (p), when the symptomatic and the asymptomatic infections develop immunity at the same rate (d=1) (see Figure 5.21D). This therefore also demonstrates that as the tolerance for the duration of progression from asymptomatic to symptomatic infection (δ^{-1}) decreases, more of the conservative samples of parameter space for the proportion of infections leading to symptoms (p), the duration of transmission in the asymptomatic class (d) and the level of transmission in the asymptomatic class (c) can also be seen as having an unfeasible duration of progression from asymptomatic to symptomatic infection (δ^{-1}) (see Figure 5.21B-D).

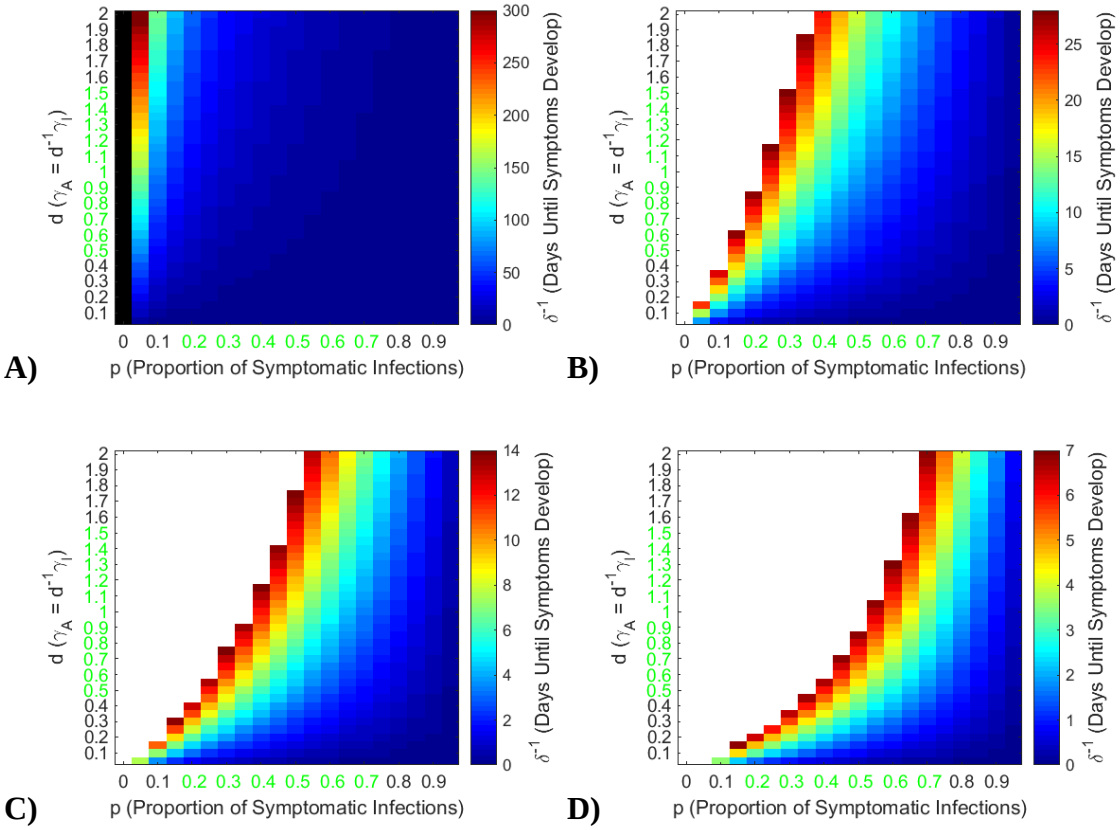


Figure 5.21: Effect of altering the duration of asymptomatic infection (d) and the proportion of infections that are symptomatic (p), on the duration of progression from asymptomatic to symptomatic infection (δ^{-1}) for Model B, values equal to infinity are in black. A) Without values above a certain number of days being coloured white. Note the colour scale represents 0-300 days. B) Values greater the 28 days are in white. Note the colour scale represents 0-28 days. C) Values greater the 14 days are in white. Note the colour scale represents 0-14 days. D) Values greater the 7 days are in white. Note the colour scale represents 0-7 days. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

5.4 Discussion

Robinson & Stilianakis (2013) do not explore how the parameter space surrounding the probability of an infection leading to symptoms (p), the level and duration of transmission from asymptomatic infections (c and d respectively) effects the outcomes of Models A and B. Instead Robinson & Stilianakis (2013) use fixed

parameters based on influenza to explore the long term behaviours of both Model A and B, then going on to modify Model B in order to explore the consequences of the evolution of a drug resistant strain of influenza. That being said there are comparisons between the results found here and those of Robinson & Stilianakis (2013) that can be made, some of which enable generalisations over the outcomes from Model A and B to be made. This study found that in terms of R_0 Models A and B produce identical results (see Figure 5.6-9). There is little comparison that can be made concerning R_0 when looking at Robinson & Stilianakis (2013) as they calculate the asymptomatic and symptomatic transmission terms (β_A and β_I, respectively) using a fixed value of R_0 from the 2009 H1N1 influenza pandemic, along with fixed values for other parameters based on influenza studies. This study found that epidemic persistence time and percentage of the population being left resistant to dengue was extremely similar between models A and B (see Figure 5.9-20), despite there been differences in the peak incidence of asymptomatic and symptomatic infections between the two models (see Figure 5.4). Likewise Robinson & Stilianakis (2013) found that epidemic persistence time was similar between the two models, despite there been differences in the peak incidence of asymptomatic and symptomatic infections between models A and B. It should be noted that Robinson & Stilianakis (2013) did not mention the percentage of the population being left resistant to influenza in their simulations, most likely because they did not think it was of relevance to their study. The findings from my study and a comparison to the finding of Robinson & Stilianakis (2013), therefore lead to two generalisations regarding models A and B. Firstly that when modelled in a deterministic frequency dependent framework there is little overall difference between modelling infection leading to an asymptomatic state or symptomatic state compared to modelling infection leading to an asymptomatic state that can possibly progress to a symptomatic state.

Secondly that this is despite differences in the peak incidence of asymptomatic and symptomatic infections between two such modelling approaches.

A caveat to this and many other observations made from using Model B, is that certain combinations of the proportion of asymptomatic infections becoming symptomatic (p) and the period of time that immunity takes to clear an asymptomatic infection (d) can produce biologically unrealistic lengths of time in the progression from an asymptomatic infection to a symptomatic infection (δ^{-1}) (see Figure 5.21). This is not only true for large parts of the parameter space explored from altering the proportion of asymptomatic infections becoming symptomatic (p) and the period of time that immunity takes to clear an asymptomatic infection (d) but the parameter space explored from altering the proportion of asymptomatic infections becoming symptomatic (p) and the transmission from asymptomatic infections (c) (see Figure 5.21). This also affects large parts of the more conservative samples of parameter space for the proportion of infections leading to symptoms (p), the duration of transmission in the asymptomatic class (d) and to a lesser extent the level of transmission in the asymptomatic class (c) (see Figure 5.21).

For both models A and B most combinations of the proportion of infections leading to symptoms (p), level and duration of transmission from asymptomatic infections (c and d respectively) produce epidemics that last little over a year or less (see Figure 5.9-14). The few epidemics lasting over several years occur for a proportion of infections that develop symptoms (p), which is towards the low end. p=0 to 0.25 for a low-level transmission setting (β_I=200/365), p=0 to 0.2 for a mid-level transmission setting (β_I=300/365), p=0 to 0.15 for a high-level transmission setting (β_I=400/365) (see Figure 5.9-13). Especially when compared to the range sampled in the more conservative sample of parameter space based on the review by Grange et al. (2014)

(thus often been excluded from this conservative sample). Grange et al. (2014) found that 3-80% of dengue infections were symptomatic with a mean rate in cohort studies of 22.9% and 25.6%, for the Americas and Asia, respectively. The mean symptomatic infection rate in cohort studies was 24% whereas in index cluster studies it was 63% (Grange et al. 2014). Cohort studies quantify the ratio of asymptomatic to symptomatic dengue infections by following a cohort of people and use case reporting, absenteeism and/or symptom questionnaires, combined with blood screening for dengue antibodies at regular intervals (Endy 2002a; Endy 2002b; Arguello et al. 2015). The other commonly used method is index cluster analysis, where people surrounding an index case of dengue illness are sampled. Their symptoms are quantified through symptom questionnaires or clinical diagnosis and there blood is screened for dengue antibodies (Singh et al. 2000; Beckett et al. 2005; Reyes et al. 2010; Wang et al. 2015). The difference in proportions of symptomatic dengue infections observed through these two methodologies led Grange et al. (2014) to point out that in cohort studies, the common use of questionnaires of perceived symptoms may well be biased against the number of dengue infections defined as symptomatic. Furthermore Grange et al. (2014) points to Yoon et al. (2012) who used both index cluster analysis and cohort methodologies finding that the cohort methodology may have underestimated the rate of symptomatic infection. Countering this Grange et al. (2014) also points out that the index cluster approach may be biased towards higher rates of symptomatic infection, as the virus responsible for the index case may be more likely to produce symptoms. The more conservative samples of parameter space for the level and duration of transmission from asymptomatic infections (c and d respectively) leads to all these longer lasting epidemics being excluded (see Figure 5.9-13). However, there was no literature

available to base the more conservative samples of parameter space for the level and duration of transmission from asymptomatic infections (c and d respectively).

The epidemics that last more than a year occur for a low proportion of infections becoming symptomatic (p) combined with either a low duration or level of transmission from asymptomatic infections (c and d, respectively). This translates as very few infections becoming symptomatic and those that are asymptomatic either becoming immune very quickly (a low value for d) or transmitting dengue to very few people (a low value of c) as such the R_0 value is only just above 1 (compare Figure 5.9-14 with Figure 5.6-8). Considering that R_0 is defined as the average number of infections produced by the arrival of an infected individual in a completely susceptible population (Anderson & May 1991a), this could mean that if these models were to be modelled stochastically instead of in a deterministic manner, the parameter settings that produce the longer lasting epidemics may produce dengue outbreaks that do not infect more than a few individuals. These few individuals would simply become immune or die before infecting someone else with dengue virus. As such, there would also be few individuals who are immune to dengue and thereby at a risk of DHF in an outbreak of a different dengue serotype.

Across most of the parameter space, epidemics lead to nearly 100% of the population being immune to the invading dengue serotype. With between 5%-98% being immune for proportions of infection leading to symptoms (p) less than 1 when combined with levels or duration of transmission from asymptomatic infections (c and d respectively) that are also less than 1 in the lower transmission setting (β_I=200/365) (see Figure 5.15-16). In the medium transmission setting (β_I=300/365) this becomes 36%-98% of the population being immune, for proportions of infection leading to symptoms (p) less than 0.7 when combined with levels or durations of transmission from

asymptomatic infections (c and d respectively), that are also less than 0.7 (see Figure 5.17-18). Likewise for the higher transmission setting (β_I=400/365) this becomes 39%-98% of the population being immune, for less than half of infections leading to symptoms ($p<0.5$) when combined with levels or durations of transmission from asymptomatic infections that are half that of symptomatic infections ($c<0.5$ and $d<0.5$ respectively) (see Figure 5.19-20). Looking at the more conservative samples of parameter space this means that even at the low level transmission setting (β_I=200/365) above 85% of the population is resistant to dengue virus at the end of an epidemic. If within 1-3 years after the initial epidemic another dengue serotype were to invade the majority of the population would be immune through cross protective immunity (Reich et al. 2013). However as time progresses past this period, the large proportion that are immune to the initial serotype would become increasingly at risk of DHF in any subsequent dengue epidemics caused by another serotype (Guzman et al. 2010; Rodenhuis-Zybert et al. 2010). Unless seroprevalence surveys had been carried out this large proportion of the population at risk of DHF would be completely unknown to a health authority, as they would be relying on reported cases from the first epidemic.

The percentage of population left immune by a dengue serotype and therefore at risk of DHF upon infection with another serotype in epidemics produced by Models A and Model B, may well explain the historical occurrence of DF and DHF in Cuba. From 1977-1979 there was a nationwide epidemic of DENV-1, with a further nationwide epidemic of DENV-2 in 1981 and an outbreak of DENV-2 restricted to the city of Santiago de Cuba in 1997. Of the 205 DHF/DSS cases in Santiago de Cuba in 1997 all but three had evidence of a prior dengue infection, and all where over 15, suggesting that they had been infected with DENV-1 during the island wide 1977-1979 outbreak (Guzmán 2000; Vaughn et al. 2000; Guzmán et al. 2000). Furthermore there was a

greater DHF/DSS mortality rate in the 1997 DENV-2 outbreak, than the 1981 DENV-2 outbreak (Guzmán et al. 2002). Some have suggested that this greater risk of disease severity seen in the second DENV-2 outbreak may be connected to a decrease in heterologous neutralizing antibody titre (Guzmán et al. 2002; Guzman et al. 2007). The results from these models would suggest that an initial outbreak of a dengue serotype would lead to a large proportion of the population becoming immune to that serotype. An epidemic by a second serotype would be unlikely in the near future, as Reich et al. (2013) approximated that cross protective immunity lasts for 1-3 years. Unfortunately as time progressed there would be an increased chance of a successful epidemic that would lead to more severe dengue infections in those that are immune to the serotype responsible for the first epidemic (Guzmán et al. 2002; Guzman et al. 2007), as cross protective immunity wanes (Reich et al. 2013). A health authority using reported dengue illnesses would not necessarily know how big a proportion of the population had become immune to the initial serotype. Results from this model suggest that even at low probabilities of developing symptoms, a large proportion of the population could be immune to this initial serotype. Therefore in order to understand the risks of DHF/DSS in countries that suffer from infrequent dengue epidemics not only is there a need for knowledge of the immunity to specific serotypes, the sequence of serotype immunity and length of time between serotype infections that leads to DHF, but also further knowledge about the proportion of primary dengue infections that are asymptomatic. On this point Grange et al. (2014) review suggested that there was no significant difference between primary and secondary dengue infections leading to asymptomatic dengue infection. It should be noted that there was significant difference between the general rates of asymptomatic dengue infection between studies (Grange et al. 2014). There has also been very few studies looking at viremia in asymptomatic dengue

infections (Beckett et al. 2005; Reyes et al. 2010; Carrington & Simmons 2014), let alone quantifying the differences in viremia in either primary or secondary dengue infections which are asymptomatic (Duong et al. 2011).

The 1997 outbreak in Santiago de Cuba highlights another observation that can be inferred from this model. The first reported cases of dengue were in January 1997, although retrospective serological evidence suggest that the initial transmission occurred in the latter half of December 1996 (Kourí et al. 1998). As dengue had not been reported since 1981 in Cuba and the first reported case was in a male who had never been outside of Cuba (Guzmán 2000), this would suggest that dengue had been introduced by an international traveller who was asymptomatically infected. Likewise subfigures A and C of Figure 5.9-14 illustrate that the arrival of an asymptomatic individual can produce a dengue epidemic. Even in a low transmission setting with low probabilities of developing symptoms (p), combined with either low levels of asymptomatic transmissibility (c) or short periods of viremia (d), the arrival of an asymptomatic infection can cause an outbreak in Models A and B (see Subfigures A and C of Figure 5.9-10). Asymptomatic infections would therefore also limit the effectiveness of airport fever screening as an dengue epidemic prevention strategies as suggested by Kuan et al. (2010), Kuan & Chang (2012) and Chastel (2012). Whilst there has been reported cases of travellers returning from dengue endemic countries with fever or developing fever (Jelinek 2000; Wichmann et al. 2005; Freedman et al. 2010; Kuan & Chang 2012; Chastel 2012), there has been little work on estimating the number of imported asymptomatic dengue infections to non-endemic countries. Autochthonous cases of dengue fever were reported in France and Croatia in 2010, furthermore there was an outbreak of dengue fever in the Portuguese islands of Madeira (WHO 2015b). In 2007 in north-eastern Italy there was an outbreak of chikungunya,

with *Ae. albopictus* being implicated as the vector (WHO 2015a). In light of these incidences Quam et al. (2015) through mathematical modelling estimated a yearly importation of 572 apparent and 1747 inapparent dengue infections through Rome's airport. If Quam et al. (2015) approximations are reliable then it may be the case that stochasticity plays a role in preventing imported dengue from causing regular epidemics from taking place in Italy. Incorporation of such stochasticity into Models A or Model B may shed further light on this.

Literature reviews by Jelinek (2000) and Chastel (2012) and the results from the models used in this study highlight two key aspects of asymptomatic infection in the risk of asymptomatically dengue infected travellers causing epidemics. These two aspects are the level and duration of transmission from asymptomatic dengue infections. To date no studies have directly researched the duration or degree of transmissibility of asymptomatic dengue virus infections to a biting mosquito. However, the degree and duration of transmissibility relate to the level and duration of dengue virus viremia in asymptomatic dengue infections, respectively. Viremia of an asymptomatic dengue infection has to be high enough to produce an infectious blood meal for a mosquito and work by Nguyet et al. (2013) suggest the higher the viral titre, the greater the likelihood of a blood meal infecting a mosquito. Figure 5.6-8 demonstrate that a very low proportion of infections developing symptoms (p) combined with either a very low asymptomatic transmission rate (c) or period of infection in asymptomatic infection (d) could produce an R_0 less than 1, meaning an epidemic could not take place (see Figure 5.9-14). This becomes more important at lower transmission levels (β_I=200/365) as the range at which the proportion of infection leading to symptoms (p) combined with levels or duration of transmission from asymptomatic infections (c and d respectively) leading to an R_0 less than 1 increase (see Figure 5.6-8) and these low level of

transmission settings may reflect the transmission settings of non-dengue endemic countries.

5.5 Conclusions

Modelled deterministically with frequency dependent transmission there is little difference regarding epidemiological aspects between Model A and Model B. This is despite Model B making unrealistic assumptions about the length of time an infection progresses from an asymptomatic state to a symptomatic state (δ^{-1}) and for many different proportions of asymptomatic infections becoming symptomatic (p) or periods of time that immunity takes to clear an asymptomatic infection (d). The addition of stochastic elements to Models A or Model B would shed greater light on the role of asymptomatic infections in terms of the likelihood of dengue epidemics taking hold in a population. Above all models A and B demonstrate the need for further research on the level and duration of transmission in asymptomatic infections, as well as the proportion of dengue infections that remain asymptomatic. This affects not only whether or not a dengue epidemic can occur in a population and how long that epidemic persists but the proportion of the population left immune to dengue that would thereby be at risk of developing DHF in any subsequent dengue epidemic. The longer lasting epidemics were either on the edge of or excluded from the more conservative sample of parameter space for the proportion dengue virus infection that are symptomatic, which was based on the review by Grange et al. (2014). Considering this the differences in the proportion dengue virus infection that are symptomatic seen in Grange et al. (2014) review highlight the need for comparative research between cohort studies and index cluster studies in order to assess which is the most accurate, followed by standardisation of methodologies in future studies assessing this. To date there have been no studies demonstrating that asymptomatically dengue virus infected humans can transmit dengue

virus to a biting mosquito, and very few studies speculating on the degree or duration of transmissibility. Therefore the findings of this study highlight these areas as a key priority for future research. These areas could be researched by studying how the level and duration viremia in asymptomatically dengue virus infected humans compares to that of symptomatically dengue virus infected humans capable of transmitting dengue virus to a mosquito.

Chapter 6: Mosquito dependent models of asymptomatic dengue infections: their relationship to epidemic success, persistence and population at risk of developing dengue haemorrhagic fever

Abstract

In order for an *Aedes* mosquito to act as a competent vector of dengue virus, the virus has to surmount several barriers, replicate and disseminate into the saliva, ready for injection at a mosquito's next blood meal. All of these processes take a period of time. This could have the effect of slowing dengue's transmission dynamics down. This slowing down of dengue's transmission could reduce the speed at which an epidemic progresses or uses up the newly born susceptible population; this in turn could lead to an epidemic becoming endemic. For these reasons I modified the two models in the previous chapter to include mosquitoes in dengue virus transmission. As in the previous chapter the level and duration of transmission from the asymptomatic class was varied over a wide range due to the lack of data. The inclusion of mosquitoes within the models produced broadly similar results, across most of the parameter space. Except for finding that the dengue virus could become endemic, provided the transmission setting was high, the probability of developing symptoms was less than 1 and there was either a high level or duration of transmission from asymptomatic infections.

6.1 Introduction

Dengue is the most important arboviral pathogen causing an estimated 390 million dengue infections per year, of which 96 million manifest as clinical illness (Bhatt et al. 2013). Being from the Flavivirus genus dengue viruses are positive-stranded RNA viruses (Guzman et al. 2010; Rodenhuis-Zybert et al. 2010). Dengue viruses are separated into four immunological serotypes (DENV-1, DENV-2, DENV-3

and DENV-4), (Guzman et al. 2010; Rodenhuis-Zybert et al. 2010). Primary infection leads to asymptomatic infection through to dengue fever (DF) (Guzman et al. 2010; Andraud et al. 2012; Grange et al. 2014; WHO 2015c). Upon recovery from primary infection an individual is immune to that serotype for life (Guzman et al. 2010; Rodenhuis-Zybert et al. 2010; Andraud et al. 2012). For an estimated 1-3 years an individual is also immune to other dengue serotypes (Reich et al. 2013). After this period of time however, secondary infection with another serotype can lead to a person developing dengue haemorrhagic fever (DHF) (Guzman et al. 2010; Rodenhuis-Zybert et al. 2010; Andraud et al. 2012; WHO 2015c). DHF can lead to dengue shock syndrome (DSS), both of which are life threatening. Often grouped together as 'severe dengue', DHF and DSS have been estimated to cause 500,000 cases of illness, mostly in children and have a mortality rate of 2.5% (WHO 2015c).

Mosquitoes of the *Aedes* genus act as the vector of dengue viruses (M. Service 2012b). *Aedes aegypti* being the principal vector of dengue viruses, with *Ae. albopictus* having less of a role as a vector of dengue (M. Service 2012b; Lambrechts et al. 2010). With *Ae. albopictus* increasing its range to include 29 states of the USA, 15 European countries, Australia and New Zealand (M. Service 2012b; Lambrechts et al. 2010; WHO 2015c), there has been a growing concern that there could be an increasing risk of outbreaks of dengue fever in these countries (Lambrechts et al. 2010).

Given that a large proportion of dengue infections are thought to be asymptomatic (Bhatt et al. 2013; Grange et al. 2014) in Chapter 5 I used two SAIR frequency dependent models previously developed by Robinson & Stilianakis (2013), to explore the role of asymptomatic dengue infections on several features of dengue virus epidemiology. The key finding of the work in Chapter 5 was that asymptomatic infections did not lead to dengue becoming endemic. Whilst epidemics usually lasted

only a year or two, when the probability of developing symptoms was low and combined with low asymptomatic transmission or short duration of transmission epidemics could extend over 2 years. Furthermore epidemics could extend to over 15 years in the low transmission setting. Extremely low probabilities of developing symptoms combined with extremely low values of asymptomatic transmission or the duration of asymptomatic transmission caused the basic reproductive number (R_0) to drop below 1 meaning dengue could not spread through the population. This was the case even in the high transmission setting. Another finding from Chapter 5 was that across most of the explored parameter space more than 90% of the population was immune to dengue at the end of an epidemic and thereby at risk of DHF in subsequent epidemics caused by a different dengue serotype. This at risk population decreased with lower transmission settings, a lower probability of being symptomatic, as well as a lower transmission and duration of transmission in the asymptomatic class.

Lacking from Chapter 5's two models was the explicit inclusion of mosquitoes in the transmission dynamics. In order for an *Aedes* mosquito to act as a competent vector of dengue, the virus has to surmount two gut barriers, disseminate to the salivary glands, replicate and disseminate into the saliva, ready for injection at a mosquitoes next blood meal (Lambrechts et al. 2010). All of these processes take a period of time often referred to as the extrinsic incubation period (EIP) (Andraud et al. 2012). Dengue's EIP could have an effect on dengue's transmission by slowing its dynamics down compared to that seen in a frequency dependent model of transmission, where EIP is not included. This slowing down of dengue's transmission could reduce the speed at which an epidemic progresses or uses up the newly born susceptible population; this in turn could lead to an epidemic becoming endemic. For these reasons I modified the two models in the previous chapter to include mosquitoes in dengue virus transmission.

6.2 Methods

6.2.1 Description of Model A under mosquito dependent transmission

The two frameworks used to Model Asymptomatic Dengue infection were adapted from the work of Robinson & Stilianakis (2013). As discussed the EIP within the mosquito may affect dengue's transmission dynamics and so affect the persistence of dengue epidemics. Therefore a mosquito dependent transmission version of the 2nd framework described in Robinson & Stilianakis (2013) (here referred to as Model A) was made and adapted to dengue transmission dynamics (see the Equation 6.1-8 below and the flow diagram in Figure 6.1). Table 6.1 contains a list of all parameter and variables in Model A under mosquito dependent transmission.

$$\frac{\Delta S_H}{\Delta t} = \mu_H N_H - \frac{S_H}{N_H}\beta_V I_V - \mu_H S_H \qquad \textbf{Equation 6.1}$$

$$\frac{\Delta A_H}{\Delta t} = (1-p)\frac{S_H}{N_H}\beta_V I_V - \gamma_A A_H - \mu_H A_H \qquad \textbf{Equation 6.2}$$

$$\frac{\Delta I_H}{\Delta t} = p\frac{S_H}{N_H}\beta_V I_V - \gamma_I I_H - \mu_H I_H \qquad \textbf{Equation 6.3}$$

$$\frac{\Delta R_H}{\Delta t} = \gamma_I I_H + \gamma_A A_H - \mu_H I_H \qquad \textbf{Equation 6.4}$$

$$\frac{\Delta S_E}{\Delta t} = \mu_V N_V - \omega S_E \qquad \textbf{Equation 6.5}$$

$$\frac{\Delta S_V}{\Delta t} = \omega S_E - S_V\frac{\beta_A A_H + \beta_I I_H}{N_H} - \mu_V S_V \qquad \textbf{Equation 6.6}$$

$$\frac{\Delta E_V}{\Delta t} = S_V\frac{\beta_A A_H + \beta_I I_H}{N_H} - \varepsilon E_V - \mu_V E_V \qquad \textbf{Equation 6.7}$$

$$\frac{\Delta I_V}{\Delta t} = \varepsilon E_V - \mu_V I_V \qquad \textbf{Equation 6.8}$$

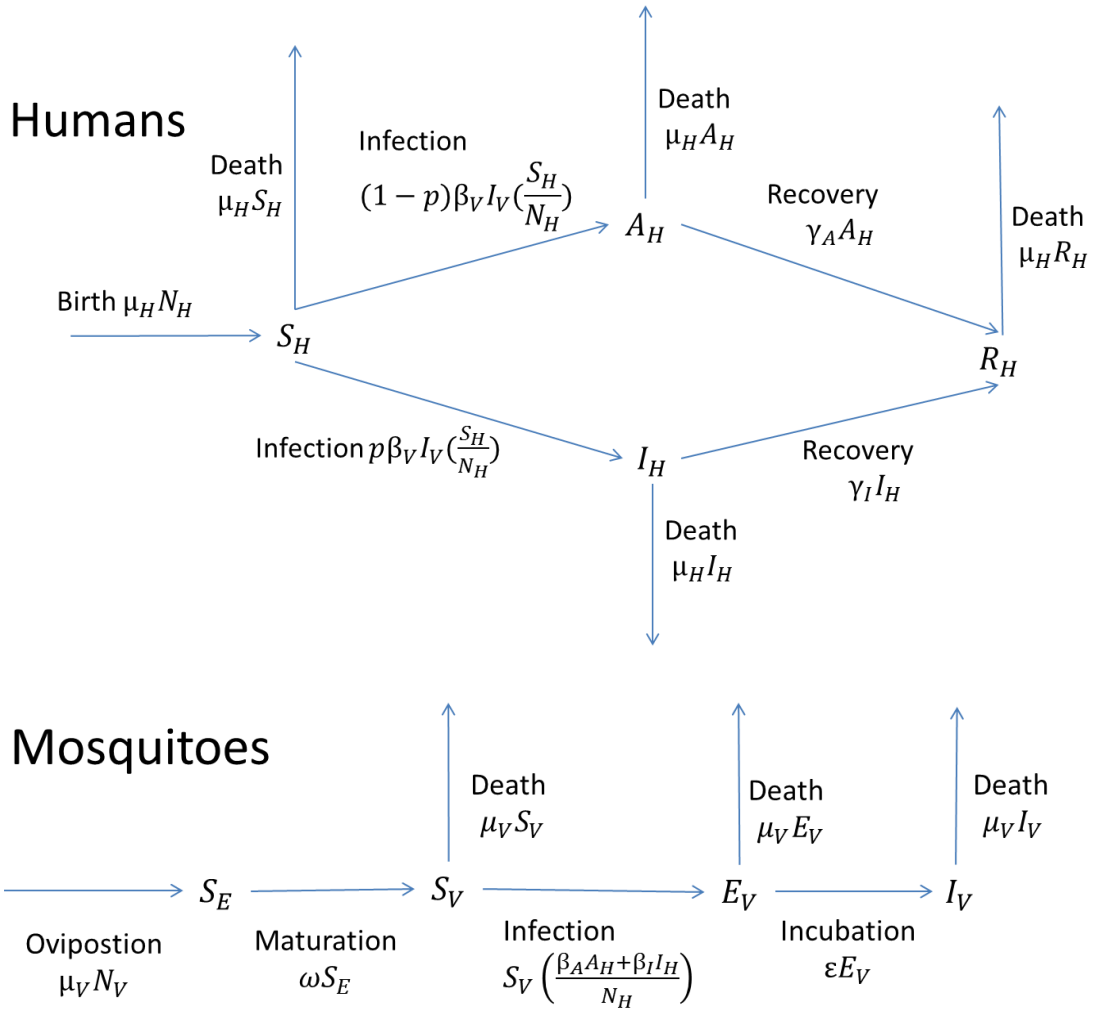


Figure 6.1: Flow diagrams representing Model A under mosquito dependent transmission

Table 6.1: Variables at starting value and parameters used in Model A under mosquito dependent transmission

Symbol	Name	Value
μ_H	Birth and death rate (per day)	$1/(60x365)$
c	Infectivity of asymptomatic infections compared to symptomatic infections	0 to 2
b	Biting rate (per day)	0.3-1
β_i	Probability of symptomatic transmission to a vector	0-1
β_I	Symptomatic transmission rate (per day)	$b\beta_i$
β_A	Asymptomatic transmission rate (per day)	$c\beta_I$
φ	Days symptomatic	8
γ_I	Symptomatic recovery rate (per day)	φ^{-1}
d	Asymptomatic recovery rate compared to symptomatic recovery rate	0.05 to 2
γ_A	Asymptomatic recovery rate (per day)	$(d\varphi)^{-1}$
p	Probability of being symptomatic	0 to 1
S_H	Susceptible population	10^6-1
A_H	Asymptomatic population	0 to 1
I_H	Symptomatic population	0 to 1
R_H	Recovered population	0
N_H	Total population	10^6
μ_V	Mosquito birth and death rate (per day)	$1/6$
ω	Mosquito maturation rate (per day)	$1/11$
β_v	Probability of vector transmission to a human	0.425
β_V	Mosquito transmission rate (per day)	$b\beta_v$
ε	Extrinsic incubation period	$1/10$
S_E	Pre-adult mosquitos	$(\omega/\mu_V)N_V$
S_V	Susceptible adult mosquito population	$9.5x10^6$
E_V	Latent adult mosquito population	0
I_V	Infectious adult mosquitoes population	0
N_V	Total adult mosquito population	$9.5x10^6$

The total human population (N_H) is divided into susceptible (S_H), asymptomatically infected (A_H), symptomatically infected (I_H) and recovered (R_H) classes. The total mosquito population (N_V) is divided into immature mosquito (S_E), susceptible mosquito (S_V), incubating mosquito (E_V) and infected mosquito (I_V) classes. Every class of human experiences loss due to a death rate μ_H, however the human

population remains constant as humans are born into the susceptible class at a birth rate of $\mu_H N_H$. Susceptible humans become infected at a mosquito dependent transmission term, given by $S_H/N_H(\beta_V I_V)$. Upon infection humans either become asymptomatic at a rate of $(1-p)S_H/N_H(\beta_V I_V)$ or symptomatically infected at a rate of $pS_H/N_H(\beta_V I_V)$. Asymptomatically infected people gain complete lifelong immunity at a rate of γ_A and symptomatically infected people gain complete lifelong immunity at a rate of γ_I, both of which then move to the recovered class. Every class of mosquito experiences loss due to death rate μ_V, however the mosquito population remains constant as mosquitoes are oviposited into the immature mosquito class at a of $\mu_V N_V$. Mosquitoes mature into adults at a rate ω and become infected at a rate of $S_V(\beta_A A_H + \beta_I I_H)/N_H$ moving to the incubating class, after a period of incubation ε mosquitoes move to the infectious class.

The symptomatic transmission term β_I is made up of two component terms, the mosquito biting rate b and probability of infection from biting a symptomatic human β_i ($\beta_I = b\beta_i$). Likewise the mosquito transmission term β_V is made up of two component terms, the mosquito biting rate b and probability of infection from an infectious mosquito β_v ($\beta_V = b\beta_v$).

6.2.2 Description of Model B under mosquito dependent transmission

A mosquito dependent transmission version of the 1st framework described in Robinson & Stilianakis (2013) (here referred to as Model B) was made and adapted to dengue transmission dynamics (see Equation 6.9-16 and the flow diagram in Figure 6.2). Table 6.2 contains a list of all parameters and variables.

$$\frac{\Delta S_H}{\Delta t} = \mu_H N_H - \frac{S_H}{N_H}\beta_V I_V - \mu_H S_H$$ **Equation 6.9**

$$\frac{\Delta A_H}{\Delta t} = \frac{S_H}{N_H}\beta_V I_V - \delta A_H - \gamma_A A_H - \mu_H A_H$$ **Equation 6.10**

$$\frac{\Delta I_H}{\Delta t} = \delta A_H - \gamma_I I_H - \mu_H I_H$$ **Equation 6.11**

$$\frac{\Delta R_H}{\Delta t} = \gamma_I I_H + \gamma_A A_H - \mu_H I_H$$ **Equation 6.12**

$$\frac{\Delta S_E}{\Delta t} = \mu_V N_V - \omega S_E$$ **Equation 6.13**

$$\frac{\Delta S_V}{\Delta t} = \omega S_E - S_V \frac{\beta_A A_H + \beta_I I_H}{N_H} - \mu_V S_V$$ **Equation 6.14**

$$\frac{\Delta E_V}{\Delta t} = S_V \frac{\beta_A A_H + \beta_I I_H}{N_H} - \varepsilon E_V - \mu_V E_V$$ **Equation 6.15**

$$\frac{\Delta I_V}{\Delta t} = \varepsilon E_V - \mu_V I_V$$ **Equation 6.16**

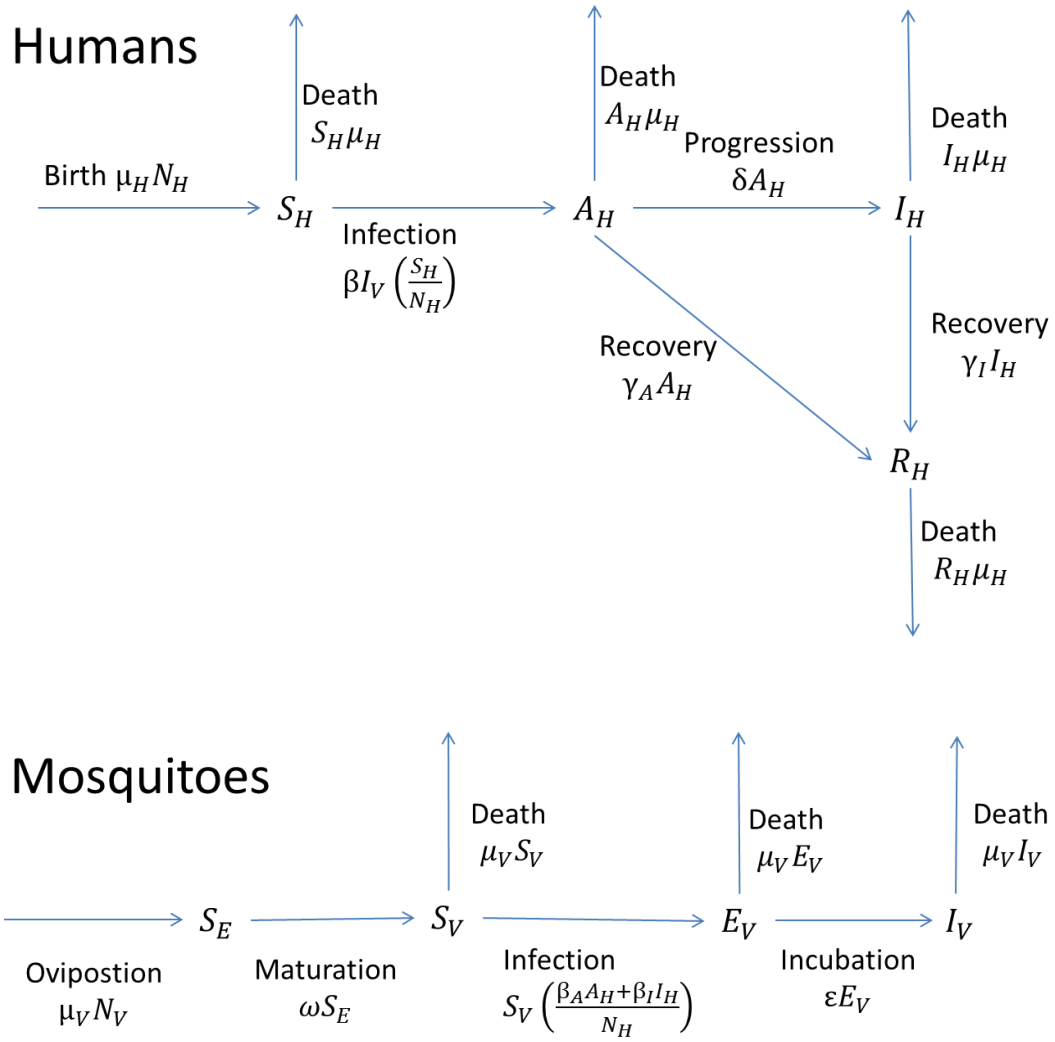


Figure 6.2: Flow diagrams representing Model B under mosquito dependent transmission

Table 6.2: Variables at starting value and parameters used in Model B under mosquito dependent transmission

Symbol	Name	Value
μ_H	Human birth and death rate (per day)	1/(60x365)
c	Infectivity of asymptomatic infections compared to symptomatic infections	0 to 2
b	Biting rate (per day)	0.3 to 1
β_i	Probability of symptomatic transmission to a Vector	0.5 to 1
β_I	Symptomatic transmission rate (per day)	$b\beta_i$
β_A	Asymptomatic transmission rate (per day)	$c\beta_I$
φ	Days symptomatic	8
γ_I	Symptomatic recovery rate (per day)	φ^{-1}
d	Asymptomatic recovery rate compared to symptomatic recovery rate	0.05 to 2
γ_A	Asymptomatic recovery rate (per day)	$(d\varphi)^{-1}$
p	Probability of being symptomatic	0 to 0.95
δ	Progression rate from asymptomatic to symptomatic (per day)	$P(\gamma_A+\mu_h)/(1-p)$
S_H	Susceptible population	10^6-1
A_H	Asymptomatic population	0 to 1
I_H	Symptomatic population	0 to 1
R_H	Recovered population	0
N_H	Total population	10^6
μ_V	Mosquito oviposition and death rate (per day)	1/6
ω	Mosquito maturation rate (per day)	1/11
β_v	Probability of vector transmission to a human	0.425
β_V	Mosquito transmission rate (per day)	$b\beta_v$
ε	Extrinsic incubation period	1/10
S_E	Pre-adult mosquitos	$(\omega/\mu_V)N_V$
S_V	Susceptible adult mosquito population	$9.5x10^6$
E_V	Latent adult mosquito population	0
I_V	Infectious adult mosquitoes population	0
N_V	Total adult mosquito population	$9.5x10^6$

The total human population (N_H) is divided into susceptible (S_H), asymptomatically infected (A_H), symptomatically infected (I_H) and recovered (R_H) classes. The total mosquito population (N_V) is divided into immature (S_E), susceptible

(S_V), incubating (E_V) and infected (I_V) classes. Every class of human experiences loss due to a death rate μ_H, however the human population remains constant as humans are born into the susceptible class at a birth rate of $\mu_H N_H$. Susceptible humans become infected at a mosquito dependent transmission term, given by $S_H/N_H(\beta_V I_V)$ and move to the asymptomatic class. Asymptomatically infected people gain complete lifelong immunity at a rate of γ_A moving to the recovered class, die μ_H or progress to symptomatic infection δ. This means that a proportion p, which is equal to $\delta/(\delta+\gamma_A+\mu_H)$, of asymptomatic infection will become symptomatically infected. Symptomatically infected people gain complete lifelong immunity at a rate of γ_I moving to the recovered class. Every class of mosquito experiences loss due to death rate μ_V, however the mosquito population remains constant as mosquitoes are oviposited into the immature mosquitoes class at a of $\mu_V N_V$. Mosquitoes mature into adults at a rate ω and become infected at a rate of $S_V(\beta_A A_H + \beta_I I_H)/N_H$ moving to the incubating class, after a period of incubation ε mosquitoes move to the infectious class.

The symptomatic transmission term β_I is made up of two component terms, the mosquito biting rate b and probability of infection from biting a symptomatic human β_i ($\beta_I=b\beta_i$). Likewise the mosquito transmission term β_V is made up of two component terms, the mosquito biting rate b and probability of infection from an infectious mosquito β_v ($\beta_V=b\beta_v$).

6.2.3 Model A's basic reproduction number (R_0) under mosquito dependent transmission

R_0 of the mosquito dependent transmission version of Model A was calculated using the next generation matrix methods as outlined in Diekmann et al. (2010) (see Equation 6.17-19). Note that in a completely susceptible population $S_H=N_H$ and $S_V=N_V$.

$$\varphi = t + \sigma$$ **Equation 6.17a**

$$\varphi = \begin{vmatrix} A_H \\ I_H \\ E_V \\ I_V \end{vmatrix},\ t = \begin{vmatrix} (1-p)\beta_V I_V \\ p\beta_V I_V \\ N_V\left(\frac{\beta_A A_H + \beta_I I_H}{N_H}\right) \\ 0 \end{vmatrix},\ \sigma = \begin{vmatrix} -\gamma_A A_H - \mu_H A_H \\ \gamma_I I_H - \mu_H I_H \\ -\varepsilon E_V - \mu_V E_V \\ \varepsilon E_V - \mu_V I_V \end{vmatrix}$$ **Equation 6.17b**

$$K_L = T *\text{-}\Sigma^{-1}$$ **Equation 6.18a**

$$T = \begin{vmatrix} 0 & 0 & 0 & (1-p)\beta_V \\ 0 & 0 & 0 & p\beta_V \\ \frac{\beta_A N_V}{N_H} & \frac{\beta_I N_V}{N_H} & 0 & 0 \\ 0 & 0 & 0 & 0 \end{vmatrix}$$ **Equation 6.18b**

$$\Sigma = \begin{vmatrix} -\gamma_A - \mu_H & 0 & 0 & 0 \\ 0 & -\gamma_I - \mu_H & 0 & 0 \\ 0 & 0 & -\varepsilon - \mu_V & 0 \\ 0 & 0 & \varepsilon & -\mu_V \end{vmatrix}$$ **Equation 6.18b continued**

$$K_L = \begin{vmatrix} 0 & 0 & -\frac{(p-1)\varepsilon\beta_V}{\mu_V(\varepsilon+\mu_V)} & -\frac{(p-1)\beta_V}{\mu_V} \\ 0 & 0 & \frac{p\varepsilon\beta_V}{\mu_V(\varepsilon+\mu_V)} & \frac{p\beta_V}{\mu_V} \\ \frac{\beta_A N_V}{N_H(\gamma_A+\mu_H)} & \frac{\beta_I N_V}{N_H(\gamma_I+\mu_H)} & 0 & 0 \\ 0 & 0 & 0 & 0 \end{vmatrix}$$ **Equation 6.18b continued**

$$Largest\ eigen\ value\ (\lambda)\ of\ K_L = R_0$$ **Equation 6.19a**

$$R_0 = \sqrt{\frac{N_V}{N_H} \times \frac{\varepsilon\beta_V}{\mu_V(\varepsilon+\mu_V)} \times \frac{(1-p)\beta_A(\gamma_I+\mu_H) + p\beta_I(\gamma_A+\mu_H)}{(\gamma_I+\mu_H)(\gamma_A+\mu_H)}}$$ **Equation 6.19b**

6.2.4 Model B's basic reproduction number (R_0) under mosquito dependent transmission

R_0 of the mosquito dependent transmission version of Model B was calculated using the next generation matrix methods as outlined in Diekmann et al. (2010) (see Equation 6.20-22). Note that in a completely susceptible population $S_H=N_H$ and $S_V=N_V$.

$$\varphi = t + \sigma \qquad \textbf{Equation 6.20a}$$

$$\varphi = \begin{vmatrix} A_H \\ I_H \\ E_V \\ I_V \end{vmatrix}, \quad t = \begin{vmatrix} \beta_V I_V \\ 0 \\ N_V\left(\frac{\beta_A A_H + \beta_I I_H}{N_H}\right) \\ 0 \end{vmatrix} \qquad \textbf{Equation 6.20b}$$

$$\sigma = \begin{vmatrix} -\gamma_A A_H - \delta A_H - \mu_H A_H \\ \delta A_H - \gamma_I I_H - \mu_H I_H \\ -\varepsilon E_V - \mu_V E_V \\ \varepsilon E_V - \mu_V I_V \end{vmatrix} \qquad \textbf{Equation 6.20b continued}$$

$$K_L = T *\text{-}\Sigma^{-1} \qquad \textbf{Equation 6.21a}$$

$$T = \begin{vmatrix} 0 & 0 & 0 & \beta_V \\ 0 & 0 & 0 & 0 \\ \frac{\beta_A N_V}{N_H} & \frac{\beta_I N_V}{N_H} & 0 & 0 \\ 0 & 0 & 0 & 0 \end{vmatrix} \qquad \textbf{Equation 6.21b}$$

$$\Sigma = \begin{vmatrix} -\delta - \gamma_A - \mu_H & 0 & 0 & 0 \\ \delta & -\gamma_I - \mu_H & 0 & 0 \\ 0 & 0 & -\varepsilon - \mu_V & 0 \\ 0 & 0 & \varepsilon & -\mu_V \end{vmatrix} \qquad \textbf{Equation 6.21b continued}$$

$$K_L = \begin{vmatrix} 0 & 0 & \frac{\varepsilon\beta_V}{\mu_V(\varepsilon+\mu_V)} & \frac{\beta_V}{\mu_V} \\ 0 & 0 & 0 & 0 \\ \frac{\beta_A N_V}{N_H(\delta+\gamma_A+\mu_H)} + \frac{\delta\beta_I N_V}{N_H(\gamma_I+\mu_H)(\delta+\gamma_A+\mu_H)} & \frac{\beta_I N_V}{N_H(\gamma_I+\mu_H)} & 0 & 0 \\ 0 & 0 & 0 & 0 \end{vmatrix} \qquad \textbf{Equation 6.21b continued}$$

$$Largest\ eigenvalue\ (\lambda)\ of\ K_L = R_0 \qquad \textbf{Equation 6.22a}$$

$$R_0 = \sqrt{\frac{N_V}{N_H} \times \frac{\varepsilon\beta_V}{\mu_V(\varepsilon+\mu_V)} \times \frac{\gamma_I\beta_A + \mu_H\beta_A + \delta\beta_I}{(\delta+\gamma_A+\mu_H)(\gamma_I+\mu_H)}} \qquad \textbf{Equation 6.22b}$$

6.2.5 Analyses of Models A and Model B under mosquito dependent transmission

Models A and B were coded in Matlab. The parameter space was then explored with ode45, under the 'RelTol', 10^{-6} setting, using Dengue parameters sourced as mid-range values from the parameters listed in Andraud et al. (2012) literature review of Dengue transmission models (unless otherwise stated) so as to make the model comparable with other models of dengue virus transmission. Mosquito oviposition and mortality rate (μ_V) where the average mortality rates of *Aedes aegypti* and *Ae. albopictus*, as sourced from a meta-analyses of marked release recapture studies (Brady et al. 2013). Mosquito maturation rate (ω) was sourced from (M. Service 2012b) (see Table 6.1 and Table 6.2).

Asymptomatic individuals could be transmitting dengue at a lower rate due to a decreased viremia. Likewise asymptomatic individuals could be transmitting dengue at a higher rate, as they may be more likely to pass through areas of high mosquito abundance, due to not exhibiting the decreased movement through illness as seen in symptomatically infected humans. For these reasons parameter c of both models, the coefficient relating β_A to β_I ($\beta_A=c\beta_I$), was varied from 0-2. For each of these settings of c parameter p was varied from 0-1 in Model A. In Model B however for each of these settings of c parameter p was varied from 0-0.95, due to the fact that altering p alters δ as $\delta=p(\gamma_A+\mu)/1-p$ and at $p=1$ $\delta=\infty$. Parameter d, the coefficient relating γ_A and γ_I ($\gamma_A=1/d\varphi$, $\gamma_I=1/\varphi$), was kept at 1.

As information on the immune recovery rate for asymptomatically dengue infected humans is unavailable. Parameter d, the coefficient relating γ_A and γ_I, was varied from 0.05-2, as at $d=0$ γ_A would equal infinity ($\gamma_A=1/d\varphi$, $\gamma_I=1/\varphi$). For each of these settings of d parameter p was varied from 0-1 in Model A. In Model B however for each of these settings of d parameter p was varied from 0-0.95, due to the fact that

altering p alters δ as $\delta=p(\gamma_A+\mu)/1-p$ and at p=1 $\delta=\infty$. Parameter c, the coefficient relating β_A to β_I ($\beta_A=c\beta_I$), was kept at 1.

The range in parameter space for the probability of a dengue virus infection leading symptoms (p) could be considered a large sample. Therefore it was decided to highlight a conservative sample of parameter space for this parameter, through labelling certain axis values regarding this parameter in green on figures that display model outputs with respect to changes in this parameter. The lower limit to this conservative parameter space p=0.2 is based on the rounded down mean possibility of symptomatic dengue virus infection from cohort studies in Grange et al. (2014). The upper limit to this conservative parameter space of p=0.7 is based on the rounded up mean possibility of symptomatic dengue virus infection from index cluster studies in Grange et al. (2014). The non-rounded mean symptomatic dengue virus infection rate (p) in cohort studies was 24% whereas in index cluster studies it was 63% (Grange et al. 2014). Cohort studies quantify the ratio of asymptomatic to symptomatic dengue infections by following a cohort of people and use case reporting, absenteeism and/or symptom questionnaires, combined with blood screening for dengue antibodies at regular intervals (Endy 2002a; Endy 2002b; Arguello et al. 2015). Index cluster studies sample people surrounding an index case of dengue illness. Sampled individuals' symptoms are quantified through symptom questionnaires or clinical diagnosis and their blood is screened for dengue antibodies (Singh et al. 2000; Beckett et al. 2005; Reyes et al. 2010; Wang et al. 2015).

Likewise, the range in parameter space for the level and duration of transmission from asymptomatic dengue virus infections (c and d, respectively) could be considered large samples. Therefore it was decided to highlight more conservative samples of parameter space for these parameters, through labelling certain axis values regarding

these parameters in green for figures that display model outputs with respect to changes in these parameters. The lower limit of these conservative parameter spaces is 0.5 and the upper limit is 1.5. Note that at the time of submission of this thesis there was no data regarding the level or duration of transmission from asymptomatic dengue virus infected humans available, which could be used to base a more conservative region of parameter space for both of these parameters.

For the exploration of the parameter space surrounding the asymptomatic class's transmission rate β_A and recovery rate γ_A, Model A and Model B were run with 999,999 susceptible humans and the arrival of a symptomatically infected individual. At a very low rate of transmission or duration of transmission for the asymptomatic class (c and d, respectively) an epidemic may start with the arrival of a symptomatic dengue infected human, but not with the arrival of an asymptomatic dengue infected human. For this reason I then reran the same simulation with 999,999 susceptible humans and the arrival of an asymptomatically infected individual.

Furthermore the exploration of the two models sets of parameter space was conducted at the low-transmission setting of b=0.3 and β_i=0.5, mid-level transmission setting of b=0.65 and β_i=0.75, high-level transmission setting of b=1 and β_i=1 (this is the range of transmission setting cited in the review by Andraud et al. (2012)).

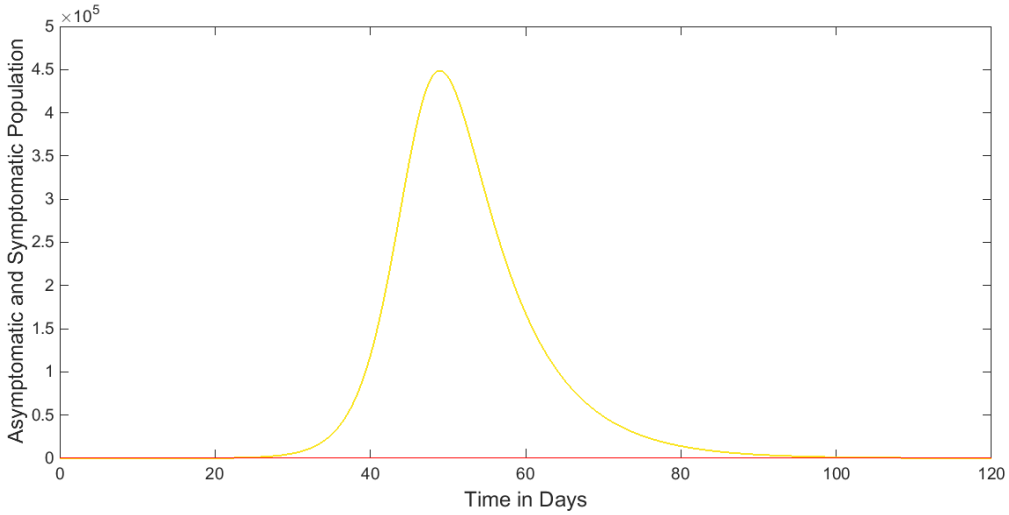
6.3 Results

6.3.1 Epidemic dynamics

When no infections led to symptoms (p=0), the level and duration of transmission in both the symptomatic and asymptomatic class is the same (c=1 and d=1 respectively) Model A and Model B both become a simple frequency dependent SIR model, with the asymptomatic class taking the place of the symptomatic class. This

demonstrates a typical SIR epidemic as the disease dramatically spreads through the population and declines as the pool of susceptible humans is used up (see Figure 6.3). As the proportion of infections leading to symptoms (p) increases in both models the symptomatic curve takes the place of the asymptomatic curve, however in Model B the symptomatic curve has a larger spread and lower peak for mid-levels of infections leading to symptoms (p) (see Figure 6.4-5). The epidemic patterns seen across Model A's and Model B's parameter space follows this pattern, except in differing heights and spreads in the symptomatic and asymptomatic curves. However epidemics with a basic reproduction number R_0 of less than 1 or started with the arrival of an asymptomatic individual when there is no transmission from that class (c=0) in Model A, fail to spread through the population (see Figure 6.6-14).

A)



B)

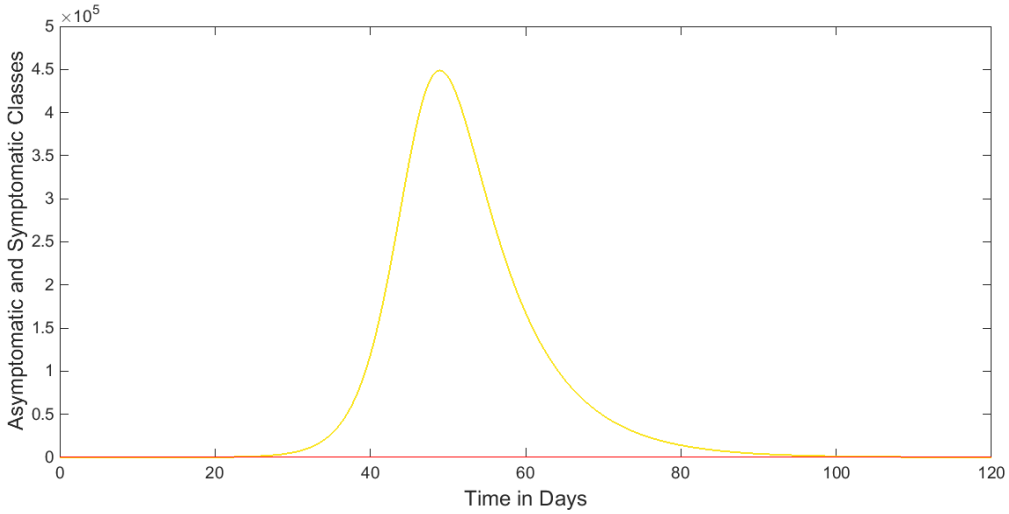
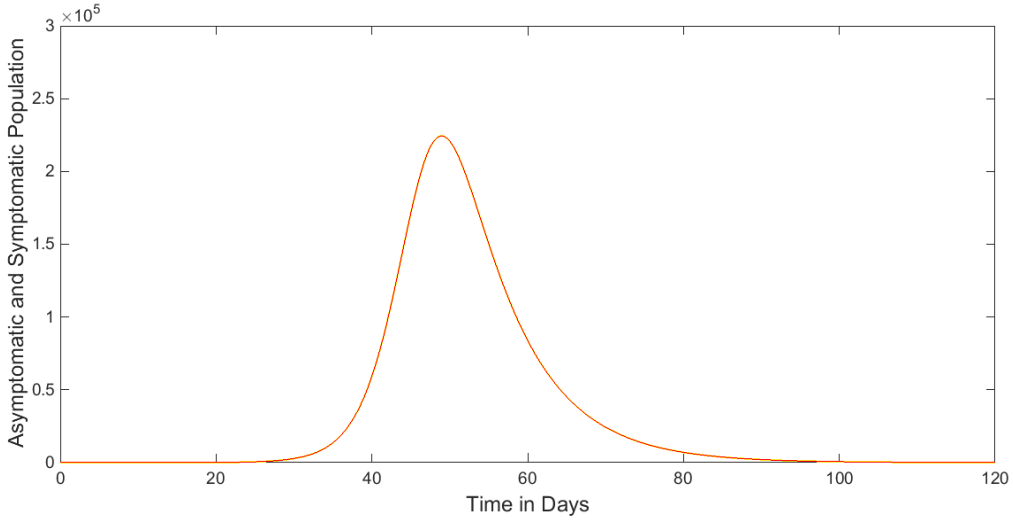


Figure 6.3: Infected human population after the arrival of symptomatically infected human at mid-level transmission (β_i=0.75 and b=0.65), with no symptomatic infections (p=0), the duration of an asymptomatic and symptomatic infection being the same (d=1) and the transmission from an asymptomatic and symptomatic infection being the same (c=1). Symptomatically infected humans are in red and asymptomatically infected humans are in yellow. A) Model A. B) Model B

A)



B)

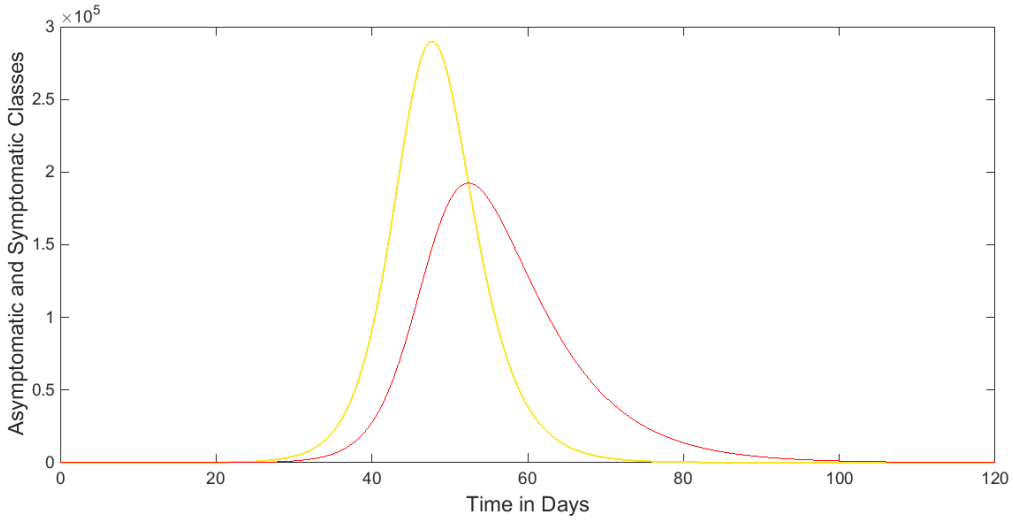
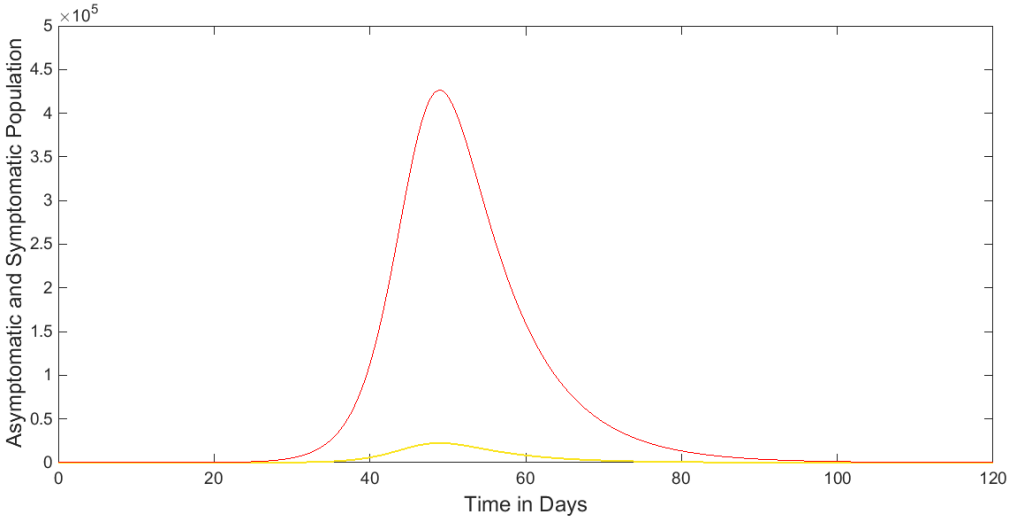


Figure 6.4: Infected human population after the arrival of symptomatically infected human at mid-level transmission (β_i=0.75 and b=0.65), with 50% of infections being symptomatic (p=0.5), the duration of an asymptomatic and symptomatic infection being the same (d=1) and the transmission from an asymptomatic and symptomatic infection being the same (c=1). Symptomatically infected humans are in red and asymptomatically infected humans are in yellow. A) Model A. B) Model B

A)



B)

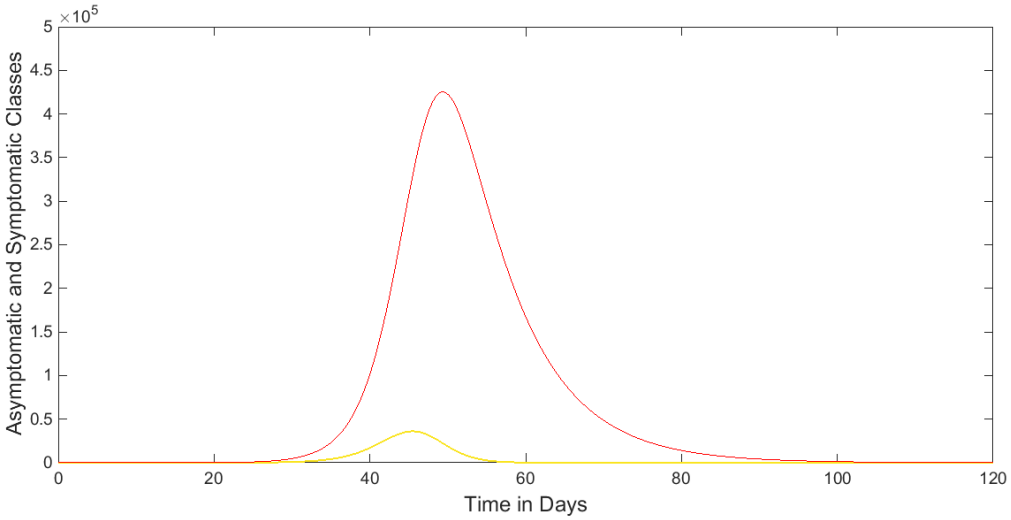


Figure 6.5: Infected human population after the arrival of symptomatically infected human at mid-level transmission (β_i=0.75 and b=0.65), with 95% of infections being symptomatic (p=0.95), the duration of an asymptomatic and symptomatic infection being the same (d=1) and the transmission from an asymptomatic and symptomatic infection being the same (c=1). Symptomatically infected humans are in red and asymptomatically infected humans are in yellow. A) Model A. B) Model B

6.3.2 Basic reproduction number R_0

As with the frequency dependent versions from Chapter 5, both Model A and Model B produce the same basic reproduction number R_0 for the same transmission settings (b and β_i), proportion of infections leading to symptoms (p) level and duration of transmission in the asymptomatic class (c and d respectively) (see Figure 6.6-8). Yet again, as a note of intuitive sense for all three transmission settings (β_i and b), when the level and duration of transmission is the same in both the asymptomatic and symptomatic class (c=1 and d=1, respectively), the R_0 value is the same no matter the proportion of infections leading to symptoms (p) (see Figure 6.6-8). Furthermore this happens to be the same R_0 value for Model A when all infections are symptomatic (p=1), no matter the level and duration of transmission in the asymptomatic class (c and d, respectively) (see Figure 6.6-8). Also similarly to Chapter 5, R_0 increases with a lower proportion of infections leading to symptoms (p) combined with a high level or duration of transmission in the asymptomatic class (c and d respectively) but decreases with a low proportion of infections leading to symptoms (p) combined with a low level or duration of transmission in the asymptomatic class (c and d respectively) (see Figure 6.6-8). This pattern in the value of R_0 is similar across all three transmission setting (b and β_i) but the overall values of R_0 increases as b and β_i increases from 0.3-1 and 0.5-1, respectively.

What is different in terms of R_0 between the frequency dependent models of Chapter 5 and the mosquito transmission models of this chapter is the threshold dynamic of R_0 being less than 1. At the low transmission setting (b=0.3 and β_i=0.5) the areas of parameter space that leads to an R_0 less than 1 is much larger with the threshold forming a line on the figures depicting epidemic effects of varying levels of transmission in the asymptomatic class (c) combined with varying the proportion of

infections leading to symptoms (p), from c=0.3 and p=0 to c=0 and p=0.3 (see Figure 6.6 A-B, Figure 6.9 and Figure 6.16). On figures depicting varying durations of transmission in the asymptomatic class (c) combined with varying the proportion of infections leading to symptoms (p), this line occurs from d=0.3 and p=0 to d=0.05 and p=0.25 (see Figure 6.6 C-D, Figure 6.10 and Figure 6.17). For mosquito dependent models this area of parameter space decreases much more rapidly to the point that for both the medium transmission setting (b=0.65 and β_i=0.75) and the high transmission setting (b=1 and β_i=1), R_0 is only less than 1 when there is no transmission from the asymptomatic class (c=0) and all infections are asymptomatic (p=0) (see Figure 6.7-8, Figure 6.11-14 and Figure 6.18-21).

The more conservative samples of parameter space for the proportion of infections leading to symptoms (p), the level and duration of transmission in the asymptomatic class (c and d respectively) do not lead to the lower values of R_0 seen in the bottom left corner of the sub-figures of Figure 6.6-8, likewise they do not lead to the higher values of R_0 seen in the top left corner of the sub-figures of Figure 6.6-8. This means that the areas of parameter space that lead to an R_0 below 1 (see Figure 6.6-8) and therefore lead to no epidemic spreading through the population are not included in the more conservative samples of parameter space (see Figure 6.9-14).

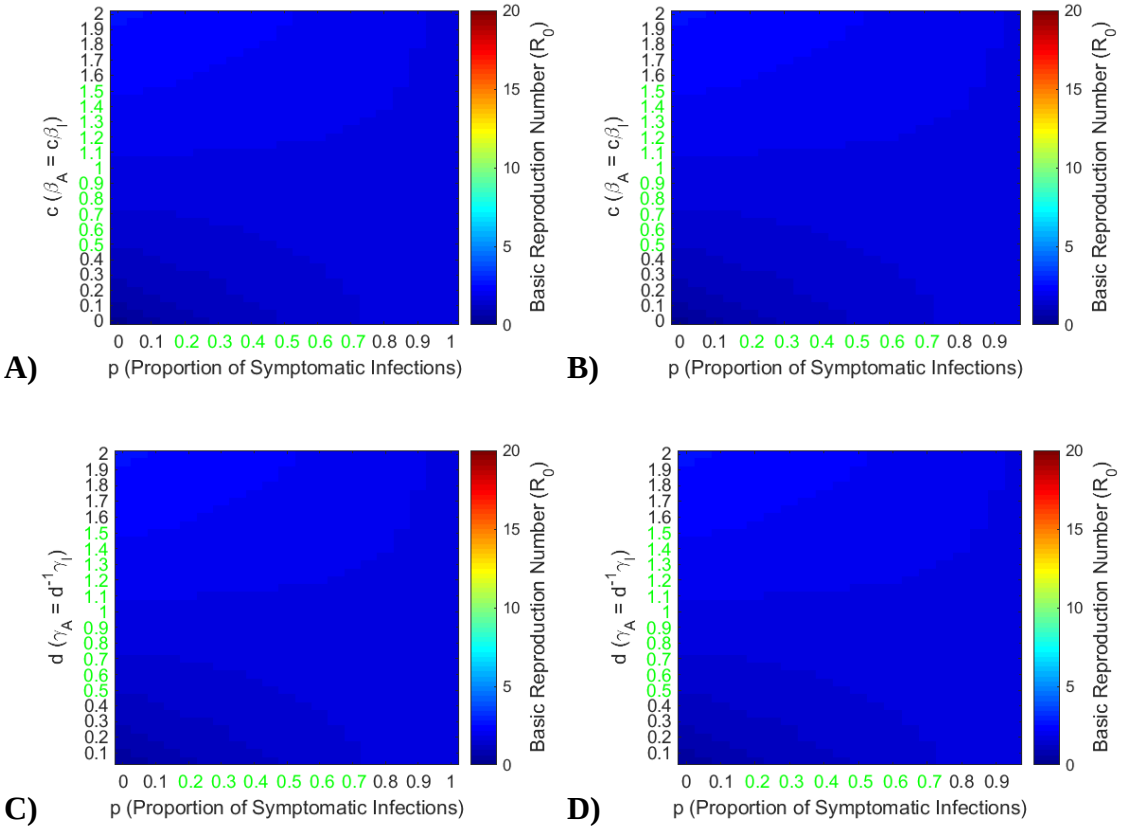


Figure 6.6: Basic reproduction number (R_0) at low-level transmission (β_i=0.5 and b=0.3). A) Model A at different proportions of infections developing symptoms (p) and different levels of transmission in asymptomatic infections (c), when the duration of asymptomatic and symptomatic infections is the same (d=1). B) Model B at different proportions of infections developing symptoms (p) and different levels of transmission in asymptomatic infections (c), when the durations of asymptomatic and symptomatic infections is the same (d=1). C) Model A at different proportions of infections developing symptoms (p) and different durations of asymptomatic infection (d), when transmission from the asymptomatic and symptomatic infections is the same (c=1). D) Model B at different proportions of infections developing symptoms (p) and different durations of asymptomatic infection (d), when transmission from the asymptomatic and symptomatic infections is the same (c=1). For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

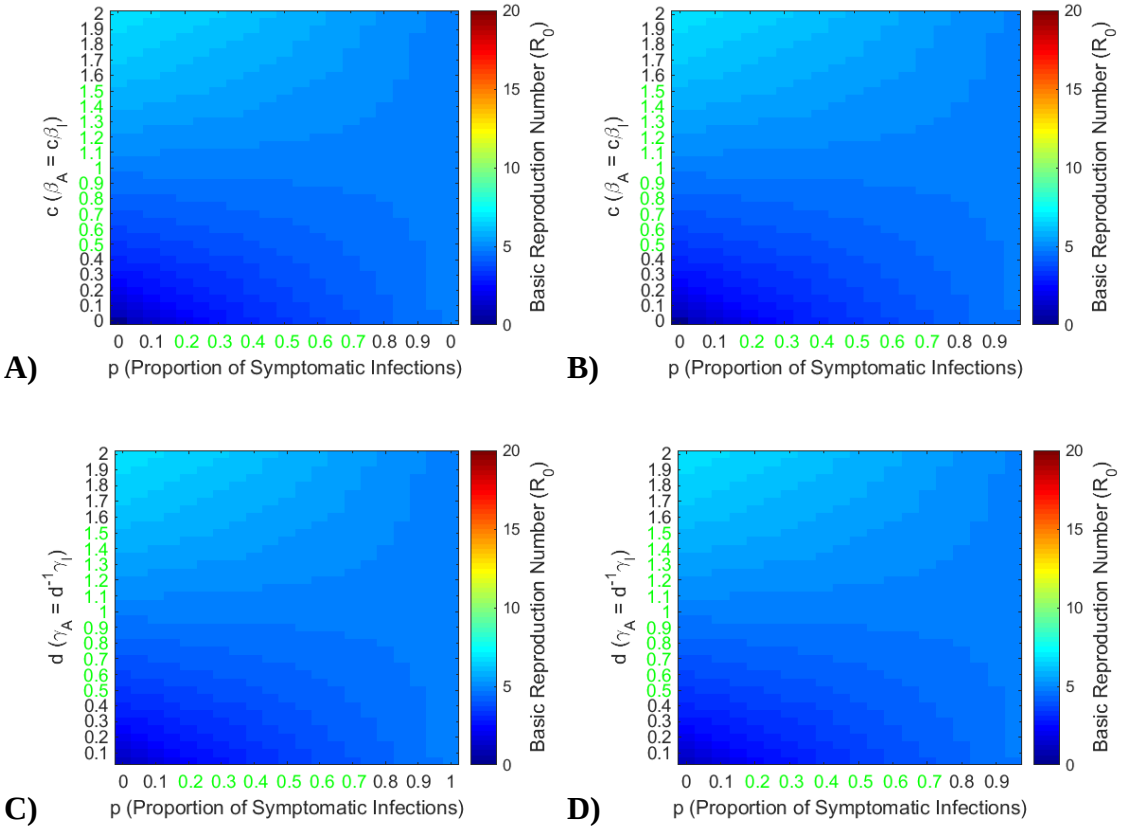


Figure 6.7: Basic reproduction number (R_0) at mid-level transmission (β_i=0.75 and b=0.65). A) Model A at different proportions of infections developing symptoms (p) and different levels of transmission in asymptomatic infections (c), when the duration of asymptomatic and symptomatic infections is the same (d=1). B) Model B at different proportions of infections developing symptoms (p) and different levels of transmission in asymptomatic infections (c), when the durations of asymptomatic and symptomatic infections is the same (d=1). C) Model A at different proportions of infections developing symptoms (p) and different durations of asymptomatic infection (d), when transmission from the asymptomatic and symptomatic infections is the same (c=1). D) Model B at different proportions of infections developing symptoms (p) and different durations of asymptomatic infection (d), when transmission from the asymptomatic and symptomatic infections is the same (c=1). For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

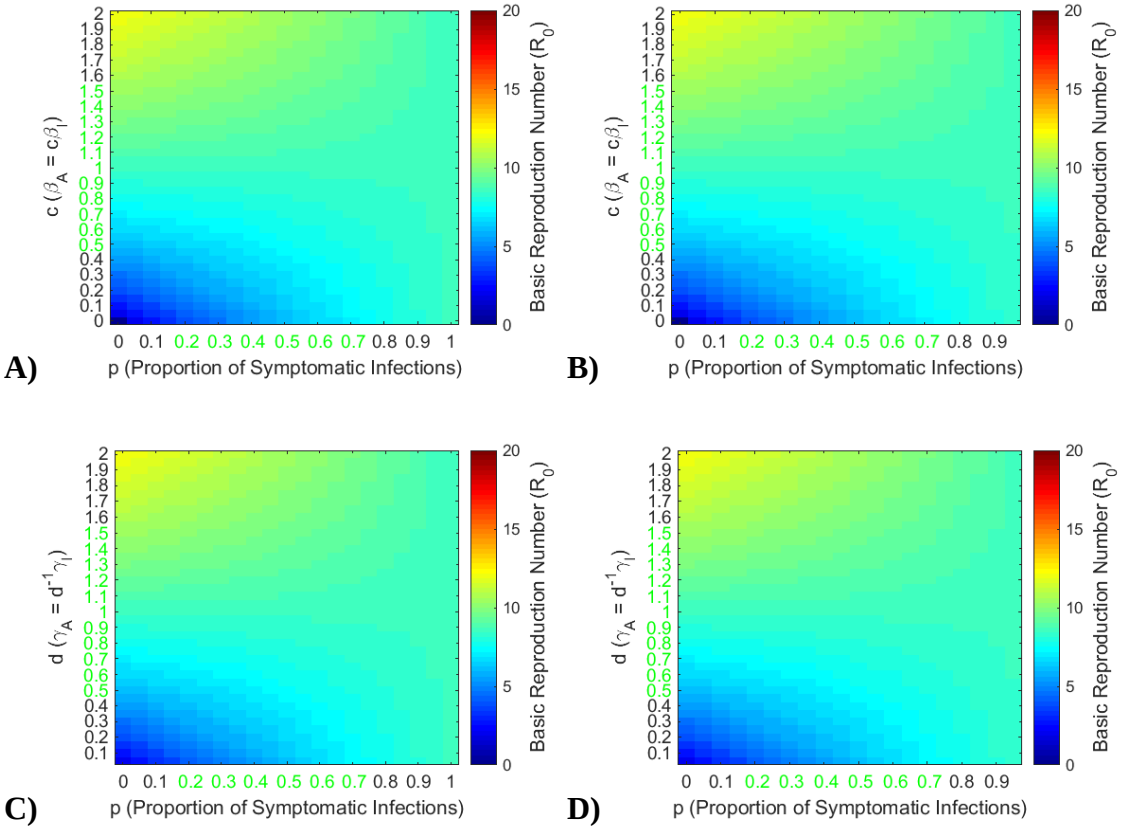


Figure 6.8: Basic reproduction number (R_0) at high-level transmission (β_i=1 and b=1). A) A) Model A at different proportions of infections developing symptoms (p) and different levels of transmission in asymptomatic infections (c), when the duration of asymptomatic and symptomatic infections is the same (d=1). B) Model B at different proportions of infections developing symptoms (p) and different levels of transmission in asymptomatic infections (c), when the durations of asymptomatic and symptomatic infections is the same (d=1). C) Model A at different proportions of infections developing symptoms (p) and different durations of asymptomatic infection (d), when transmission from the asymptomatic and symptomatic infections is the same (c=1). D) Model B at different proportions of infections developing symptoms (p) and different durations of asymptomatic infection (d), when transmission from the asymptomatic and symptomatic infections is the same (c=1). For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

6.3.3 Epidemic extinction

At the low to mid-level transmission settings (b and β_i) in the vast majority of epidemics asymptomatic and symptomatic dengue infections die out after a year or two, regardless of the proportion of infections leading to symptoms (p), the level and duration of transmission in the asymptomatic class (c and d respectively) or model (see Figure 6.9-12). Epidemics that last more than a year occur for a lower proportion of infections leading to symptoms (p) combined with low level or duration of transmission in the asymptomatic class (c and d respectively). For the lower transmission settings (b and β_i) the longer lasting epidemics last even longer. Epidemics that last more than 5 and in some cases longer than 10 years occur at the lowest transmission setting (b=0.3 and β_i=0.5) (see Figure 6.9-10) or in the mid-level transmission setting for a level of asymptomatic transmission at 5% of the symptomatic transmission (c=0.05) with no one developing symptoms (p=0) and no transmission from asymptomatic infections (c=0) with only 5% of infections being symptomatic (p=0.05). These longer lasting epidemics also coincide with the Basic Reproduction Number R_0 being only slightly over 1 or put another way, for the parameter values just above the threshold lines described in section 6.3.2 above (compare Figure 6.9-12 with Figure 6.6-7). This suggests that whilst at these parameter settings dengue virus can spread through the population, R_0 being greater than 1, dengue virus spreads slowly, due to a low force of infection. It should be noted that the more conservative samples of parameter space for the proportion of infections leading to symptoms (p), the level and duration of transmission in the asymptomatic class (c and d respectively) did not produce the longer lasting epidemics (see Figure 6.9-12). This was in a similar fashion to the more conservative samples of parameter space not producing the lower values of R_0 above (compare Figure 6.9-12

with Figure 6.6-7). These finding were also observed for the frequency dependent versions of Model A and Model B seen Chapter 5.

At the higher transmission setting (b=1 and β_i=1) epidemics tend to last from 5-17 months. However for levels or durations of transmission in the asymptomatic class greater than those of the symptomatic class (c>1 and d>1 respectively) combined with all but the highest proportions of infections leading to symptoms (p) the epidemics are still extant by 30 years (see Figure 6.13-14). This is because as seen by Figure 6.15 the epidemic pattern has shifted to a system of low level endemic persistence of dengue viral infections. A large area of the more conservative samples of parameter space for the proportion of infections leading to symptoms (p), the level and duration of transmission in the asymptomatic class (c and d respectively) includes these regions where the dengue virus becomes endemic (see Figure 6.13-14).

This epidemic pattern is broadly similar between models A and B, whether an epidemic is started by the arrival of an asymptomatic individual or symptomatic individual. However an epidemic started by the arrival of an asymptomatic individual lengthens the course of the longer lasting epidemics (see Figure 6.9-12).

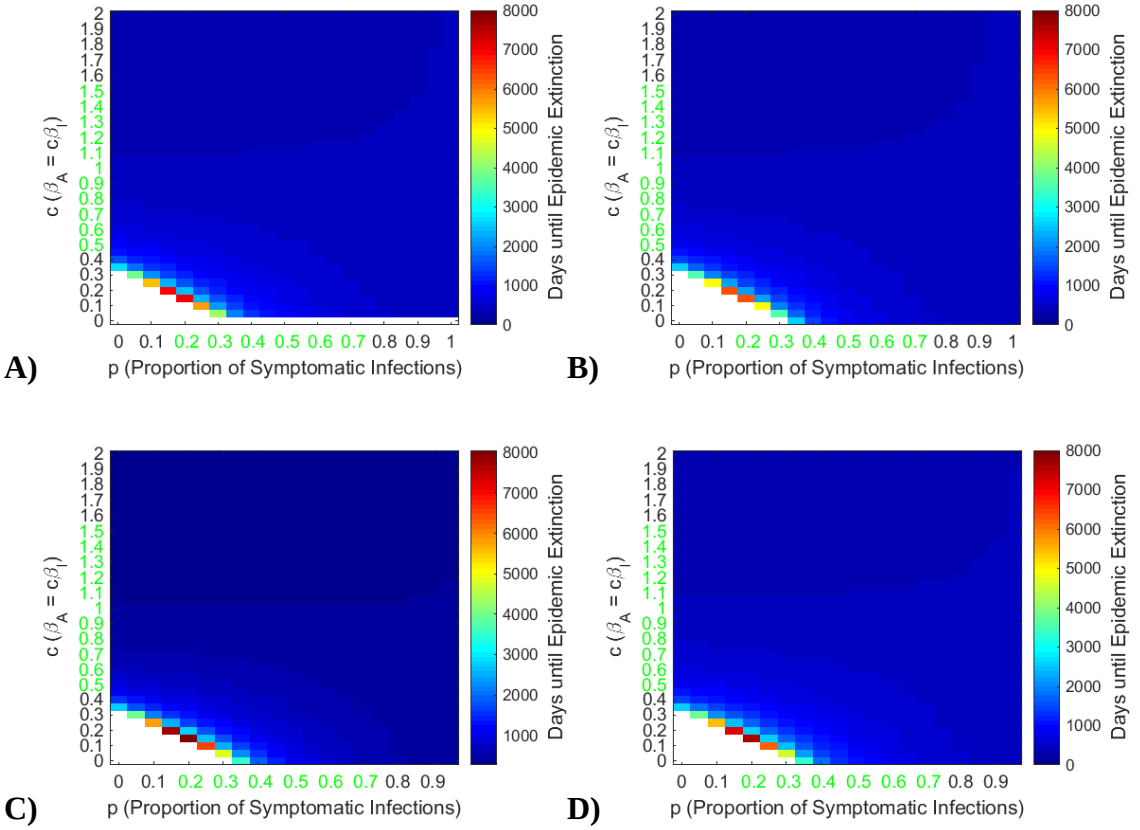


Figure 6.9: Days until epidemic extinction against different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under low-level transmission (β_i=0.5 and b=0.3). Days until epidemic extinction is defined as the first occurrence of the asymptomatic and symptomatic classes being less than 1 after the peak total of symptomatic and asymptomatic infections. Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. Note the colour scale represents 0-8000 days for subfigures A-D. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

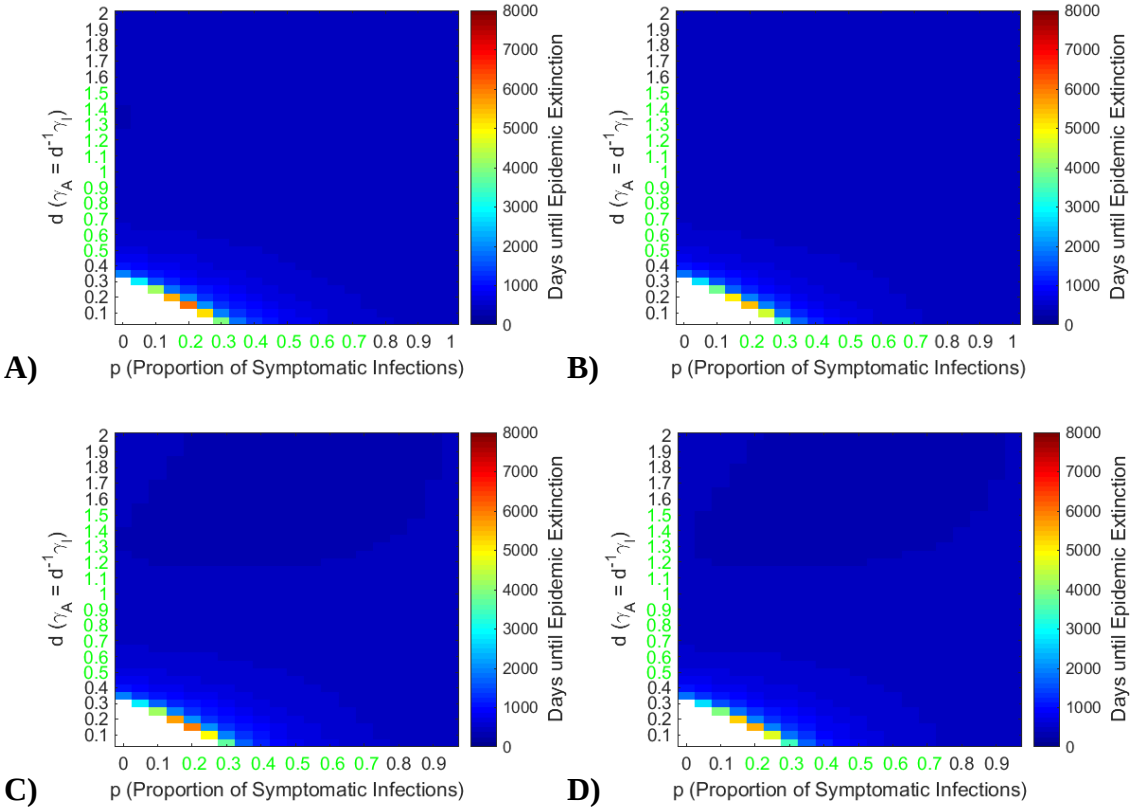


Figure 6.10: Days until epidemic extinction against different durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under low-level transmission (β_i=0.5 and b=0.3). Days until epidemic extinction is defined as the first occurrence of the asymptomatic and symptomatic classes being less than 1 after the peak total of symptomatic and asymptomatic infections. Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. Note the colour scale represents 0-8000 days for subfigures A-D. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

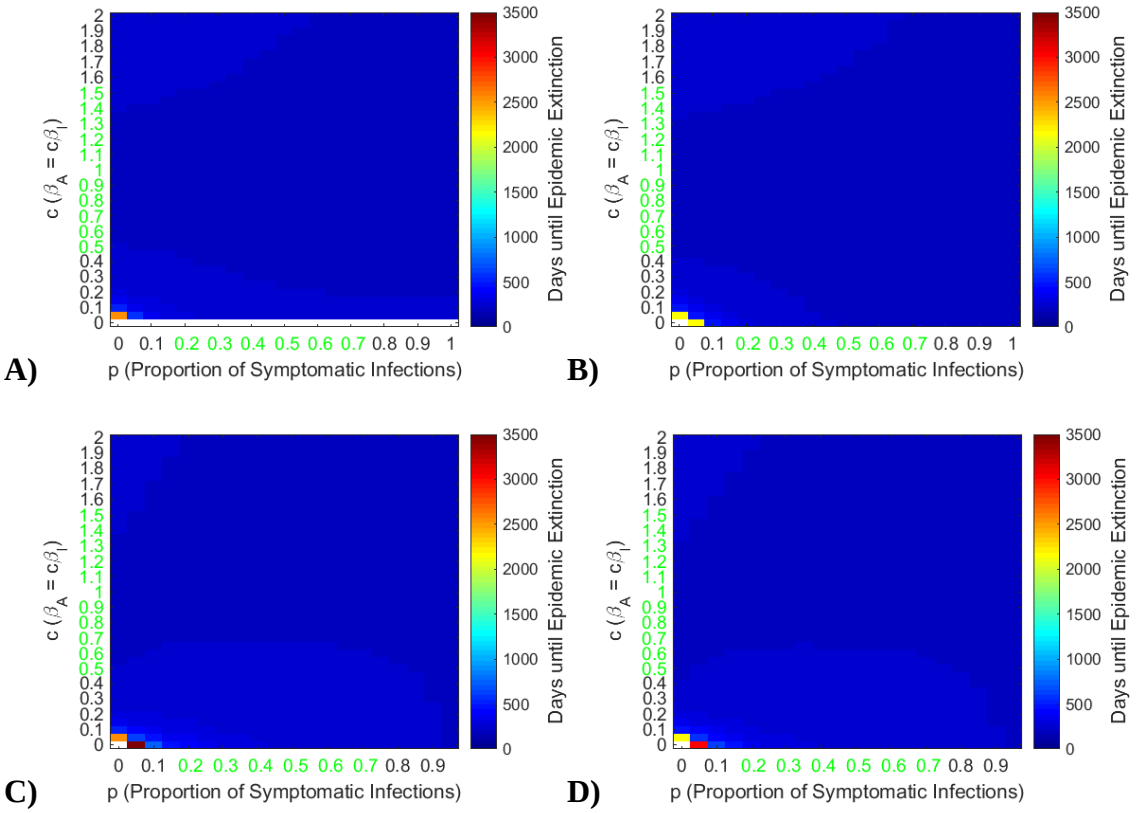


Figure 6.11: Days until epidemic extinction against different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under mid-level transmission (β_i=0.75 and b=0.65). Days until epidemic extinction is defined as the first occurrence of the asymptomatic and symptomatic classes being less than 1 after the peak total of symptomatic and asymptomatic infections. Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. Note the colour scale represents 0-3500 days for subfigures A-D. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

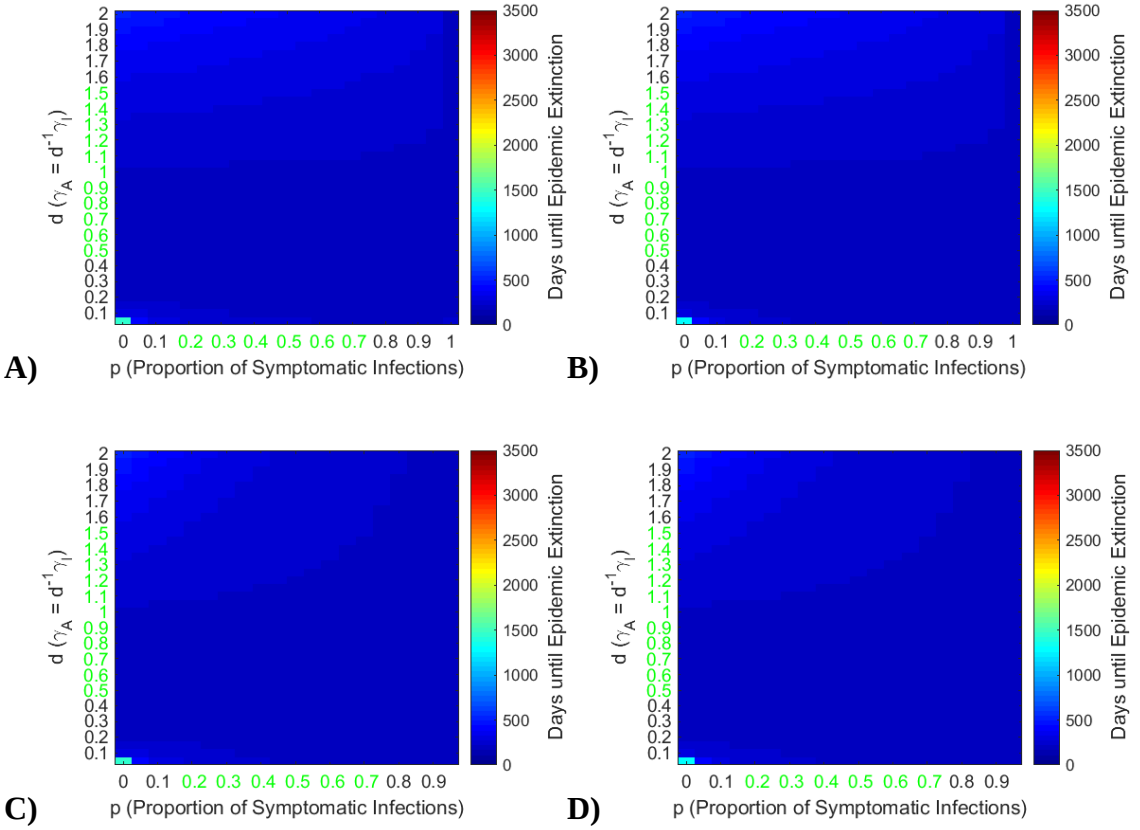


Figure 6.12: Days until epidemic extinction against different durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under mid-level transmission (β_i=0.75 and b=0.65). Days until epidemic extinction is defined as the first occurrence of the asymptomatic and symptomatic classes being less than 1 after the peak total of symptomatic and asymptomatic infections. Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. Note the colour scale represents 0-3500 days for subfigures A-D. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

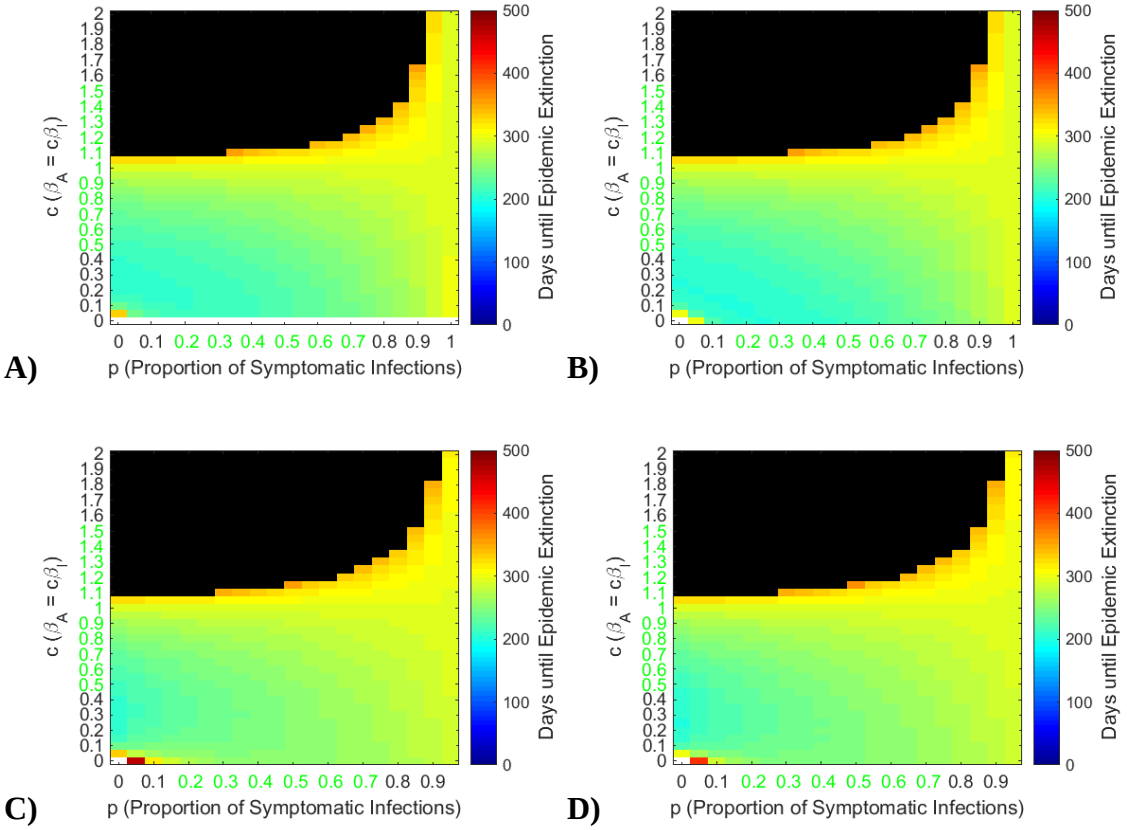


Figure 6.13: Days until epidemic extinction against different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under high-level transmission (β_i=1 and b=1). Days until epidemic extinction is defined as the first occurrence of the asymptomatic and symptomatic classes being less than 1 after the peak total of symptomatic and asymptomatic infections. Epidemics that had an R_0 of less than 1 or last less than 20 days are in white, epidemics where asymptomatic and symptomatic classes never reached less than 1 in 30 years are in black. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. Note the colour scale represents 0-500 days for subfigures A-D. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

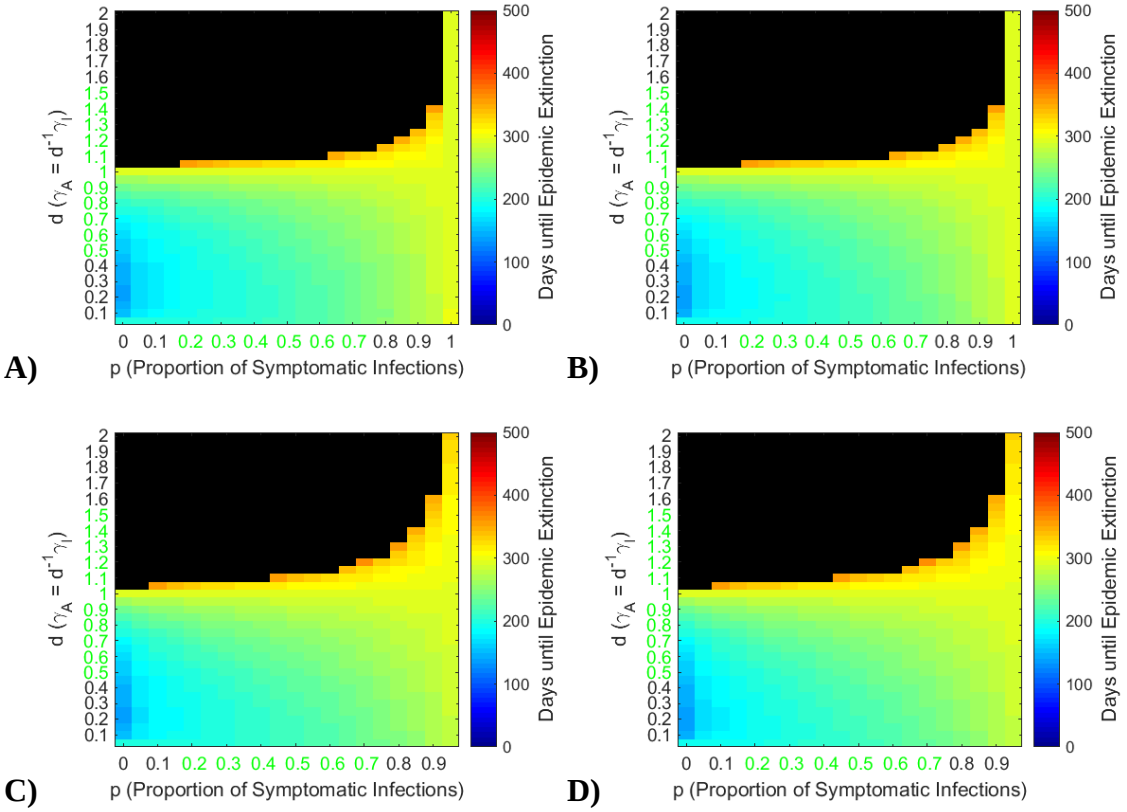


Figure 6.14: Days until epidemic extinction against different durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under high-level transmission (β_i=1 and b=1). Days until epidemic extinction is defined as the first occurrence of the asymptomatic and symptomatic classes being less than 1 after the peak total of symptomatic and asymptomatic infections. Epidemics that had an R_0 of less than 1 or last less than 20 days are in white, epidemics where asymptomatic and symptomatic classes never reached less than 1 in 30 years are in black. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. Note the colour scale represents 0-500 days for subfigures A-D. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

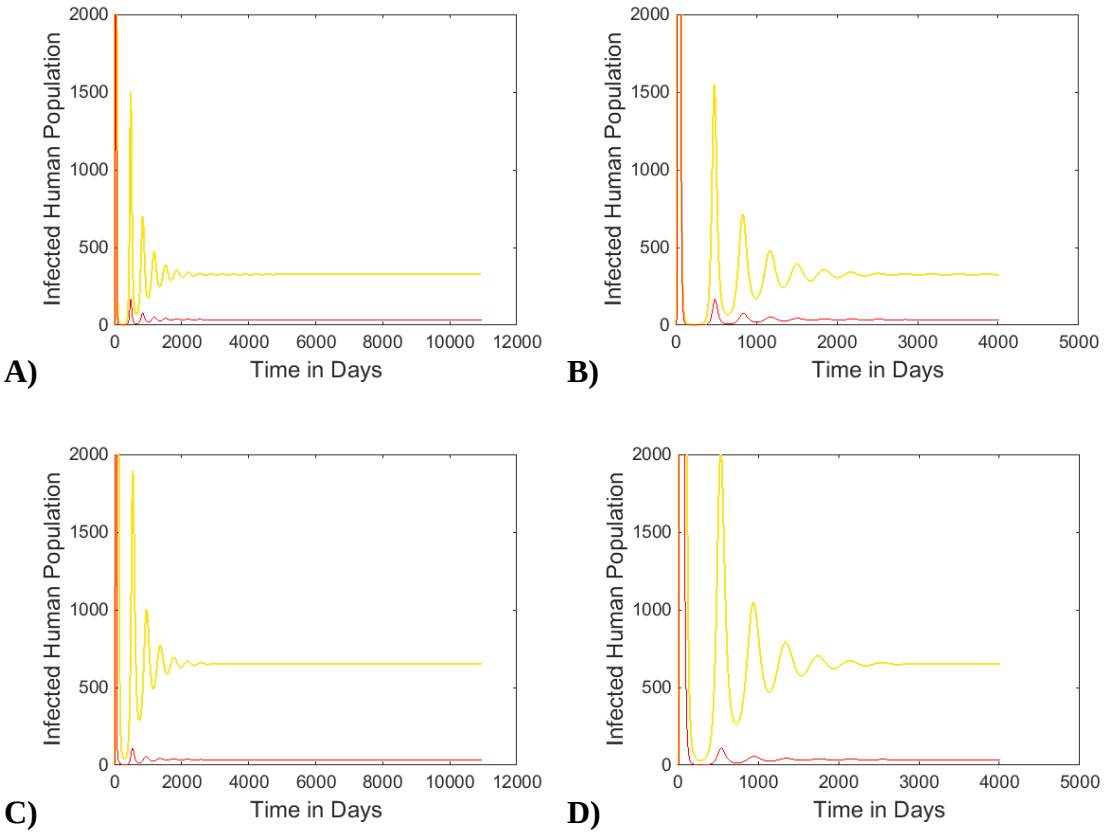


Figure 6.15: Infected human population in epidemics started by the arrival of an asymptomatic human in a high-level transmission setting (β_i=1, b=1). Symptomatically infected humans are in red and asymptomatically infected humans are in yellow. A) Model A when transmission from asymptomatic infections is twice that of symptomatic infections (c=2), the duration of asymptomatic and symptomatic infections is the same (d=1) and the proportion of infections that develop symptoms is 10% (p=0.1). B) Model B when transmission from asymptomatic infections is twice that of symptomatic infections (c=2), the duration of asymptomatic and symptomatic infections is the same (d=1) and the proportion of infections that develop symptoms is 10% (p=0.1). C) Model A when transmission from asymptomatic infections is same as symptomatic infections (c=1), the duration of asymptomatic infections twice that of symptomatic infections (d=2) and the proportion of infections that develop symptoms is 10% (p=0.1). D) Model B when transmission from asymptomatic infections is same as symptomatic infections (c=1), the duration of asymptomatic infections twice that of symptomatic infections (d=2) and the proportion of infections that develop symptoms is 10% (p=0.1).

6.3.4 Percentage of the population resistant at the end of an epidemic or when an epidemic becomes endemic

Both Model A and Model B produce an extremely similar percentage of population resistant to dengue by the end of the initial epidemic, for each of the transmission settings (b and β_i), proportion of infections leading to symptoms (p), level and duration of transmission in the asymptomatic class (c and d respectively). This is regardless if an outbreak is started by the arrival of a symptomatic or asymptomatic individual (see Figure 6.16-21). Across the different transmission settings most combinations of the proportion of infections leading to symptoms (p), level and duration of transmission in the asymptomatic class (c and d respectively) produce epidemics where above 90% of the population are immune to dengue.

In the lower transmission setting of b=0.3 and β_i=0.5, levels of transmission in the asymptomatic class (c) less than 0.85 combined with proportions of infections leading to symptoms (p) less than 0.85 lead to the percentages of population resistant to dengue dropping from 90% to 8% as these parameters decrease. This is also true for the duration of transmission in the asymptomatic class in proportion to the symptomatic class (d) (see Figure 6.16-7). Meaning that at the low-level transmission setting (b=0.3 and β_i=0.5) the more conservative samples of parameter space for the proportion of infections leading to symptoms (p), the level and duration of transmission in the asymptomatic class (c and d respectively) produce epidemics where above 80% of the population is resistant to dengue virus (see Figure 6.16-7).

In the medium transmission setting of b=0.65 and β_i=0.75, for a level of transmission in the asymptomatic class (c) less than 0.15 combined with a proportion of infections leading to symptoms of less than 0.15, the percentage of population resistant to dengue drops to 85% and further until 24% as values of these parameters decrease.

This is also true for the duration of transmission in the asymptomatic class in proportion to the symptomatic class (d) (see Figure 6.18-19). The pattern means that at the mid-level transmission setting (b=0.65 and β_i=0.75) the more conservative samples of parameter space for the proportion of infections leading to symptoms (p), the level and duration of transmission in the asymptomatic class (c and d respectively) produce epidemics where above 95% of the population is resistant to dengue virus (see Figure 6.18-19).

In the higher transmission setting of b=1 and β_i=1, provided an epidemic happened at all, above 90% of the population become resistant to dengue by the end of the initial epidemic, regardless of the proportion of infections that developed symptoms or the duration and level of transmission of the asymptomatic class (see Figure 6.20-21). At this high-level transmission setting (b=1 and β_i=1) the more conservative samples of parameter space for the proportion of infections leading to symptoms (p), the level and duration of transmission in the asymptomatic class (c and d respectively) produce epidemics where above 95% of the population is resistant to dengue virus (see Figure 6.20-21).

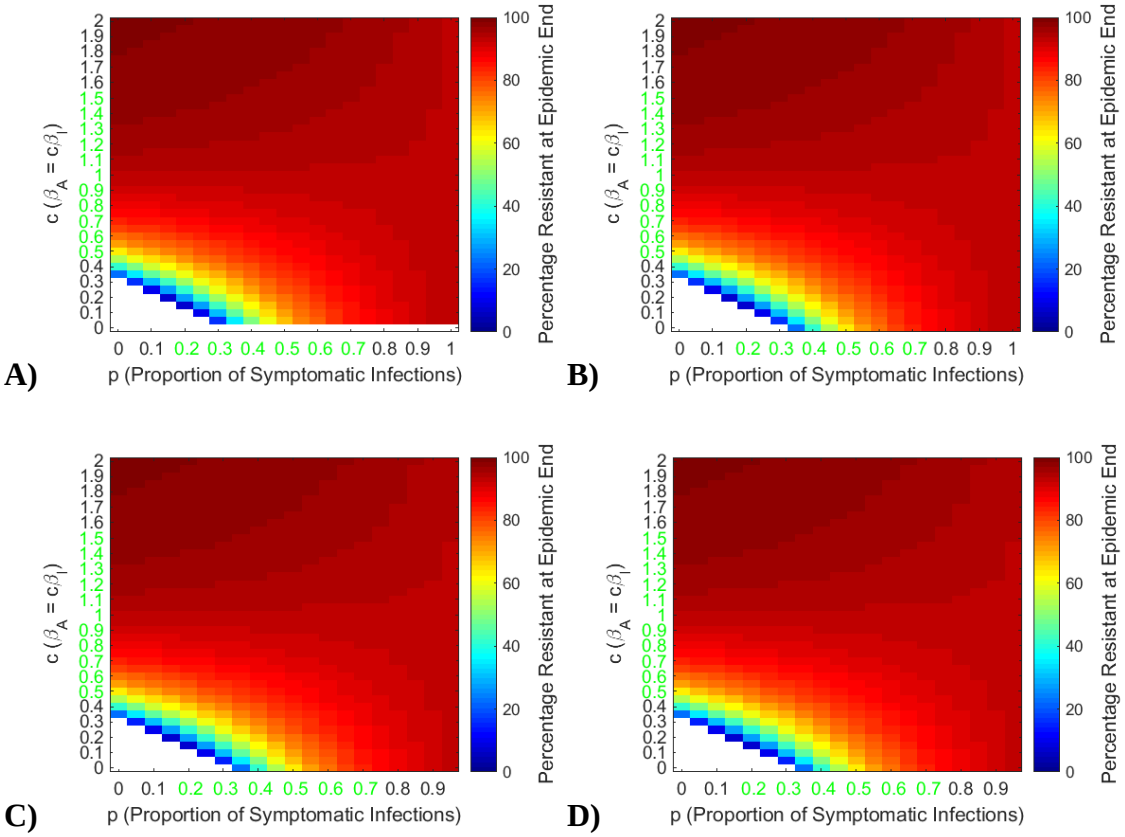


Figure 6.16: Percentage of the population resistant at the end of the epidemic, against different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under low-level transmission (β_i=0.5 and b=0.3). Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

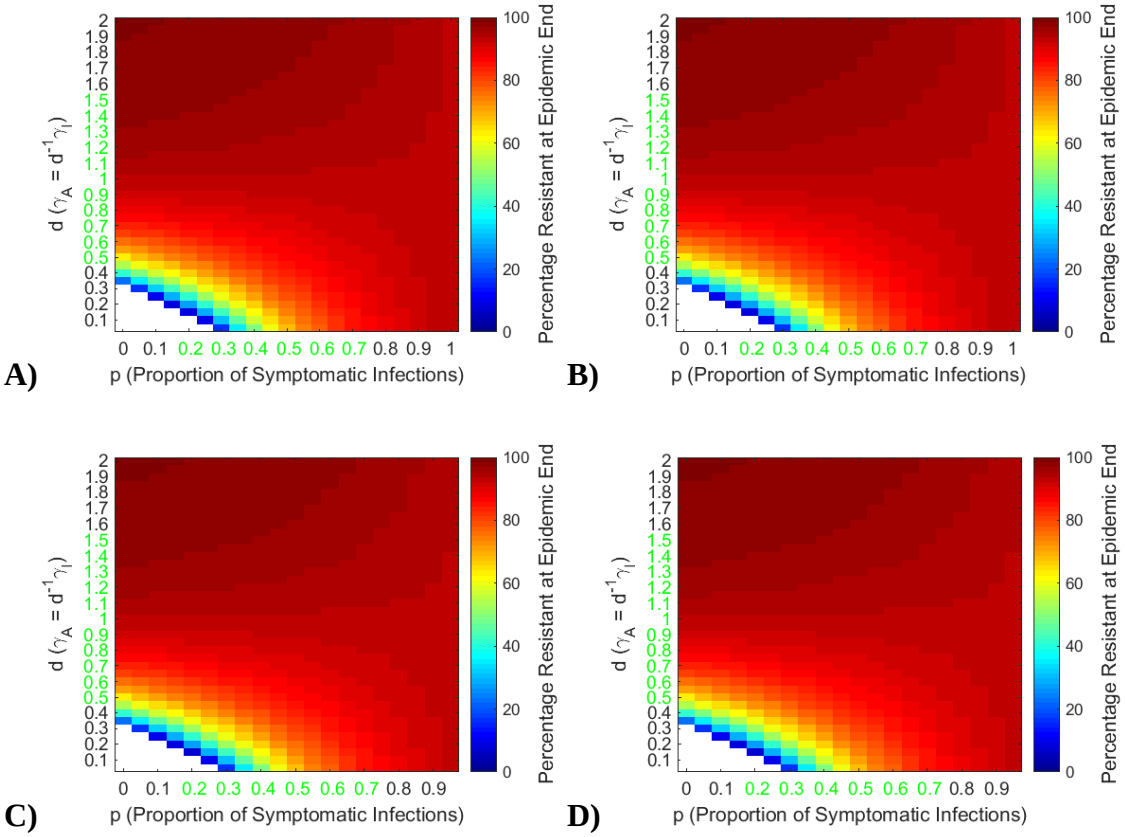


Figure 6.17: Percentage of the population resistant at the end of the epidemic, against different durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under low-level transmission (β_i=0.5 and b=0.3). Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

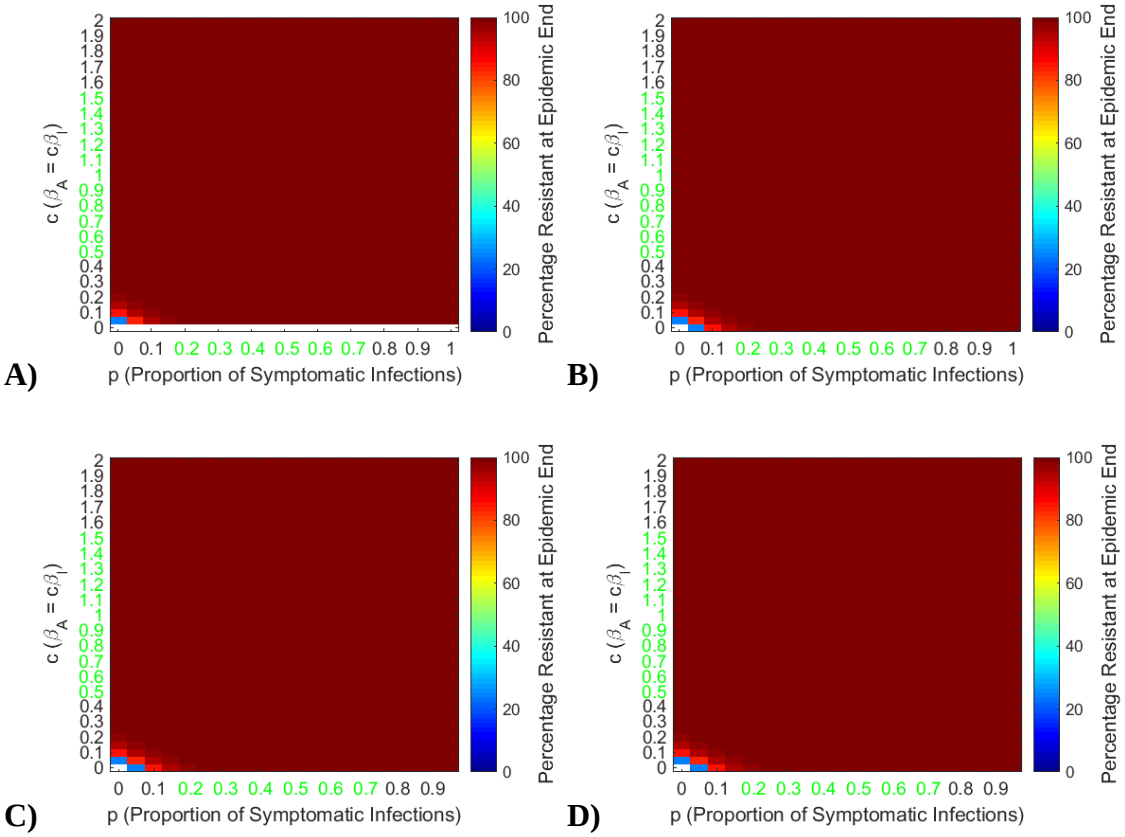


Figure 6.18: Percentage of the population resistant at the end of the epidemic, against different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under mid-level transmission (β_i=0.75 and b=0.65). Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

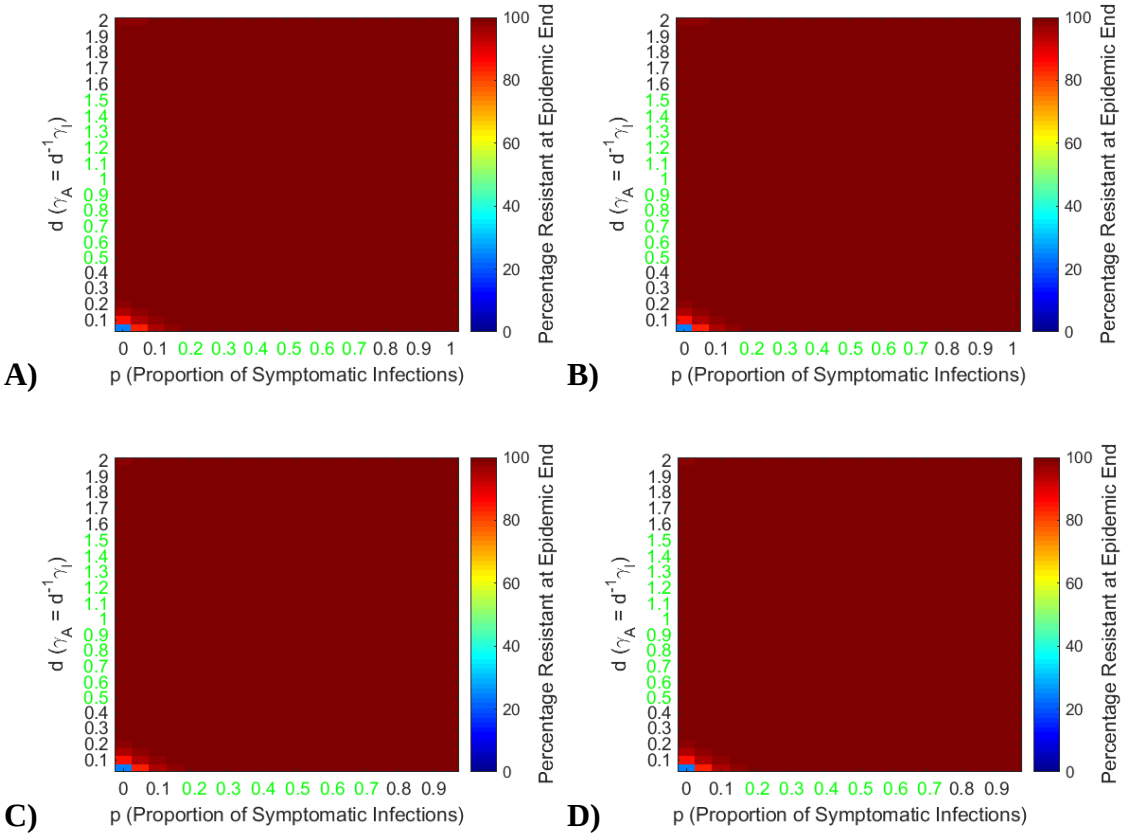


Figure 6.19: Percentage of the population resistant at the end of the epidemic, against durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under mid-level transmission (β_i=0.75 and b=0.65). Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

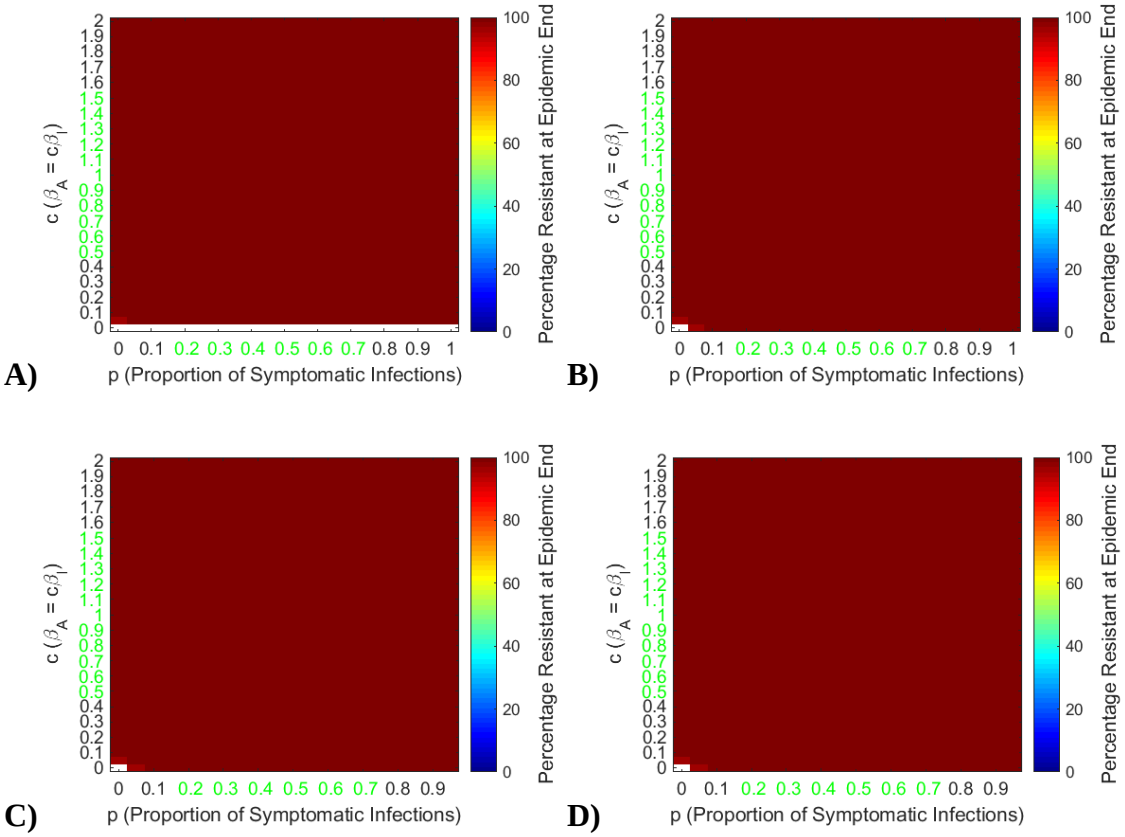


Figure 6.20: Percentage of the population resistant at the end of the epidemic, against different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under high-level transmission (β_i=1 and b=1). Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

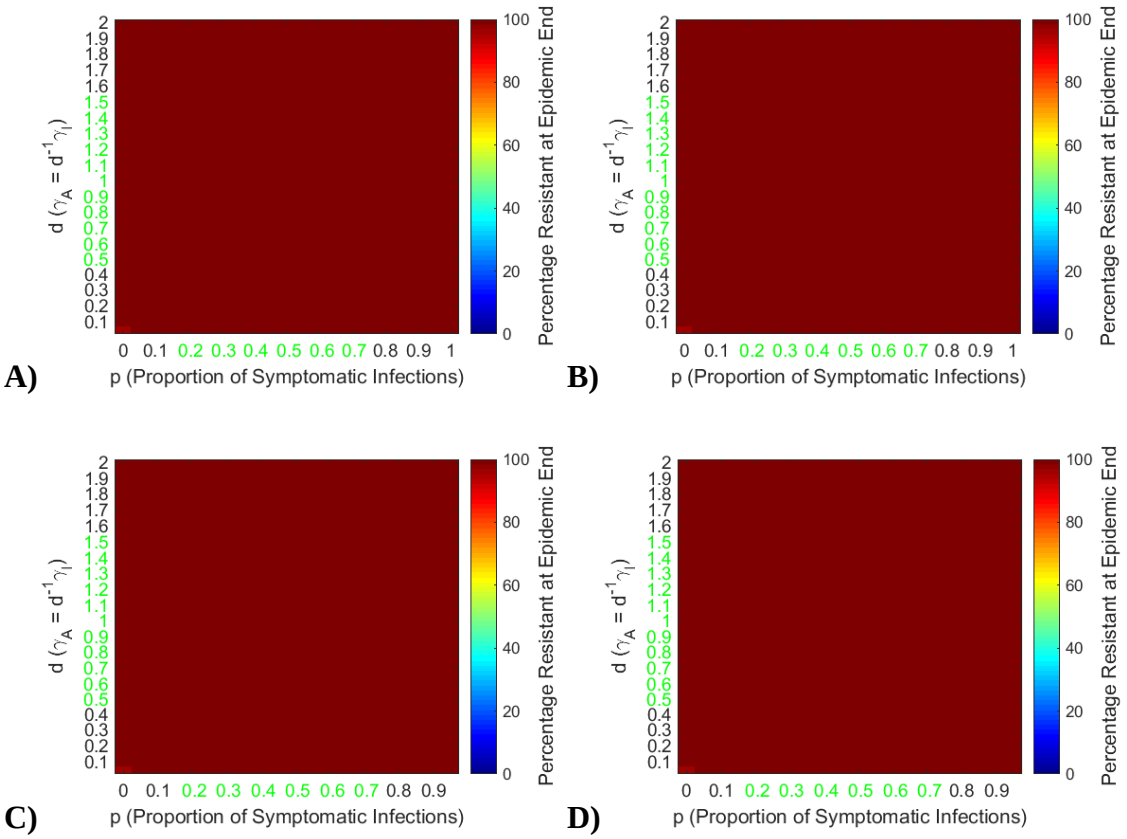


Figure 6.21: Percentage of the population resistant at the end of the epidemic, against durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under high-level transmission (β_i=1 and b=1). Epidemics that had an R_0 of less than 1 or last less than 20 days are in white. A) Model A with an epidemic started by the arrival of an asymptomatic individual. B) Model A with an epidemic started by the arrival of a symptomatic individual. C) Model B with an epidemic started by the arrival of an asymptomatic individual. D) Model B with an epidemic started by the arrival of a symptomatic individual. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

6.3.5 Biological unrealistic time lengths for progression from an asymptomatic infection to a symptomatic infection (δ^{-1}) within Model B

As discussed in Chapter 5 the results from Model B should be interpreted with a cautionary note in terms of what is biologically realistic. Recalling Equation 6.10 and

Equation 6.11, δ represents the rate at which an infected individual develops symptoms and moves from the asymptomatic class to the symptomatic class (Robinson & Stilianakis 2013). Therefore δ^{-1} represents the number of days an individual would spend asymptomatically infected if they do not die (μ) or their immune system fails to clear the infection (γ_A) (Robinson & Stilianakis 2013). Considering that $p=\delta/(\delta+\gamma_A+\mu)$ and $\gamma_A=1/d\varphi$, where φ is the number of days until immunity clears infection in a symptomatic infection and d represents how long immunity clears infection in an asymptomatic infection in proportion to a symptomatic infection, Equation 6.23 can be derived for the mosquito dependent version of Model B. Note Equation 6.23 is the same as Equation 5.11 from Chapter 5.

$$\delta^{-1} = \frac{1-p}{p((d\varphi)^{-1}+\mu)} \qquad \textbf{Equation 6.23}$$

As with the frequency dependent version of Model B (see Chapter 5), Equation 6.23 demonstrates that the number of days spent with an asymptomatic infection before progression to a symptomatic infection (δ^{-1}) can be affected by both the proportion of infections that are symptomatic (p) and the time until immunity clears all dengue virus in an asymptomatic infection in proportion to a symptomatic infection (d). As demonstrated by Figure 6.22 this means that certain combinations of these two proportions can lead to the period of time spent from becoming asymptomatic infected until progressing to a symptomatic infection (δ^{-1}), being biologically unfeasibly long, (depending if longer than a month, two weeks or a week is seen as being biologically unfeasible) (See Figure 6.22 B, C and D respectively). As observed in the frequency dependent version of Model B (see Chapter 5), this not only affects the biological realism of the results obtained from using Model B when altering the time until immunity clears all dengue virus in an asymptomatic infection (d) but the biological realism of the results when varying the transmission from asymptomatic infection (c),

along with the proportion of infections that lead to development of symptoms (p) (see Figure 6.22). Also as in Chapter 5, as the tolerance for the duration of progression from asymptomatic to symptomatic infection (δ^{-1}) decreases, more of the conservative samples of parameter space for the proportion of infections leading to symptoms (p), the duration of transmission in the asymptomatic class (d) and the level of transmission in the asymptomatic class (c) can also be seen as having an unfeasible duration of progression from asymptomatic to symptomatic infection (δ-1) (see Figure 6.22 B-D).

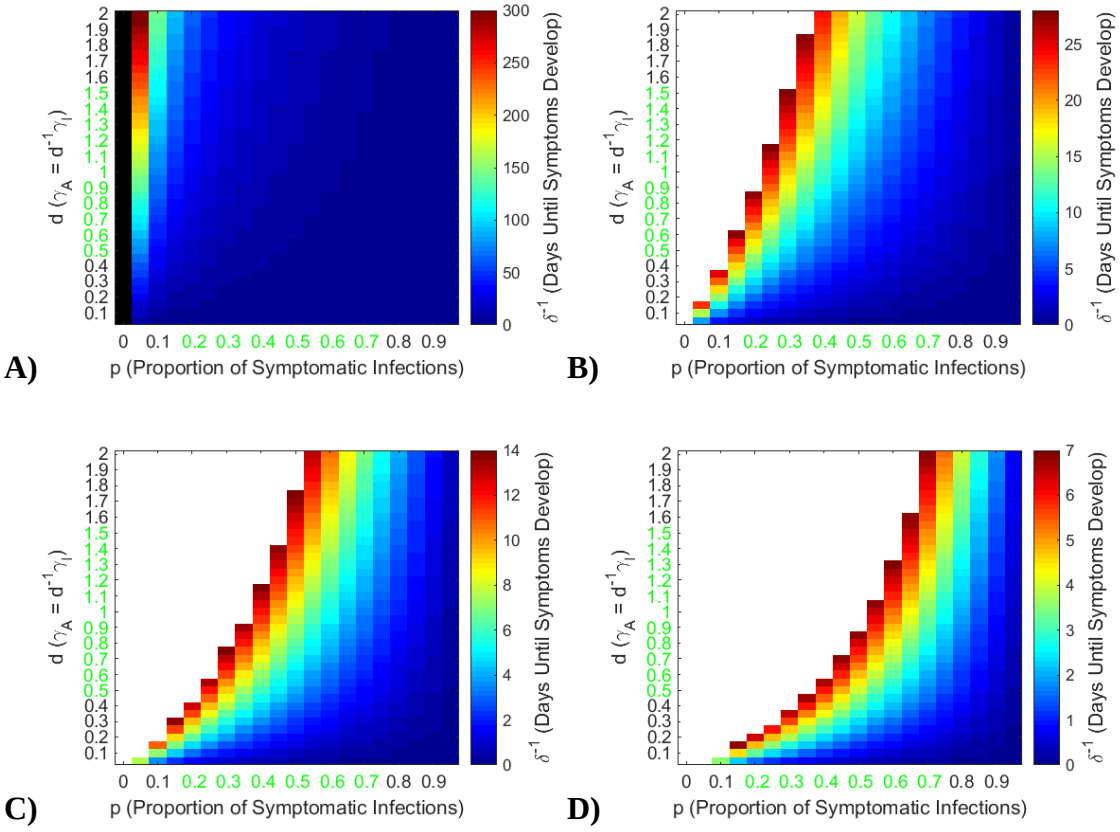


Figure 6.22: Effect of altering the duration of asymptomatic infection (d) and the proportion of infections that are symptomatic (p), on the duration of progression from asymptomatic to symptomatic infection (δ^{-1}) for Model B, values equal to infinity are in black. A) Without values above a certain number of days being coloured white. Note the colour scale represents 0-300 days. B) Values greater the 28 days are in white. Note the colour scale represents 0-28 days. C) Values greater the 14 days are in white. Note the colour scale represents 0-14 days. D) Values greater the 7 days are in white. Note the colour scale represents 0-7 days. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

6.4 Discussion

From comparing the results of this chapter with those of Chapter 5, it can be seen that for the most part moving from a frequency dependent transmission model to one that explicitly includes mosquitoes in transmission, changes few of the epidemiological patterns, caused by asymptomatic dengue infections. A striking

similarity is that whether modelled with frequency dependent transmission or mosquito dependent transmission, Model A and Model B produce extremely similar results across the different proportions of infections leading to symptoms (p), levels and durations of transmission in the asymptomatic class (c and d respectively) for all of the transmission setting barring the highest values of b and β_i (see Figure 6.6-14 and Figure 6.16-21). The mosquito dependent transmission version of Model B still has the limitation from certain combinations of the proportion of asymptomatic infections becoming symptomatic (p) and the period of time that immunity takes to clear an asymptomatic infection (d) thus producing biologically unrealistic lengths of time in the progression from an asymptomatic infection to a symptomatic infection (δ^{-1}) (see Figure 6.22). As in Chapter 5 this is not only true for large parts of the parameter space explored from altering the proportion of asymptomatic infections becoming symptomatic (p) and the period of time that immunity takes to clear an asymptomatic infection (d) but the parameter space explored from altering the proportion of asymptomatic infections becoming symptomatic (p) and the transmission from asymptomatic infections (c), as well (see Figure 6.22).

Moving beyond the technical similarities between the two types of transmission there are three remaining similarities that relate more widely to dengue virus epidemiology. The first of which is that the explicit inclusion of mosquitoes does not change the fact that at extremely low proportions of infections developing symptoms (p) combined with low levels or durations of transmission in the asymptomatic class (c and d, respectively) causes R_0 to drop below 1, meaning that an epidemic of dengue could not occur. In the lowest transmission setting this occurs for proportions of infections developing symptoms (p) that are less than 0.3 combined with levels or durations of transmission in the asymptomatic class (c and d, respectively) less than 0.3 (see Figure

6.6, Figure 6.9 and Figure 6.10). This therefore excludes these values of R_0 from the more conservative samples of parameter space concerning level and duration of transmission in the asymptomatic class (c and d, respectively) but not concerning the proportions of infections developing symptoms (p) (see Figure 6.6, Figure 6.9 and Figure 6.10). There is a difference in the mosquito dependent transmission versions of Model A and Model B, in that in the medium and high transmission setting this only occurs when no one develops symptoms (p=0) and asymptomatic infections do not transmit infection (c=0) (see Figure 6.7-8, Figure 6.11 and Figure 6.13). Therefore excluding these values of R_0 from the more conservative samples of parameter space for proportions of infections developing symptoms (p), the level and duration of transmission in the asymptomatic class (c and d, respectively) (see Figure 6.7-8, Figure 6.11 and Figure 6.13).

The second similarity is that in the low and medium transmission setting epidemics usually lasted only a year or two. However in very low proportions of infections developing symptoms (p) combined with very low levels or durations of transmission in the asymptomatic class (c and d, respectively) epidemics could extend to over 2 years, extending to over 20 years in the low transmission settings for mosquito dependent models. As in Chapter 5 this therefore excludes these longer lasting epidemics from the more conservative samples of parameter space concerning levels and durations of transmission in the asymptomatic class (c and d, respectively) but not concerning the proportions of infections developing symptoms (p) (see Figure 6.9-10). Furthermore, as with the frequency dependent model these longer lasting epidemics have an R_0 of just over 1 (see Chapter 5). This could mean that if these models were to be modelled stochastically instead of in a deterministic manner, the parameter settings that produce the longer lasting epidemics may produce dengue outbreaks that do not

infect more than a few individuals, who simply become immune or die before infecting a mosquito with dengue virus. These low numbers of infections would also produce very few individuals who are immune to dengue and therefore at risk of developing DHF, in any future epidemic caused by a differing dengue viral serotype.

The third similarity is that the frequency dependent models and mosquito transmission models produce similar proportions of the population left immune to the invading dengue serotype and therefore at risk of developing DHF in an epidemic caused by another invading serotype (see Chapter 5). As in Chapter 5 high proportions of the population are left immune from epidemics simulated using the more conservative samples of parameter space for the proportion of infections leading to symptoms (p), the level and duration of transmission in the asymptomatic class (c and d respectively). This therefore adds further validity to the point made in the previous chapter that after a dengue epidemic a larger proportion of the population may be at risk of DHF in a future epidemic caused by a different dengue serotype, than would be known to a health authority relying on reported cases from the first epidemic. As stated in Chapter 5 the DHF epidemic pattern in Cuba would suggest that this at risk group may persist for quite some time (Guzmán 2000; Vaughn et al. 2000; Guzmán et al. 2000).

There is a major key difference between the frequency dependent transmission models and the models that explicitly include mosquitoes. In the mosquito transmission models at the high transmission setting for levels or durations of transmission in the asymptomatic class that are higher than those of the symptomatic class ($c>1$ and $d>1$, respectively), combined with most of the proportions of infections that lead to symptoms (p) that are less than 1, dengue can become endemic (see Figure 6.13-15). This includes large regions of the more conservative samples of parameter space for the

proportion of infections leading to symptoms (p), the level and duration of transmission in the asymptomatic class (c and d respectively) (see Figure 6.13-14). Further demonstrating that asymptomatic dengue infections may provide a means by which dengue could become endemic in areas of high transmission.

A dengue serotype becoming endemic in this high transmission setting represents more of an ongoing risk in terms of proportion of the population at risk of developing DHF should another dengue viral serotype be introduced. Where the original dengue serotype has become endemic, Model A and Model B have reached an equilibrium point (see Figure 6.15). This means that the proportion of the population resistant to the original dengue serotype and therefore at risk of developing DHF should a different dengue serotype be introduced remains constant. This at risk group represents above 98% of the population (compare Figure 6.13-14 to Figure 6.20-21) and may be a larger proportion of the population then would be known to a health authority relying on reported cases of dengue illness.

Asymptomatic infections causing dengue virus persistence in a high transmission setting probably represents the case in areas where dengue is already endemic. In such areas there is a high abundance of *Ae. aegypti*, the primary vector of dengue viruses (Lambrechts et al. 2010; WHO 2015c). In recent years, another vector of dengue *Ae. albopictus* has become established in many European countries, 29 states of the USA and the Australasian continent (M. Service 2012b; WHO 2015c). *Ae. albopictus* is more of a catholic feeder than *Ae. aegypti*, feeding much more readily on other animals and less suited to the urban environment then *Ae. aegypti* (Lambrechts et al. 2010). Therefore the low transmission setting, with a low biting rate and vector transmission is probably indicative of the areas where *Ae. albopictus* has recently been introduced. This would suggest from the results of Model A and Model B in the low

transmission setting, that an epidemic may well occur in area where *Ae. albopictus* has been introduced but it would not become endemic.

Knowing whether a mosquito can become infected through feeding on an asymptomatic dengue infected human is key to understanding whether asymptomatic dengue infections play a role in dengue's epidemiology. Whilst no studies have researched this directly (possibly due to logistical feasibility), there have been a limited number of studies that have detected viremia in asymptomatic dengue infections (Beckett et al. 2005; Reyes et al. 2010; Duong et al. 2011; Chastel 2012; Carrington & Simmons 2014). Duong et al. (2011) has so far been the only study to quantify the level of viremia in asymptomatic dengue infections, finding no significant difference between the levels of viremia in asymptomatic dengue infections then symptomatic dengue infections. However Duong et al. (2011) had a small sample size and to date no one has tested whether or not mosquitoes feeding on viremic asymptomatically dengue infected humans can become infected with dengue virus (Carrington & Simmons 2014). Nguyet et al. (2013) found in reported symptomatic dengue infections that ambulatory dengue fever cases had a lower viremia, but still infectious viremia, than hospitalised cases. This work would suggest that asymptomatic infections may have a lower viremia leading them to being less infectious to blood feeding mosquitoes (Nguyet et al. 2013). However Nguyet et al. (2013) suggested that mildly fibril dengue cases could have a greater contact with mosquitoes due to greater movement, thereby leading to greater transmission, this could also be the case for asymptomatic dengue infections. In Model A and Model B such greater transmission through greater movement in the asymptomatic class was represented in values of c that were more than 1. It can be seen that such values of c could lead to dengue becoming endemic in the higher transmission setting (see Figure 6.13).

Related to whether or not a mosquito can become infected from feeding of an asymptomatically dengue infected human, is how long would such an asymptomatic dengue infection be infectious. Both Model A and Model B demonstrate that the duration of viremia of asymptomatic dengue infections (d) can have large effects on dengue epidemiology, even leading to its persistence. There have to my knowledge been no studies quantifying the duration of viremia of asymptomatic dengue infections. Furthermore, several reviews on the different epidemiological roles of asymptomatic dengue infection (Chastel 2012; Carrington & Simmons 2014; Grange et al. 2014) do not mention any such studies.

Model A and Model B demonstrate that large scale dengue epidemics can happen, with very few dengue infections being symptomatic. This presents a risk to the blood supply as asymptomatically dengue virus infected people may make a donation without realising they are viremic. The evidence from Model A and Model B, as well as the detection of dengue viruses in blood donations in several countries (Mohammed et al. 2008; Linnen et al. 2008) would suggest that the expense of screening of blood for dengue viruses needs to be considered (Teo et al. 2009).

6.5 Conclusion

As with Chapter 5's frequency dependent models, the addition of stochastic elements to Models A or Model B would shed greater light on the role of asymptomatic infections in terms of the likelihood of dengue epidemics taking hold in a population. The major finding of this chapter was that at high transmission setting higher levels and durations of transmission from asymptomatic dengue virus infections could lead to the dengue virus becoming endemic. Therefore further research into these two aspects of asymptomatic dengue virus infections needs to be made. Direct research via screening mosquitoes fed on asymptomatic dengue virus infected humans for dengue virus has so

far been completely lacking (possibly due to feasibility). There has been some related research on the viremia of asymptomatic dengue virus infected humans but this so far has been limited (Beckett et al. 2005; Reyes et al. 2010; Duong et al. 2011; Chastel 2012; Carrington & Simmons 2014). This research could be improved upon by studying how the level and duration viremia in asymptomatically dengue virus infected humans compares to symptomatically dengue virus infected humans. Asymptomatic dengue virus infected humans could be greater transmitters of dengue virus through their greater ambulatory nature leading to a high number of mosquito bites when compared to symptomatic dengue virus infected humans. Therefore, if possible, differences in the mosquito biting rates experienced by asymptomatic and symptomatic dengue virus infected humans needs to be assessed. As this may not be feasible, this could be inferred by comparing the mosquito biting rates experienced by more and less ambulatory non-dengue virus infected humans.

Chapter 7: Stochastic susceptible asymptomatic infectious recovered (SAIR) models of the transmission of dengue viruses

Abstract

In both previous chapters, epidemics that occurred over 2 years coincided with a Basic Reproductive Number (R_0) just over 1. R_0 is defined as the average number of infections caused by an average primary infectious case over the lifetime of that infection. Therefore, when R_0 is just over 1 in a stochastic setting there is a greater chance of an infected individual either becoming immune or dying before infecting someone else. For this reason in this chapter, one of the SAIR models is modelled stochastically in both the frequency transmission dependent and mosquito transmission dependent forms. Where combinations of parameters led to R_0 being only just over 1, epidemics often did not occur due to stochasticity. Furthermore, under parameter settings where dengue virus had become endemic in the previous chapter the inclusion of stochasticity meant that the dengue virus did not always become endemic. Higher durations of asymptomatic dengue virus infection showed this result to a lesser extent, suggesting that the duration of asymptomatic dengue virus infection may be more of a key determinant for a dengue virus epidemic becoming endemic than the transmission from such infections.

7.1 Introduction

There are an estimated 390 million dengue infections per year, of which 96 million manifest as clinical illness (Bhatt et al. 2013), making dengue viruses the most important of the arboviruses. Dengue viruses are of the Flavivirus genome, with a RNA positive genome and are separated into four immunological serotypes (DENV-1, DENV-2, DENV-3 and DENV-4), (Guzman et al. 2010; Rodenhuis-Zybert et al. 2010).

Infection with a single serotype can either lead to asymptomatic infection or dengue fever (DF) (Guzman et al. 2010; Andraud et al. 2012; Grange et al. 2014; WHO 2015c). Immunity to a single serotype is for life (Guzman et al. 2010; Rodenhuis-Zybert et al. 2010; Andraud et al. 2012). However immunity to other serotypes only lasts for an estimated 1-3 years (Reich et al. 2013). Secondary infection with another serotype, after this period of time can lead to a person developing Dengue Haemorrhagic Fever (DHF) (Guzman et al. 2010; Rodenhuis-Zybert et al. 2010; Andraud et al. 2012; WHO 2015c). A clinical complication can occur in DHF called dengue shock syndrome (DSS). Grouped together as severe dengue, DHF and DSS have been estimated to cause 500,000 cases of illness, mostly in children and have a mortality rate of 2.5% (WHO 2015c).

Aedes aegypti is the principal vector of dengue viruses, with *Aedes albopictus* having a lesser role as a vector of dengue (M. Service 2012b; Lambrechts et al. 2010). Other mosquitoes of the *Aedes* genus also occasionally acting as dengue vectors (M. Service 2012b). In recent years, *Ae. albopictus* has increased its range to include southern US states, 15 southern European countries, Australia and New Zealand (M. Service 2012b; Lambrechts et al. 2010; WHO 2015c), and therefore there has been a growing concern that there could be an increasing risk of outbreaks of DF in these areas (Lambrechts et al. 2010).

Because many studies suggest that asymptomatic dengue infections are common (Bhatt et al. 2013; Grange et al. 2014), Chapter 5 and Chapter 6 used two SAIR models (previously used by Robinson & Stilianakis (2013) to model influenza) to explore the role of asymptomatic infections in dengue's epidemiology. In Chapter 5 dengue viruses were transmitted through frequency dependent transmission whereas in the Chapter 6 mosquitoes were explicitly modelled in transmission. Both forms of transmission led to

four similar results. Firstly there were little differences between the various epidemiological outcomes in the two SAIR models. Secondly extremely low probabilities of developing symptoms combined with extremely low rates or durations of transmission in the asymptomatic class caused the basic reproductive number (R_0) to drop below 1 meaning dengue virus could not spread in a population. This was the case even in the high transmission setting. Thirdly whilst epidemics usually lasted only a year or two, under lower transmission settings when the probability of developing symptoms was low combined with low transmission or a short duration of transmission in the asymptomatic class, epidemics could extend over 2 years. (Even to over 15 and 20 years in the low transmission setting, for the frequency and mosquito transmission dependent models respectively.) The final similarity was that across most of the explored parameter space above 90% of the population was immune to dengue at the end of an epidemic and thereby at risk of DHF in subsequent epidemics caused by a different serotype of dengue virus. This population at risk of DHF did decrease with lower transmission setting, lower probability of being symptomatic, as well as lower transmission and duration of transmission in the asymptomatic class.

The striking difference between the two previous chapters was that dengue epidemics could become endemic in the mosquito dependent models provided the transmission setting was high, the probability of developing symptoms was less than 1 and there was either a high rate or duration of transmission in the symptomatic class. Dengue becoming endemic also meant that the population at risk of developing DHF should any other dengue virus serotype be introduced would remain constant through time.

In Chapter 5 and Chapter 6 it was pointed out that the epidemics that occurred over 2 years coincided with an R_0 just over 1. If the outbreak of dengue was modelled

stochastically instead of deterministically this could lead to an epidemic not spreading beyond a few individuals. This is as R_0 is defined as the average number of infections caused by an infectious individual in a completely susceptible population over the lifetime of that infection. As such when R_0 is just over 1 in a stochastic setting there is a greater chance of an infected individual either dying or more likely becoming immune before infecting someone else (Anderson & May 1991a).

In both of the previous chapters these longer lasting epidemics with an R_0 just over 1 are more common in the low transmission setting. It was suggested that this lower transmission setting may be indicative of the more climatically temperate countries where *Ae. albopictus* has recently been established. This is due to *Ae. albopictus* feeding much more readily on other animals and being less suited to the urban environment than *Ae. aegypti* (Lambrechts et al. 2010). Quam et al. (2015) give the yearly importation of approximately 572 symptomatic and 1747 asymptomatic dengue infections through Rome's airport. Should Quam et al. (2015) approximation be typical for the importation of dengue infections, then it may be the case that stochasticity plays a role in preventing imported dengue from causing regular epidemics from taking place in developed countries where *Ae. albopictus* has become established. As such in this chapter one of the SAIR models is modelled stochastically in both the frequency transmission dependent and mosquito transmission dependent forms, in order to ascertain whether stochasticity could affect any of the insights into dengue's epidemiology gained in Chapter 5 and Chapter 6.

7.2 Methods

7.2.1 Model of asymptomatic dengue virus infection.

7.2.1.1 Model A under frequency dependent transmission.

A frequency dependent version of the 2nd framework described in Robinson & Stilianakis (2013) (here to referred to as Model A) is expressed as a series of ordinary differential equations (see Equation 7.1-4) and the flow diagram in Figure 8.1. Model A was chosen as unlike Model B it does not make biologically unfeasible assumptions about the length of time an infected person can remain asymptomatically infected (see Chapter 5 and Chapter 6). Table 8.1 contains a list of all parameters and variables used in Model A under frequency dependent transmission.

$$\frac{\Delta S}{\Delta t} = \mu N - \frac{S(\beta_A A + \beta_I I)}{N} - \mu S \qquad \textbf{Equation 7.1}$$

$$\frac{\Delta A}{\Delta t} = (1-p)\frac{S(\beta_A A + \beta_I I)}{N} - \gamma_A A - \mu A \qquad \textbf{Equation 7.2}$$

$$\frac{\Delta I}{\Delta t} = p\frac{S(\beta_A A + \beta_I I)}{N} - \gamma_I I - \mu I \qquad \textbf{Equation 7.3}$$

$$\frac{\Delta R}{\Delta t} = \gamma_I I + \gamma_A A - \mu R \qquad \textbf{Equation 7.4}$$

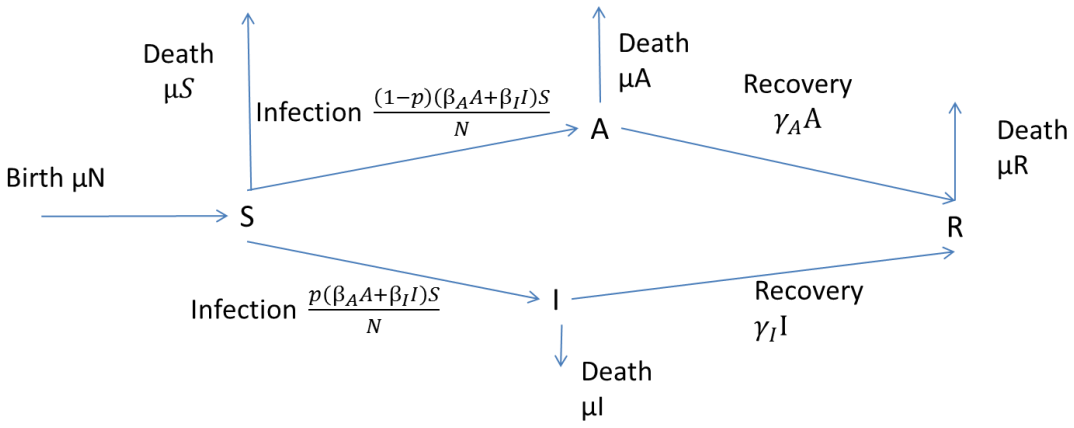


Figure 7.1: Flow diagram representing Model A.

Table 7.1: Variables at starting value and parameters used in Model A under frequency dependent transmission.

Symbol	Name	Value
μ	Birth and death rate (per day)	$1/(60*365)$
c	Infectivity of asymptomatic infections compared to symptomatic infections	0 to 2
β_I	Symptomatic transmission rate (per day)	$200/365$ to $400/365$
β_A	Asymptomatic transmission rate (per day)	$c*\beta_I$
φ	Days symptomatic	8
γ_I	Symptomatic recovery rate (per day)	φ^{-1}
d	Asymptomatic recovery rate compared to symptomatic recovery rate	0.05 to 2
γ_A	Asymptomatic recovery rate (per day)	$(d\varphi)^{-1}$
p	Probability of being symptomatic	0 to 1
S	Susceptible population	10^6-1
A	Asymptomatic population	0 to 1
I	Symptomatic population	0 to 1
R	Recovered population	0
N	Total population	10^6

The total human population (N) is divided into susceptible (S), asymptomatically infected (A), symptomatically infected (I) and recovered (R) classes. Every class of human experiences loss due to a constant death rate μ, however the human population remains constant as humans are born into the susceptible class at a birth rate of μN. Susceptible humans become infected at a frequency dependent transmission term that is the sum of transmission from asymptomatically and symptomatically infected humans, given by $S(\beta_A A+ \beta_I I)/N$. Upon infection humans either become asymptomatic at a rate of $(1-p)S(\beta_A A+ \beta_I I)/N$ or symptomatically infected at a rate of $pS(\beta_A A+ \beta_I I)/N$. Asymptomatically infected people gain complete lifelong immunity at a rate of γ_A and symptomatically infected people gain complete lifelong immunity at a rate of γ_I, in both cases moving to the recovered class (R).

The formula for Model A's basic reproduction number (R_0) under frequency dependent transmission is listed in Equation 7.5 (Robinson & Stilianakis 2013).

$$R_0 = (1-p)\left(\frac{\beta_A}{\gamma_A+\mu}\right) + p\frac{\beta_I}{\gamma_I+\mu} \quad \textbf{Equation 7.5}$$

7.2.2 Model A under mosquito dependent transmission.

The incubation period within the mosquito may cause a lag in dengue's transmission dynamics and so affect the persistence of dengue epidemics. Therefore a mosquito dependent transmission version of the 2nd framework described in Robinson & Stilianakis (2013) (here to referred to as Model A) is expressed as a series of ordinary differential equations (see Equation 7.6-13) and the flow diagram in Figure 7.2. Model A was chosen as unlike Model B it does not make biologically unfeasible assumptions about the length of time an infected person can remain asymptomatic (see Chapter 5 and Chapter 6). Table 7.2 contains a list of all the parameters and variables.

$$\frac{\Delta S_H}{\Delta t} = \mu_H N_H - \frac{S_H}{N_H}\beta_V I_V - \mu_H S_H \quad \textbf{Equation 7.6}$$

$$\frac{\Delta A_H}{\Delta t} = (1-p)\frac{S_H}{N_H}\beta_V I_V - \gamma_A A_H - \mu_H A_H \quad \textbf{Equation 7.7}$$

$$\frac{\Delta I_H}{\Delta t} = p\frac{S_H}{N_H}\beta_V I_V - \gamma_I I_H - \mu_H I_H \quad \textbf{Equation 7.8}$$

$$\frac{\Delta R_H}{\Delta t} = \gamma_I I_H + \gamma_A A_H - \mu_H I_H \quad \textbf{Equation 7.9}$$

$$\frac{\Delta S_E}{\Delta t} = \mu_V N_V - \omega S_E \quad \textbf{Equation 7.10}$$

$$\frac{\Delta S_V}{\Delta t} = \omega S_E - S_V\frac{\beta_A A_H + \beta_I I_H}{N_H} - \mu_V S_V \quad \textbf{Equation 7.11}$$

$$\frac{\Delta E_V}{\Delta t} = S_V\frac{\beta_A A_H + \beta_I I_H}{N_H} - \varepsilon E_V - \mu_V E_V \quad \textbf{Equation 7.12}$$

$$\frac{\Delta I_V}{\Delta t} = \varepsilon E_V - \mu_V I_V \quad \textbf{Equation 7.13}$$

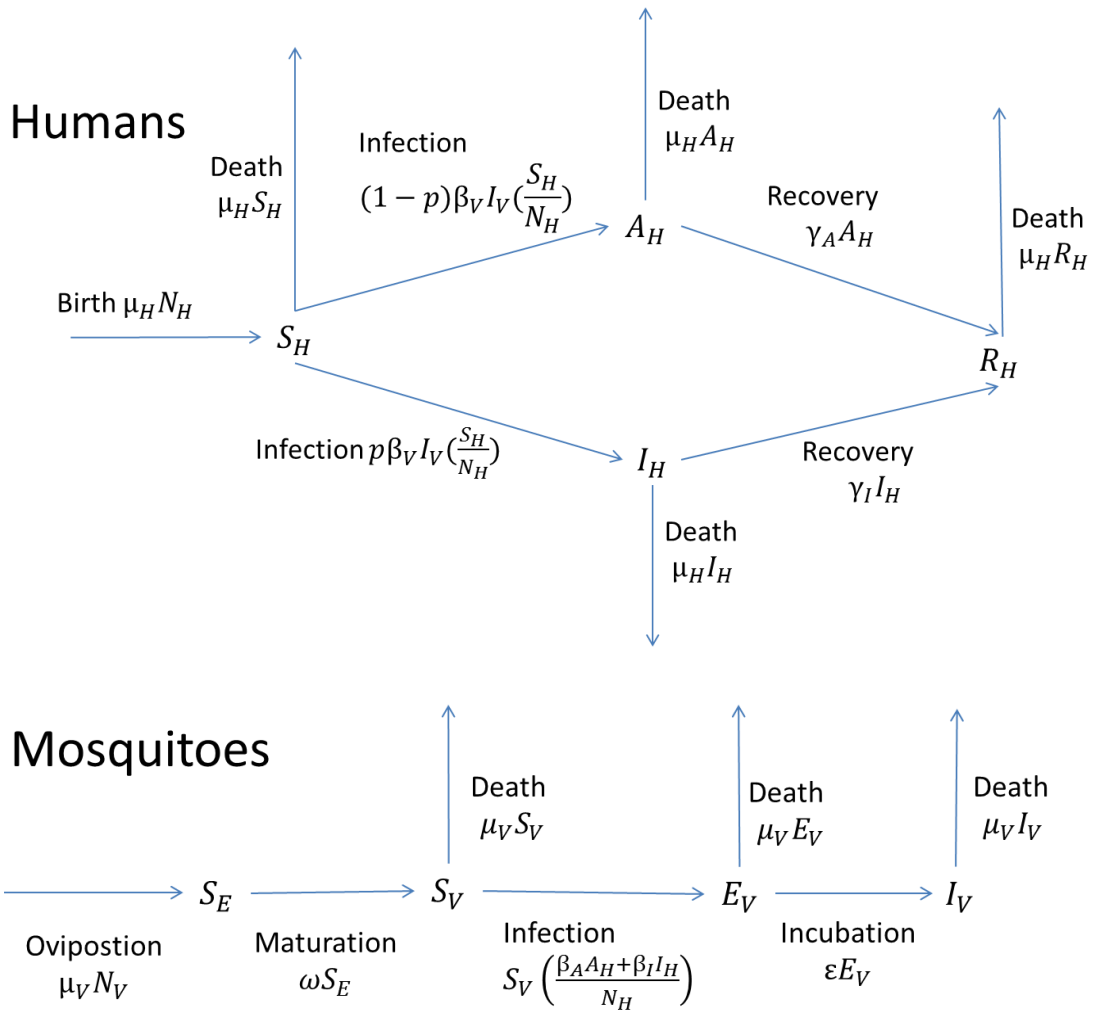


Figure 7.2 Flow diagrams representing Model A under mosquito dependent transmission.

Table 7.2: Variables at starting value and parameters used in Model A under mosquito dependent transmission.

Symbol	Name	Value
μ_H	Birth and death rate (per day)	$1/(60x365)$
c	Infectivity of asymptomatic infections compared to symptomatic infections	0 to 2
b	Biting rate (per day)	0.3-1
β_i	Probability of symptomatic Transmission to a Vector	0-1
β_I	Symptomatic transmission rate (per day)	$b\beta_i$
β_A	Asymptomatic transmission rate (per day)	$c\beta_I$
φ	Days symptomatic	8
γ_I	Symptomatic recovery rate (per day)	φ^{-1}
d	Asymptomatic recovery rate compared to symptomatic recovery rate	0.05 to 2
γ_A	Asymptomatic recovery rate (per day)	$(d\varphi)^{-1}$
p	Probability of being symptomatic	0 to 1
S_H	Susceptible population	10^6-1
A_H	Asymptomatic population	0 to 1
I_H	Symptomatic population	0 to 1
R_H	Recovered population	0
N_H	Total population	10^6
μ_V	Mosquito oviposition and death rate (per day)	$1/6$
ω	Mosquito maturation rate (per day)	$1/11$
β_v	Probability of vector transmission to a human	0.425
β_V	Mosquito transmission rate (per day)	$b\beta_v$
ε	Extrinsic incubation period	$1/10$
S_E	Pre-adult mosquitos	$(\omega/\mu_V)N_V$
S_V	Susceptible adult mosquito population	$9.5x10^6$
E_V	Latent adult mosquito population	0
I_V	Infectious adult mosquitoes population	0
N_V	Total adult mosquito population	$9.5x10^6$

The total human population (N_H) is divided into susceptible (S_H), asymptomatically infected (A_H), symptomatically infected (I_H) and recovered (R_H) classes. The total mosquito population (N_V) is divided into immature mosquito (S_E), susceptible mosquito (S_V), incubating mosquito (E_V) and infected mosquito (I_V) classes.

Every class of human experiences loss due to a death rate μ_H, however the human population remains constant as humans are born into the susceptible class at a birth rate of $\mu_H N_H$. Susceptible humans become infected at a mosquito dependent transmission term, given by $S_H/N_H(\beta_V I_V)$. Upon infection humans either become asymptomatic at a rate of $(1-p)S_H/N_H(\beta_V I_V)$ or symptomatically infected at a rate of $pS_H/N_H(\beta_V I_V)$. Asymptomatically infected people gain complete lifelong immunity at a rate of γ_A and symptomatically infected people gain complete lifelong immunity at a rate of γ_I, both of which then move to the recovered class. Every class of mosquito experiences loss due to death rate μ_V, however the mosquito population remains constant as mosquitoes are oviposited into the immature mosquito class at a of $\mu_V N_V$. Mosquitoes mature into adults at a rate ω and become infected at a rate of $S_V(\beta_A A_H + \beta_I I_H)/N_H$ moving to the incubating class, after a period of incubation ε mosquitoes move to the infectious class.

The symptomatic transmission term β_I is made up of two component terms, the mosquito biting rate b and probability of infection from biting a symptomatic human β_i ($\beta_I = b\beta_i$). Likewise the mosquito transmission term β_V is made up of two component terms, the mosquito biting rate b and probability of infection from an infectious mosquito β_v ($\beta_V = b\beta_v$).

Formula for the basic reproductive number R_0 is listed is Equation 7.14-16 and is sourced from Chapter 6.

$$\varphi = t + \sigma$$ **Equation 7.14a**

$$\varphi = \begin{vmatrix} A_H \\ I_H \\ E_V \\ I_V \end{vmatrix},\ t = \begin{vmatrix} (1-p)\beta_V I_V \\ p\beta_V I_V \\ N_v\left(\frac{\beta_A A_H + \beta_I I_H}{N_{Hh}}\right) \\ 0 \end{vmatrix},\ \sigma = \begin{vmatrix} -\gamma_A A_H - \mu_H A_H \\ \gamma_I I_H - \mu_H I_H \\ -\varepsilon E_V - \mu_V E_V \\ \varepsilon E_V - \mu_V I_V \end{vmatrix}$$ **Equation 7.14b**

$$K_L = T *\text{-}\Sigma^{-1}$$ **Equation 7.15a**

$$T = \begin{vmatrix} 0 & 0 & 0 & (1-p)\beta_V \\ 0 & 0 & 0 & p\beta_V \\ \frac{\beta_A N_V}{N_H} & \frac{\beta_I N_V}{N_H} & 0 & 0 \\ 0 & 0 & 0 & 0 \end{vmatrix}$$ **Equation 7.15b**

$$\Sigma = \begin{vmatrix} -\gamma_A - \mu_H & 0 & 0 & 0 \\ 0 & -\gamma_I - \mu_H & 0 & 0 \\ 0 & 0 & -\varepsilon - \mu_V & 0 \\ 0 & 0 & \varepsilon & -\mu_V \end{vmatrix}$$ **Equation 7.15b continued**

$$K_L = \begin{vmatrix} 0 & 0 & -\frac{(p-1)\varepsilon\beta_V}{\mu_v(\varepsilon+\mu_V)} & -\frac{(p-1)\beta_V}{\mu_V} \\ 0 & 0 & \frac{p\varepsilon\beta_V}{\mu_v(\varepsilon+\mu_V)} & \frac{p\beta_V}{\mu_V} \\ \frac{\beta_A N_V}{N_H(\gamma_A+\mu_H)} & \frac{\beta_I N_V}{N_H(\gamma_I+\mu_H)} & 0 & 0 \\ 0 & 0 & 0 & 0 \end{vmatrix}$$ **Equation 7.15b continued**

$$Largest\ eigen\ value\ (\lambda)\ of\ K_L = R_0$$ **Equation 7.16a**

$$R_0 = \sqrt{\frac{N_V}{N_H} \times \frac{\varepsilon\beta_V}{\mu_v(\varepsilon+\mu_V)} \times \frac{(1-p)\beta_A(\gamma_I+\mu_H) + p\beta_I(\gamma_A+\mu_H)}{(\gamma_I+\mu_H)(\gamma_A+\mu_H)}}$$ **Equation 7.16b**

7.2.3 Analyses Model A under frequency and mosquito dependent transmission in a stochastic framework.

In order to explore the effects of differing proportions of infection leading to symptoms, as well as the levels and durations of transmission in the asymptomatic class on epidemiology of dengue virus, in a stochastic based setting; Model A under frequency dependent transmission and mosquito dependent transmission was coded using τ-leap methodology within Matlab (Keeling & Rohani 2008b). Table 7.3 contains a list of the various rates of change used in the τ-leap methodology in the frequency dependent transmission version of Model A and Table 7.4A-B contains a list of the various rates of change used in the τ-leap methodology in the mosquito dependent transmission version of Model A. Dengue parameters were sourced as the mid-range values from the parameters listed in Andraud et al. (2012) literature review of dengue virus transmission models (unless otherwise stated), so as to make the model comparable with other models of dengue virus transmission (see Table 7.1 and Table 7.2). Mosquito oviposition and mortality rate (μ_V) where the average mortality rates of *Ae. aegypti* and *Ae. albopictus*, as sourced from a meta-analyses of marked release recapture studies (Brady et al. 2013), mosquito maturation rate (ω) was sourced from (M. Service 2012b) (see Table 7.2).

Table 7.3 Tau leap rates of change in classes for Model A under frequency dependent transmission

Rate of Change	Change in Susceptible Class (S)	Change in Asymptomatic Class (A)	Change in Symptomatic Class (I)	Change in Resistant Class (R)
μN	+1	0	0	0
$\frac{pS(\beta_A A + \beta_I I)}{N}$	-1	+1	0	0
$\frac{(1-p)S(\beta_A A + \beta_I I)}{N}$	-1	0	+1	0
$\gamma_A A$	0	-1	0	+1
$\gamma_I I$	0	0	-1	+1
μS	-1	0	0	0
μA	0	-1	0	0
μI	0	0	-1	0
μR	0	0	0	-1

Table 7.4A: Tau leap rates of change in human classes for Model A

Rate of Change	Change in Susceptible Class (S_h)	Change in Asymptomatic Class (A_h)	Change in Symptomatic Class (I_h)	Change in Resistant Class (R_h)
$\mu_H N_H$	+1	0	0	0
$\frac{p\beta_V I_V S_H}{N_H}$	-1	+1	0	0
$\frac{(p-1)\beta_V I_V S_H}{N_H}$	-1	0	+1	0
$\gamma_A A_H$	0	-1	0	+1
$\gamma_I I_H$	0	0	-1	+1
$\mu_H S_H$	-1	0	0	0
$\mu_H A_H$	0	-1	0	0
$\mu_H I_H$	0	0	-1	0
$\mu_H R_H$	0	0	0	-1

Table 7.4B Tau leap rates of change in mosquito classes for Model A

Rate of Change	Change in Immature Class (S_e)	Change in Susceptible Class (S_v)	Change in Latent Class (E_v)	Change in Infected Class (I_v)
$\mu_V N_V$	+1	0	0	0
ωS_E	-1	+1	0	0
$S_V \frac{\beta_A A_H + \beta_I I_H}{N_H}$	0	-1	+1	0
εE_V	0	0	-1	+1
$\mu_V S_V$	0	-1	0	0
$\mu_V E_V$	0	0	-1	0
$\mu_V I_V$	0	0	0	-1

Asymptomatic individuals could be transmitting dengue at a lower rate due to a decreased viremia. Likewise asymptomatic individuals could be transmitting dengue at a higher rate, as they may be more likely to pass through areas of high mosquito abundance, due to not exhibiting the decreased movement, through illness, seen in symptomatically infected humans. For these reasons parameter c of both Model A, the coefficient relating β_A to β_I (β_A=c β_I) was varied from 0-2. For each of these settings of c the proportion of infections developing symptoms (p) was varied from 0-1. Parameter d, the coefficient relating γ_A and γ_I (γ_A=1/dφ, γ_I=1/φ), was kept at 1.

As information on the immune recovery rate for asymptomatically dengue infected humans was unavailable. Parameter d, the coefficient relating γ_A and γ_I (γ_A=1/dφ, γ_I=1/φ), was varied from 0.05-2, as at d=0 γ_A would equal infinity (γ_A=1/dφ, γ_I=1/φ). For each of these settings of d parameter p was varied from 0-1. Parameter c the coefficient relating β_A to β_I (β_A=cβ_I), was kept at 1.

The range in parameter space for the probability of a dengue virus infection leading symptoms (p) could be considered a large sample. Therefore it was decided to highlight a conservative sample of parameter space for this parameter through labelling certain axis values regarding this parameter in green on figures that display model outputs with respect to changes in this parameter. The lower limit to this conservative parameter space p=0.2 is based on the rounded down mean possibility of symptomatic dengue virus infection from cohort studies in Grange et al. (2014). The upper limit to this conservative parameter space of p=0.7 is based on the rounded up mean possibility of symptomatic dengue virus infection from index cluster studies in Grange et al. (2014). The non-rounded mean symptomatic dengue virus infection rate (p) in cohort studies was 24% whereas in index cluster studies it was 63% (Grange et al. 2014). Cohort studies quantify the ratio of asymptomatic to symptomatic dengue infections by

following a cohort of people and use case reporting, absenteeism and/or symptom questionnaires, combined with blood screening for dengue antibodies at regular intervals (Endy 2002a; Endy 2002b; Arguello et al. 2015). Index cluster studies sample people surrounding an index case of dengue illness. Sampled individuals' symptoms are quantified through symptom questionnaires or clinical diagnosis and their blood is screened for dengue antibodies (Singh et al. 2000; Beckett et al. 2005; Reyes et al. 2010; Wang et al. 2015).

Likewise, the range in parameter space for the level and duration of transmission from asymptomatic dengue virus infections (c and d, respectively) could be considered large samples. Therefore it was decided to highlight more conservative samples of parameter space for these parameters through labelling certain axis values regarding these parameters in green for figures that display model outputs with respect to changes in these parameters. The lower limit of these conservative parameter spaces is 0.5 and the upper limit is 1.5. Note that at the time of submission of this thesis there was no data regarding the level or duration of transmission from asymptomatic dengue virus infected humans available, which could be used to base a more conservative region of parameter space for both of these parameters.

For two reasons, for both the variations in the asymptomatic class transmission rate β_A and recovery rate γ_A, 100 trials of Model A under frequency dependent transmission and mosquito dependent transmission were run with 999,999 susceptible humans and the arrival of an asymptomatically infected human. Firstly, as the previous two chapters established there was little difference between epidemics started by the arrival of an asymptomatically infected human and those started by a symptomatically infected human. Secondly, as due to their more ambulatory nature an asymptomatically infected human is more likely to travel to a new area and start an epidemic than a

symptomatically infected human. In order to test whether there was an effect of transmission setting, the 100 trials for each parameter setting were conducted at a low-transmission setting, a mid-level transmission setting and a high-level transmission setting (this is the range of transmission setting cited in the review by Andraud et al. (2012)) (see Table 7.1-2 and Table 7.5).

Table 7.5: Transmission settings at which trials where run.

Version of Model B	Low Transmission	Medium Transmission	High Transmission
Frequency Dependent Transmission	$\beta_I=200/365$	$\beta_I=300/365$	$\beta_I=400/365$
Mosquito Dependent Transmission	$\beta_i=0.5$ and $b=0.3$	$\beta_i=0.75$ and $b=0.65$.	$\beta_i=1$ and $b=1$.

7.3 Results

7.3.1 Trials were epidemics of dengue virus did not occur.

A number of trials produce epidemics that failed to spread through the population. For this reason a cut off was set whereby trials where dengue infections did not manage to infect more than 100 humans, as measured by the maximum total of humans in the asymptomatic, symptomatic and resistance classes, were excluded in calculating the mean extinction time of an epidemic. Subfigure C of Figure 7.3-14 show the number of trials excluded by this cut-off and subfigure D shows the mean dengue extinction time of these excluded trials (which never exceeds 40 days). The parameter settings where a higher proportion of dengue infection trials fail to spread through the population follow three trends across all three transmission settings. The first trend is for the lowest levels of transmission in the asymptomatic class (c) combined with the lowest proportion of infections developing symptoms (p) (see Figure 7.3c, Figure 7.5c,

Figure 7.7c, Figure 7.9c, Figure 7.11c and Figure 7.13c). This first non-epidemic trend is either at the low end or excluded from the more conservative samples of parameter space for the proportion of infections developing symptoms (p) and level of transmission from the asymptomatic class (c) (see Figure 7.3c, Figure 7.5c, Figure 7.7c, Figure 7.9c, Figure 7.11c and Figure 7.13c). The second trend is for the lowest durations of transmission in the asymptomatic class (d) combined with the lowest proportion of infections developing symptoms (p) (see Figure 7.4c, Figure 7.6c, Figure 7.8c, Figure 7.10c, Figure 7.12c and Figure 7.14c). This second non-epidemic trend is either at the low end or excluded from the more conservative samples of parameter space for the proportion of infections developing symptoms (p) and duration of transmission from the asymptomatic class (d) (see Figure 7.4c, Figure 7.6c, Figure 7.8c, Figure 7.10c, Figure 7.12c and Figure 7.14c). Of note is that both of these two trends also occur when R_0 is slightly over 1 or below 1 (see Subfigure A of Figure 7.3-14). The third trend occurs when there is no transmission from the asymptomatic class (c=0), at this parameter setting no epidemic occurs in all 100 trials. This is logical since the arrival of an asymptomatically infected human will not cause an epidemic of dengue fever if such an infection does not transmit dengue virus to another host (see Figure 7.3c, Figure 7.5c, Figure 7.7c, Figure 7.9c, Figure 7.11c and Figure 7.13c). This third non-epidemic trend is excluded from the more conservative samples of parameter space for the level of transmission from the asymptomatic class (c) (see Figure 7.3c, Figure 7.5c, Figure 7.7c, Figure 7.9c, Figure 7.11c and Figure 7.13c).

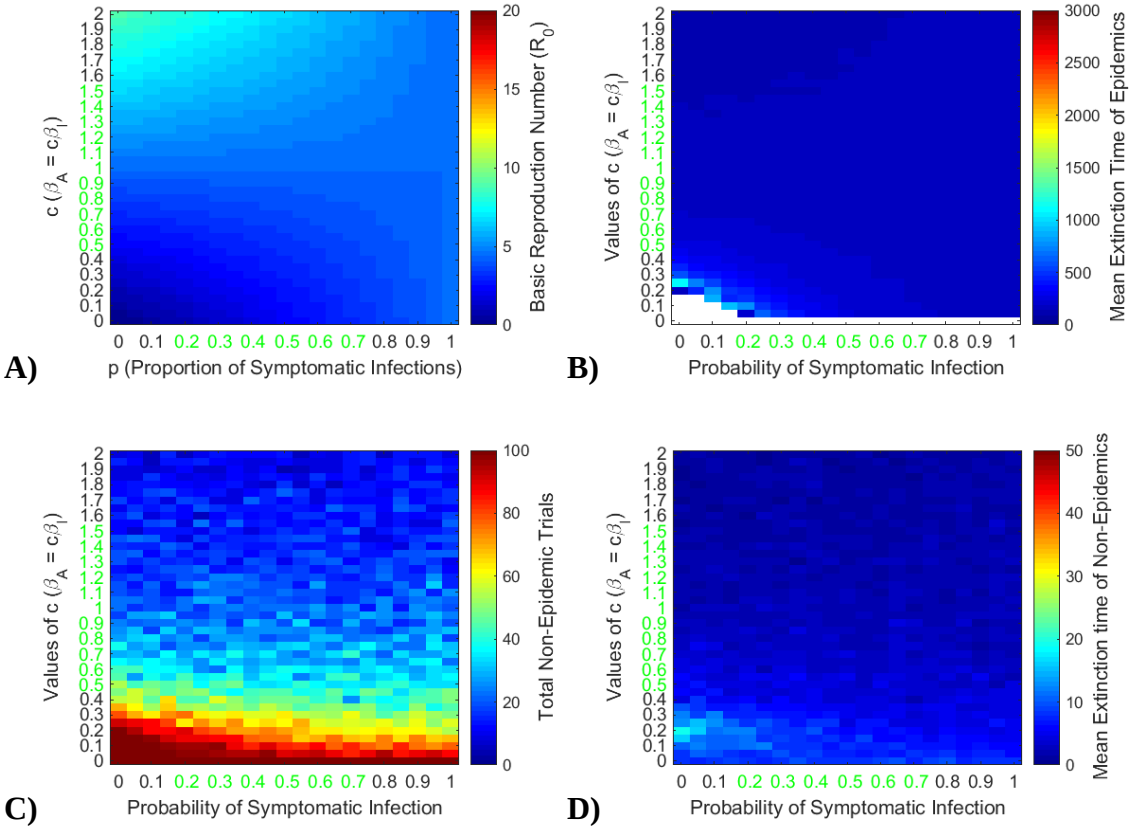


Figure 7.3: The effects of different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under low-level transmission (β_I=200/365), on the frequency dependent transmission version of Model A. A) Basic reproductive number (R_0). B) Mean dengue virus extinction time, excluding trials that do not reach a total of 100 individuals in the Asymptomatic, Symptomatic and Resistant classes combined (i.e. non-epidemic trials). C) Total number of non-epidemic trials. D) Mean dengue virus extinction time of non-epidemic trials. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

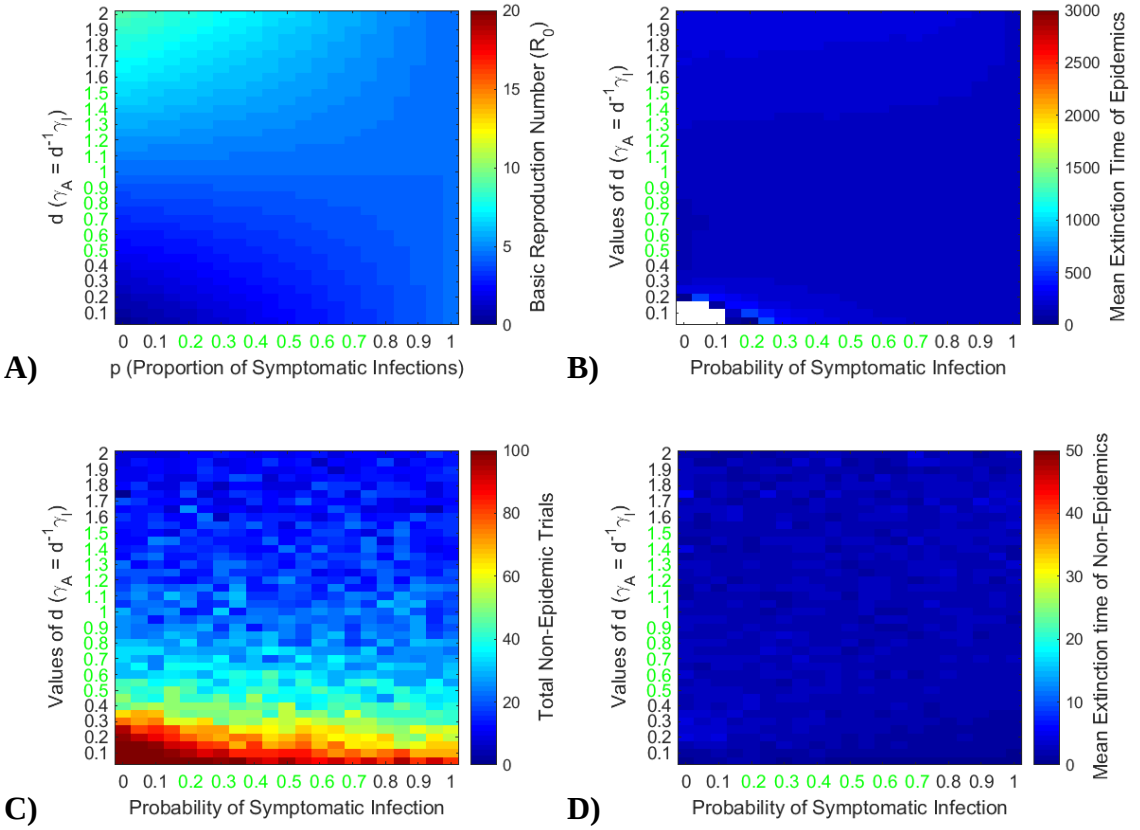


Figure 7.4: The effects of different durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under low-level transmission (β_I=200/365), on the frequency dependent transmission version of Model A. A) Basic reproductive number (R_0). B) Mean dengue virus extinction time, excluding trials that do not reach a total of 100 individuals in the Asymptomatic, Symptomatic and Resistant classes combined (i.e. non-epidemic trials). C) Total number of non-epidemic trials. D) Mean dengue virus extinction time of non-epidemic trials. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

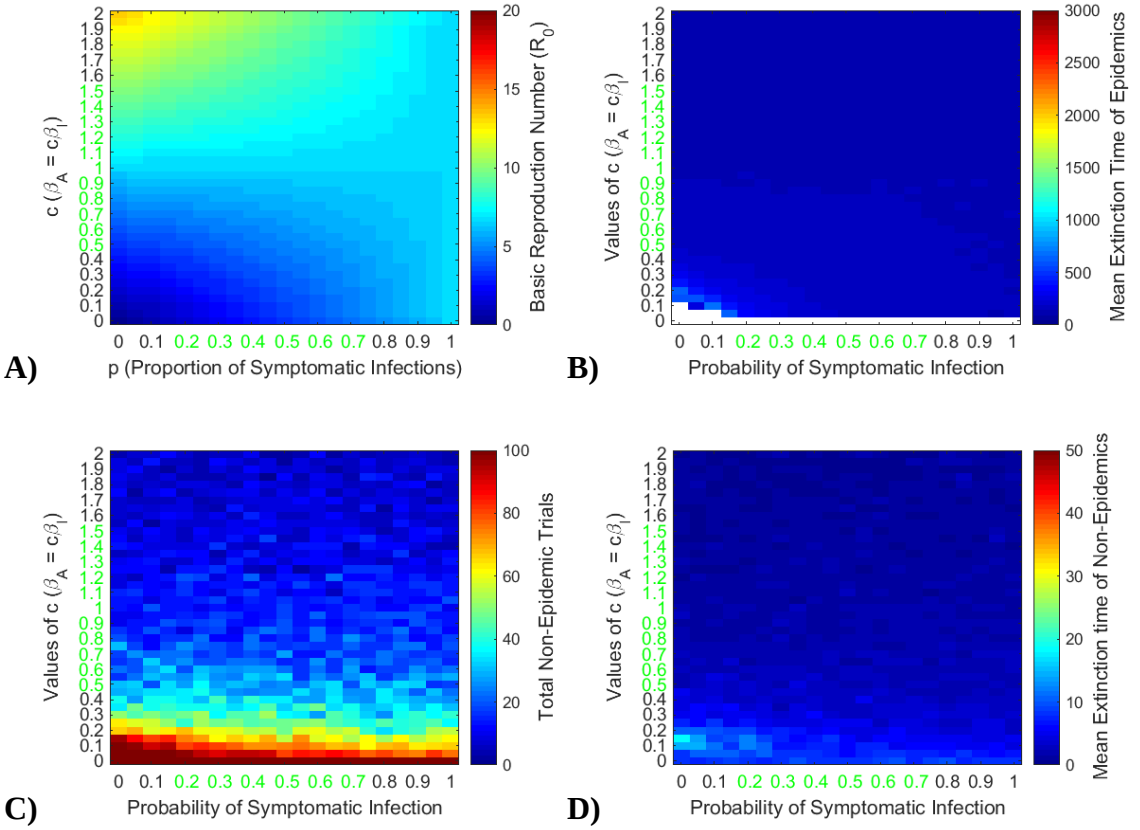


Figure 7.5: The effects of different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under mid-level transmission (β_I=300/365), on the frequency dependent transmission version of Model A. A) Basic reproductive number (R_0). B) Mean dengue virus extinction time, excluding trials that do not reach a total of 100 individuals in the Asymptomatic, Symptomatic and Resistant classes combined (i.e. non-epidemic trials). C) Total number of non-epidemic trials. D) Mean dengue virus extinction time of non-epidemic trials. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

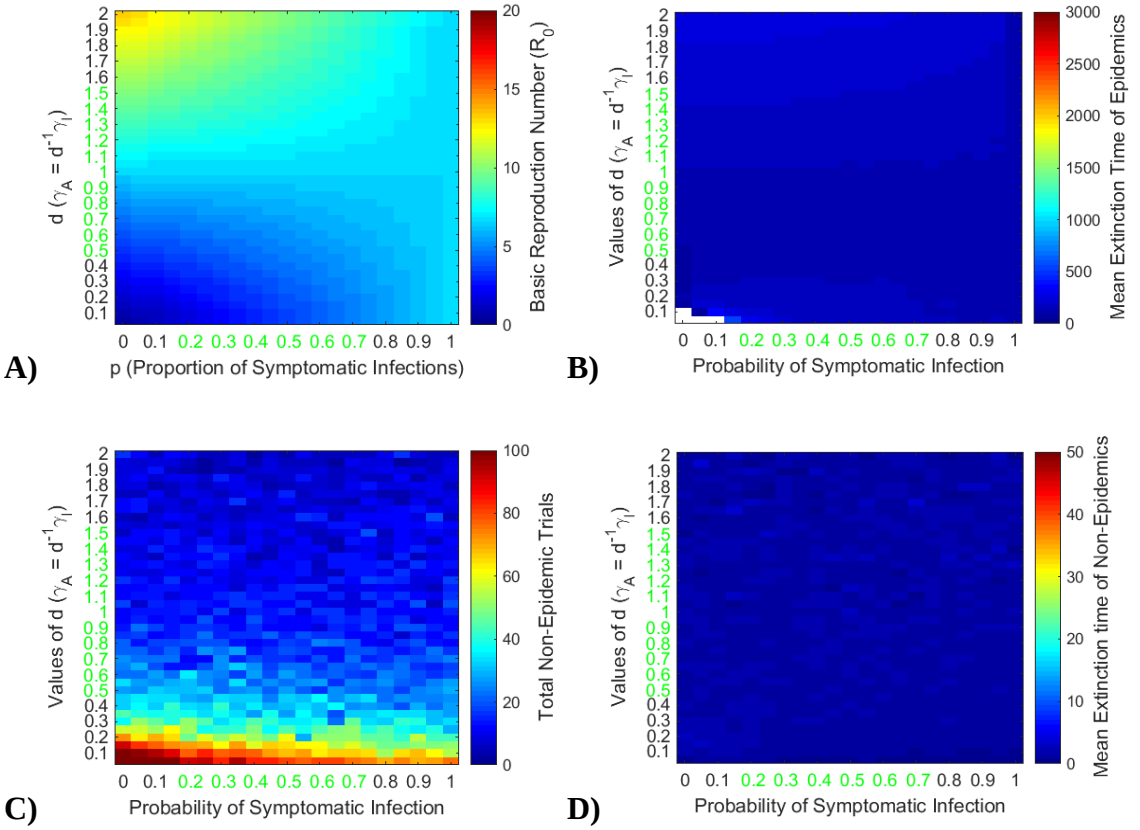


Figure 7.6: The effects of different durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under mid-level transmission (β_I=300/365), on the frequency dependent transmission version of Model A. A) Basic reproductive number (R_0). B) Mean dengue virus extinction time, excluding trials that do not reach a total of 100 individuals in the Asymptomatic, Symptomatic and Resistant classes combined (i.e. non-epidemic trials). C) Total number of non-epidemic trials. D) Mean dengue virus extinction time of non-epidemic trials. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

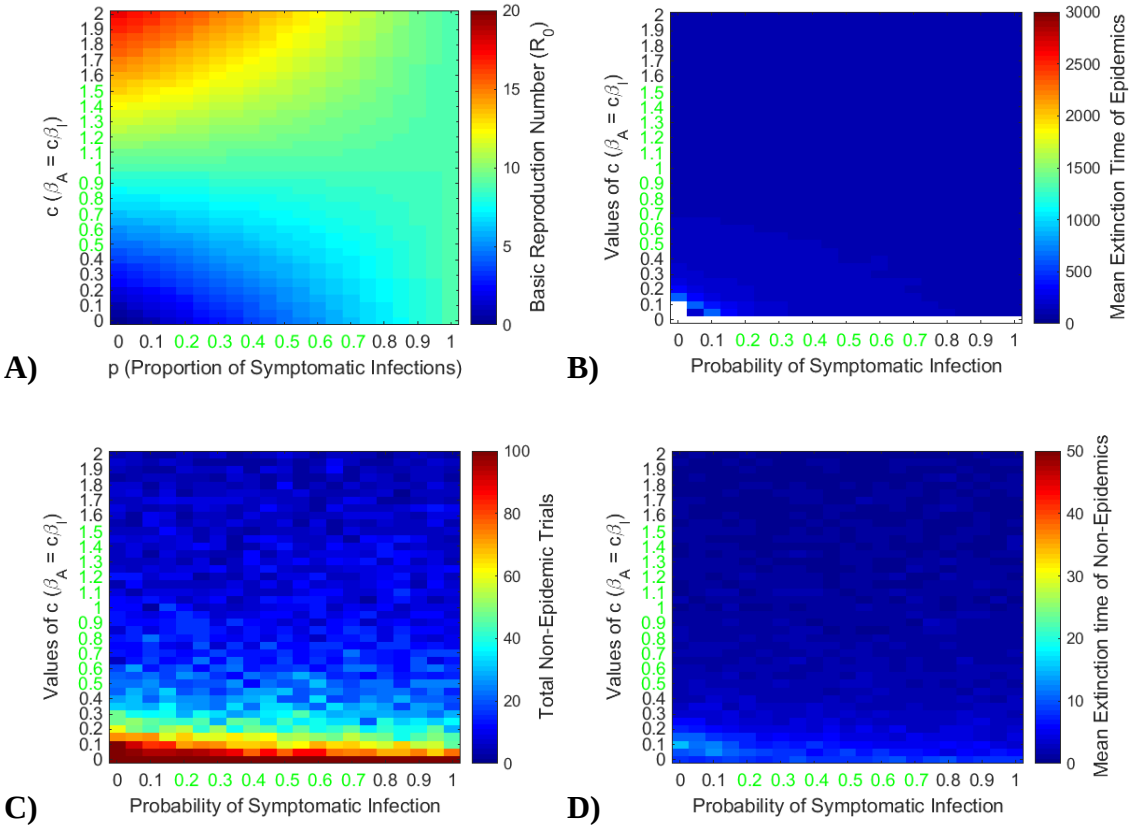


Figure 7.7: The effects of different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under high-level transmission (β_I=400/365), on the frequency dependent transmission version of Model A. A) Basic reproductive number (R_0). B) Mean dengue extinction time, excluding trials that do not reach a total of 100 individuals in the Asymptomatic, Symptomatic and Resistant classes combined (i.e. non-epidemic trials). C) Total number of non-epidemic trials. D) Mean dengue virus extinction time of non-epidemic trials. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

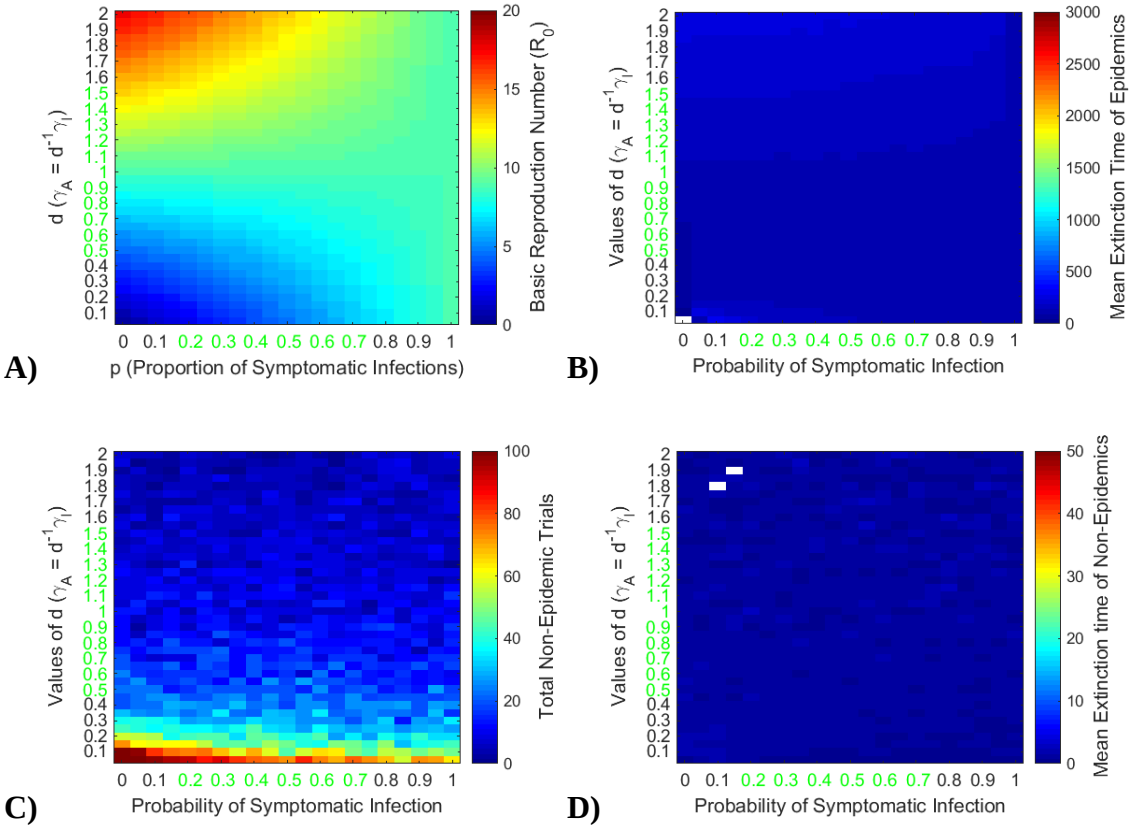


Figure 7.8: The effects of different durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under high-level transmission (β_I=400/365), on the frequency dependent transmission version of Model A. A) Basic reproductive number (R_0). B) Mean dengue extinction time, excluding trials that do not reach a total of 100 individuals in the Asymptomatic, Symptomatic and Resistant classes combined (i.e. non-epidemic trials). C) Total number of non-epidemic trials. D) Mean dengue virus extinction time of non-epidemic trials. For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

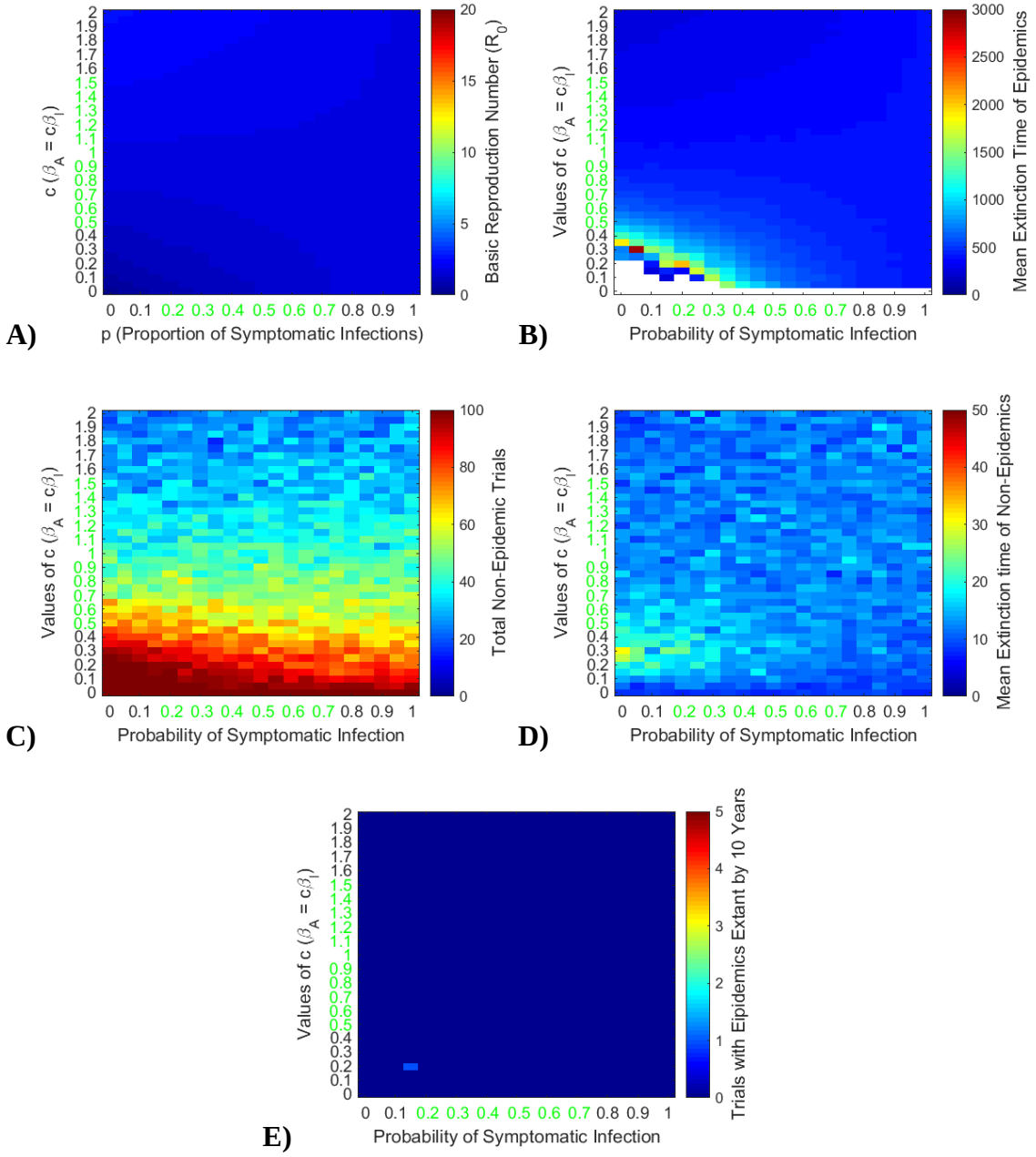


Figure 7.9: The effects of different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under low-level transmission (β_i=0.5 and b=0.3), on the mosquito dependent transmission version of Model A. A) Basic reproductive number (R_0). B) Mean dengue extinction time, excluding trials that do not reach a total of 100 individuals in the Asymptomatic, Symptomatic and Resistant classes combined (i.e. non-epidemic trials). C) Total number of non-epidemic trials. D) Mean epidemic extinction time of non-epidemic trials. E) Number of trials with extant epidemics at 10 years (Note colour scale is from 0-5). For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

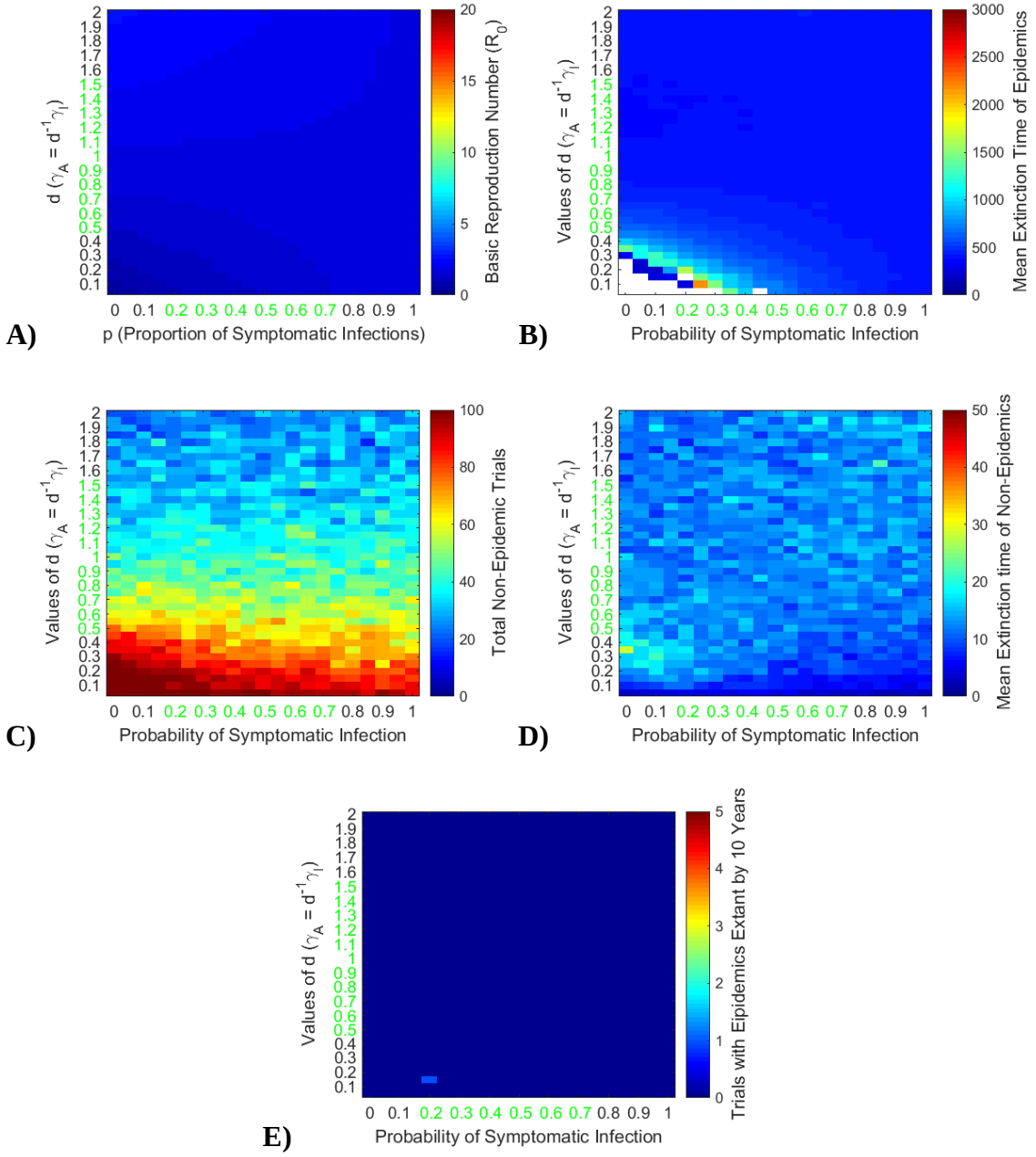


Figure 7.10: The effects different durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under low-level transmission (β_i=0.5 and b=0.3), on the mosquito dependent transmission version of Model A. A) Basic reproductive number (R_0). B) Mean dengue extinction time, excluding trials that do not reach a total of 100 individuals in the Asymptomatic, Symptomatic and Resistant classes combined (i.e. non-epidemic trials). C) Total number of non-epidemic trials. D) Mean epidemic extinction time of non-epidemic trials. E) Number of trials with extant epidemics at 10 years (Note colour scale is from 0-5). For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

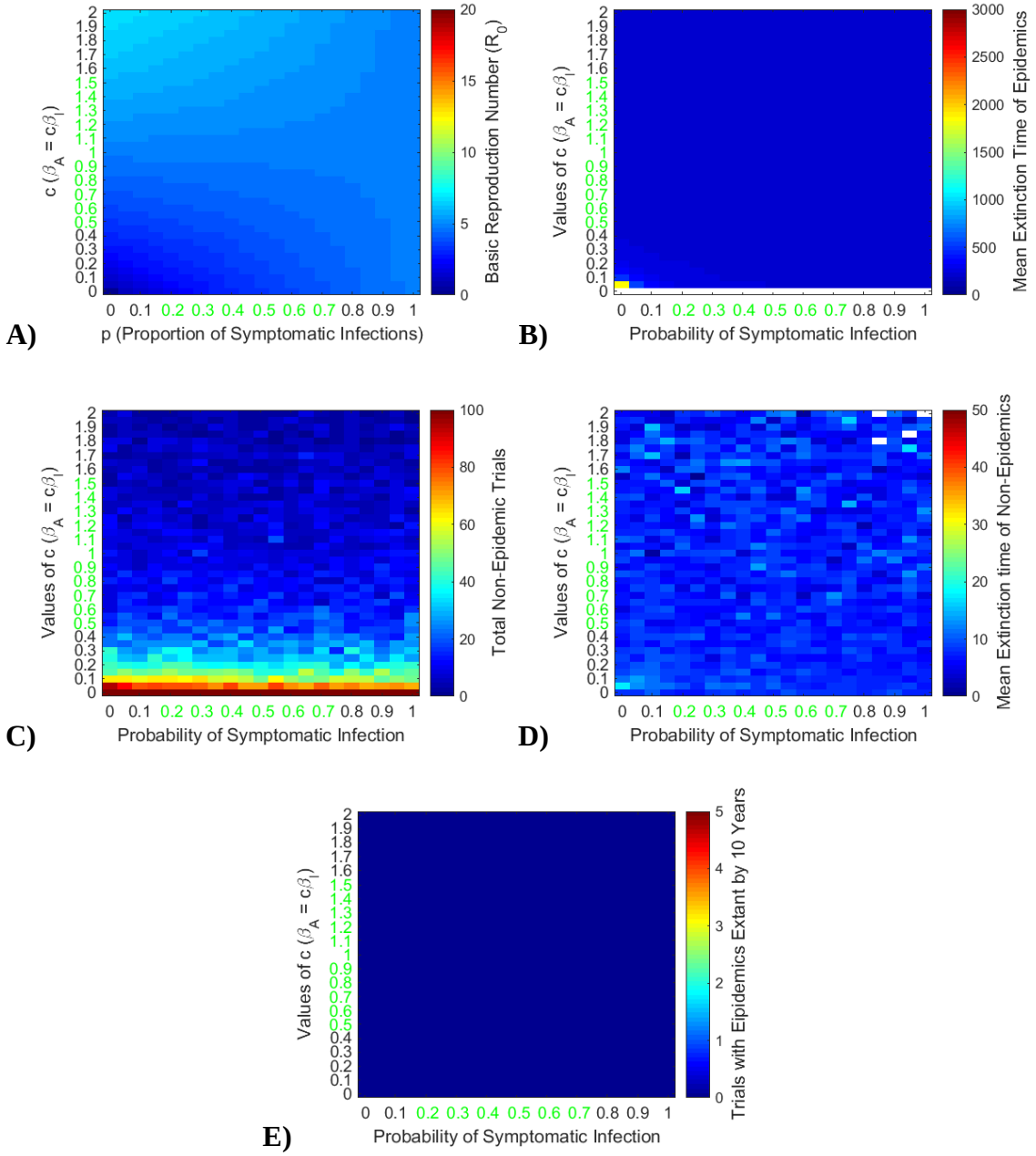


Figure 7.11: The effects of different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under mid-level transmission (β_i=0.75 and b=0.65), on the mosquito dependent transmission version of Model A. A) Basic reproductive number (R_0). B) Mean dengue extinction time, excluding trials that do not reach a total of 100 individuals in the Asymptomatic, Symptomatic and Resistant classes combined (i.e. non-epidemic trials). C) Total number of non-epidemic trials. D) Mean epidemic extinction time of non-epidemic trials. E) Number of trials with extant epidemics at 10 years (Note colour scale is from 0-5). For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

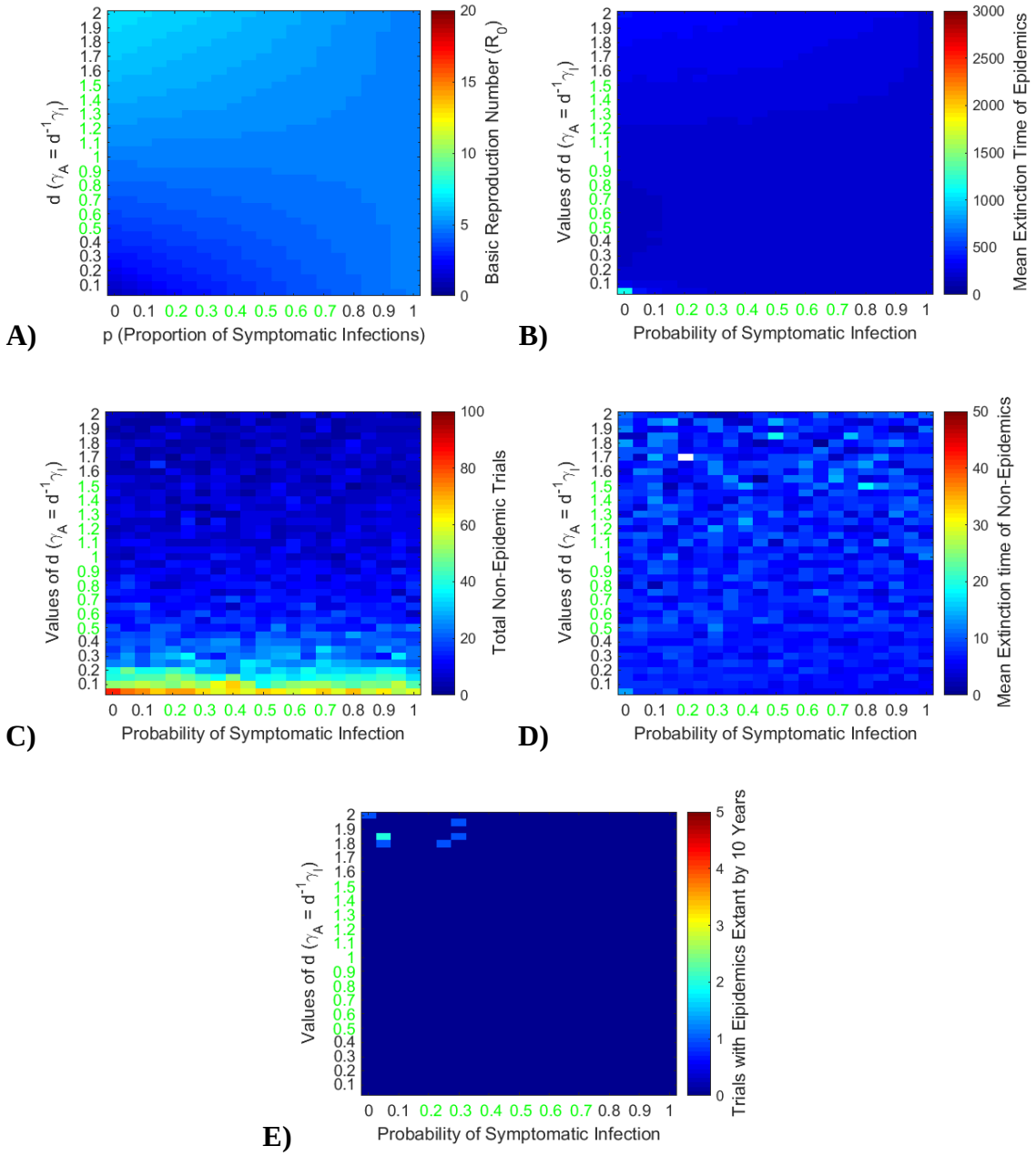


Figure 7.12: The effects different durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under mid-level transmission (β_i=0.75 and b=0.65), on the mosquito dependent transmission version of Model A. A) Basic reproductive number (R_0). B) Mean dengue extinction time, excluding trials that do not reach a total of 100 individuals in the Asymptomatic, Symptomatic and Resistant classes combined (i.e. non-epidemic trials). C) Total number of non-epidemic trials. D) Mean epidemic extinction time of non-epidemic trials. E) Number of trials with extant epidemics at 10 years (Note colour scale is from 0-5). For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

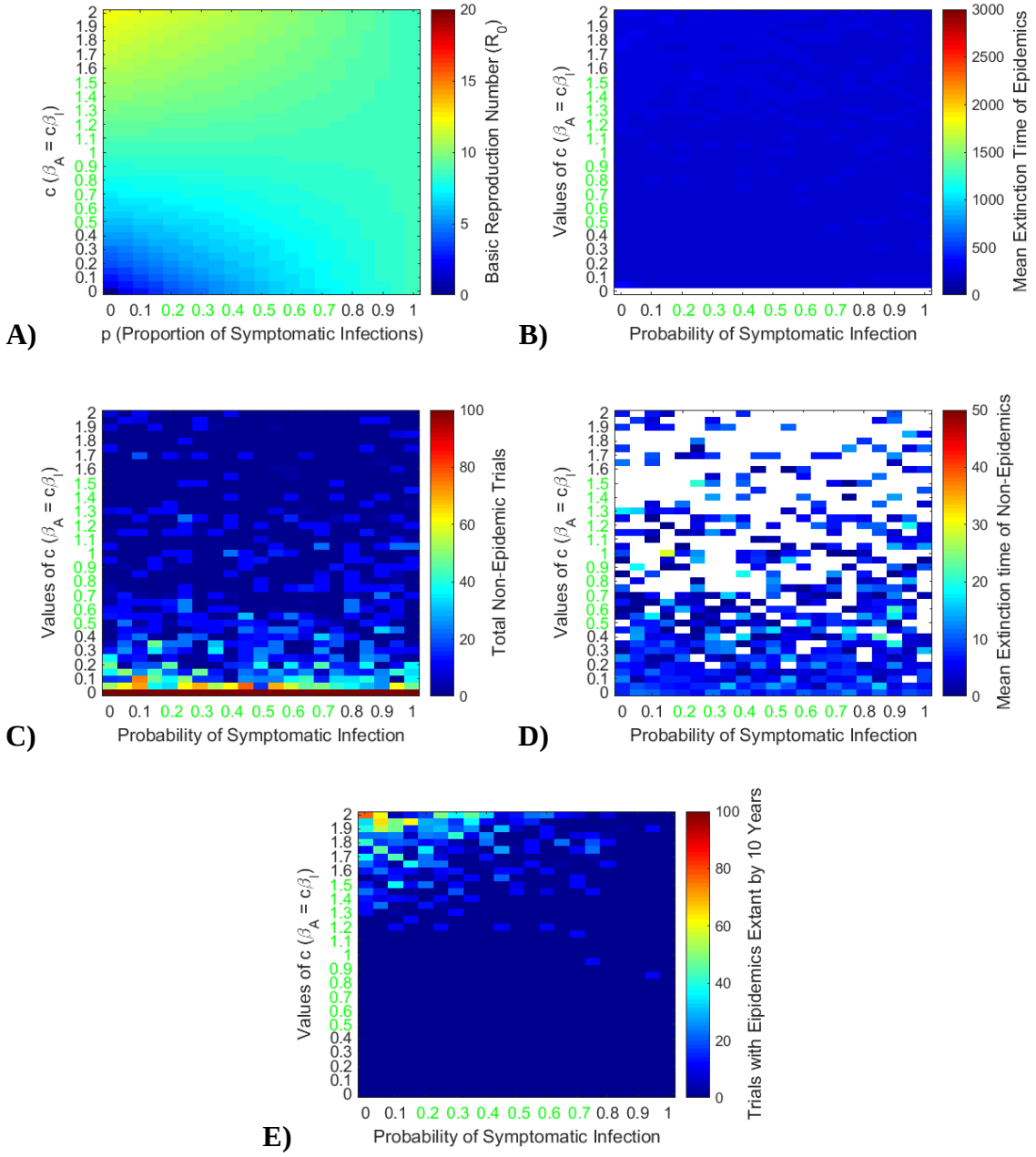


Figure 7.13: The effects of different levels of transmission from asymptomatic infections (c) and different proportions of infections developing symptoms (p), when durations of asymptomatic and symptomatic infections were the same (d=1), under high-level transmission (β_i=1 and b=1), on the mosquito dependent transmission version of Model A. A) Basic reproductive number (R_0). B) Mean dengue extinction time, excluding trials that do not reach a total of 100 individuals in the Asymptomatic, Symptomatic and Resistant classes combined (i.e. non-epidemic trials). C) Total number of non-epidemic trials. D) Mean epidemic extinction time of non-epidemic trials. E) Number of trials with extant epidemics at 10 years (Note colour scale is from 0-100). For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

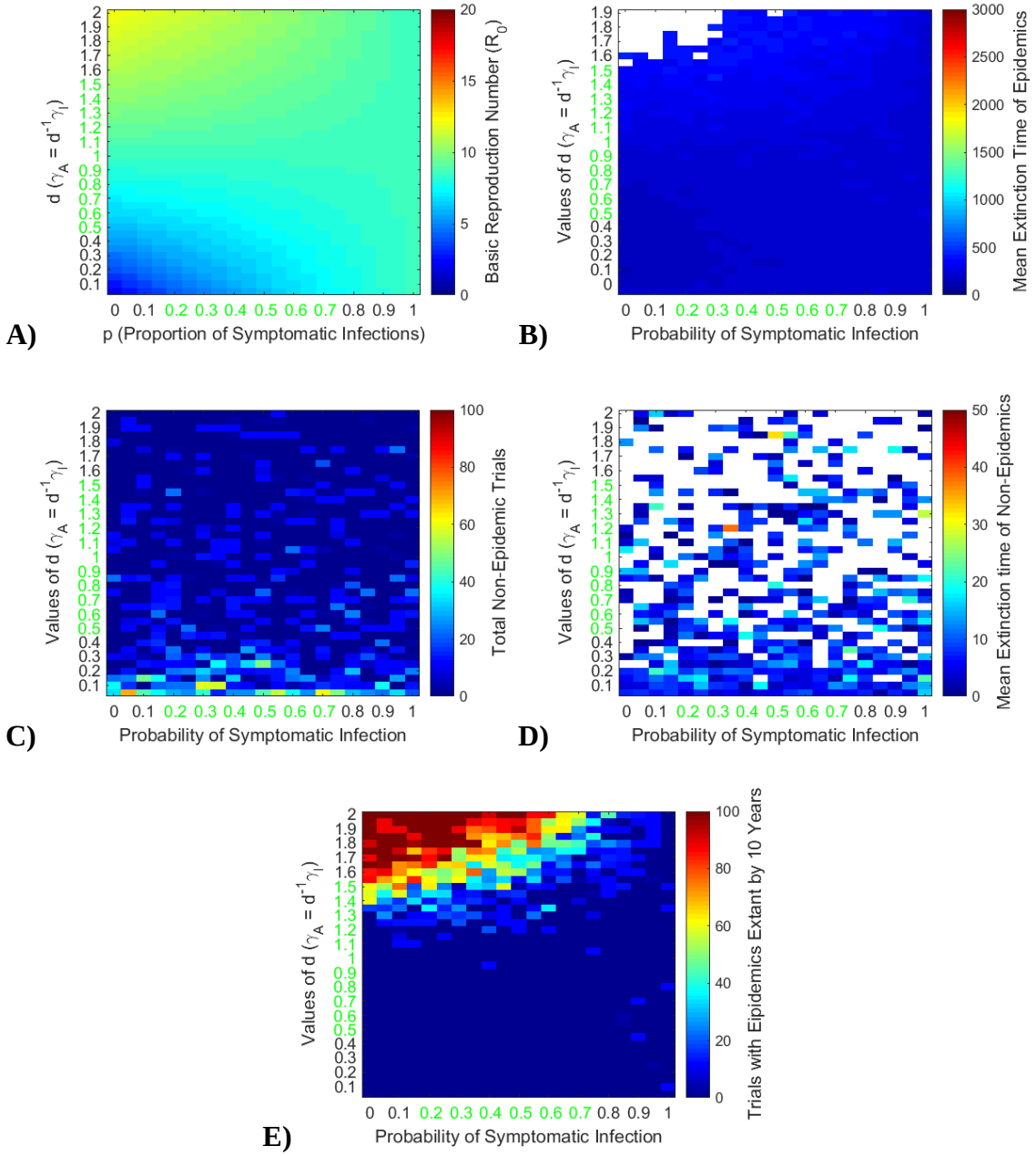


Figure 7.14: The effects different durations of asymptomatic infection (d) and different proportions of infections developing symptoms (p), when levels of transmission from asymptomatic and symptomatic infections were the same (c=1), under high-level transmission (β_i=1 and b=1), on the mosquito dependent transmission version of Model A. A) Basic reproductive number (R_0). B) Mean dengue extinction time, excluding trials that do not reach a total of 100 individuals in the Asymptomatic, Symptomatic and Resistant classes combined (i.e. non-epidemic trials). C) Total number of non-epidemic trials. D) Mean epidemic extinction time of non-epidemic trials. E) Number of trials with extant epidemics at 10 years (Note colour scale is from 0-100). For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

7.3.2 General trends in epidemics of DF

For the vast majority of parameter settings, when dengue viruses succeed in spreading through the population, the model produces on average epidemics that last at most a year and a half. Across the low, mid and high level transmission settings the epidemics that last more than a year and a half on average follow two similar trends to the parameter settings where a higher proportion of dengue infection trials fail to spread through the population. The first trend is for low levels of transmission from the asymptomatic class (c), combined with low proportions of infections leading to symptoms (p) (see Figure 7.3, Figure 7.5, Figure 7.7, Figure 7.9 and Figure 7.11). This first on average longer lasting epidemic trend is either at the low end or excluded from the more conservative samples of parameter space for the proportion of infections developing symptoms (p) and level of transmission from the asymptomatic class (c) (see Figure 7.3, Figure 7.5, Figure 7.7, Figure 7.9 and Figure 7.11). The second trend is for low durations of transmission in asymptomatic class (d), combined with low proportions of infections leading to symptoms (p) (see Figure 7.4, Figure 7.6, Figure 7.10 and Figure 7.12). This second on average longer lasting epidemic trend is either at the low end or excluded from the more conservative samples of parameter space for the proportion of infections developing symptoms (p) and duration of transmission from the asymptomatic class (d) (see Figure 7.4, Figure 7.6, Figure 7.10 and Figure 7.12). Of note is that both of these two trends also occur when R_0 is slightly over 1 (see Subfigure A of Figure 7.3-7 and Figure 7.9-12).

The fact that the longer lasting epidemics have overlapping parameter values in which a high proportion of trials have dengue viruses failing to spread through the population; suggests that whilst at these parameter settings dengue viruses can spread through the population, R_0 being greater than 1, dengue spreads slowly, due to a low

force of infection. However, at the parameter settings which cause this low force of infection, many individuals who become infected often become immune before transmitting the infection, which can lead to a dengue epidemic dying out before it spreads beyond a few individuals.

7.3.3 Epidemics that are extant by 10 years

For reasons of expediency model runtime of trials was limited to 10 years. None of the simulated epidemics in the frequency dependent versions of Model A ran over this limit. A few trials in the mosquito dependent versions of Model A had dengue persisting over this limit (see subfigures E of Figure 7.9-14). In order to ascertain why certain parameter settings caused dengue viruses to persist in the population for at least 10 years for some of those parameter settings Model A was run repeatedly until a trial had the dengue virus persisting in the population over 10 years. Figures of the infected human population over time were then plotted (see Figure 7.15-20). The trials that had dengue persisting over this limit fall into five trends in terms of parameter space.

The first trend is for low probabilities of an infection leading to symptoms (p) combined with either low levels or durations of transmission from the asymptomatic class (c and d, respectively) at the low transmission setting (β_i=0.5 and b=0.3) (see Figure 7.9E and Figure 7.10E). Here as seen by Figure 7.15 dengue viruses have simply persisted in the population beyond 10 years, but do eventually die out. These two greater than 10 year epidemics are excluded from the more conservative samples of parameter space for the level or duration of transmission from the asymptomatic class (c and d respectively). They are also either at the low end of or excluded from the more conservative samples of parameter space for the proportion of infections developing symptoms (p).

In the four other trends dengue viruses become endemic to the human and mosquito population. There is an initial spike in dengue infections followed by dengue's persistence at a low level (see subfigures A of Figure 7.16-20). The population of infected humans then begins to oscillate around a set value, with amplitude that decreases to a point (see subfigures B of Figure 7.16-20). This would suggest that for these trials the reason why the dengue virus is still within the human and mosquito population by 10 years is that the number of dengue infections is fluctuating around an endemic equilibrium of dengue infections, and will continue to do so.

The first of the trends in parameter space where dengue becomes endemic occurs when the transmission in the asymptomatic class is equal to that in the symptomatic class (c=1), at medium transmission level (β_i=0.75 and b=0.65), for high lengths of time spent in the asymptomatic class (d) combined with low proportions of infections that lead to symptoms (p), such as d=2 and p=0, d=1.85 and p=0.05, d=1.8 and p=0.05, d=1.8 and p=0.25, d=1.95 and p=0.3, as well as d=1.85 and p=0.3 (see Figure 7.12E and Figure 7.16). This happened for 1-2 of the 100 trials for each of these parameter settings (see Figure 7.12E and Figure 7.16). All of the trials for this trend of epidemics becoming endemic are excluded from the more conservative samples of parameter space for the duration of transmission from the asymptomatic class (d) (see Figure 7.12E and Figure 7.16). They are also either at the low end of or excluded from the more conservative samples of parameter space for the proportion of infections developing symptoms (p) (see Figure 7.12E and Figure 7.16).

The second of the trends in parameter space where dengue becomes endemic occurs at the highest transmission setting (β_i=1 and b=1) where the model essentially becomes an SIR type model, due to all infections being symptomatic (p=1) or Model A closely resembles an SIR model, due to nearly all infections developing symptoms (p is

close to 1), the level and duration of transmission from the asymptomatic class being similar to the symptomatic class (c and d are 1 or close to it) (see Figure 7.13E, Figure 7.14E and Figure 7.19-20). Many trials from this second trend are excluded from the more conservative sample of parameter space for the proportion of infections developing symptoms (p), as they occur when $p<0.7$.

The third of the trends in parameter space where dengue becomes endemic occurs for proportions of infection becoming symptomatic (p) that are lower than 1 combined with greater transmission from the asymptomatic class than the symptomatic class ($c>1$), when the durations of transmission in the symptomatic and asymptomatic class are the same ($d=1$), at the highest transmission setting ($\beta_i=1$ and $b=1$) (see Figure 7.13E and Figure 7.17). As the transmission from the asymptomatic class increases to reach double of that from the symptomatic class ($c=2$) and the proportion of infections developing symptoms (p) decreases to 0, at this high transmission setting ($\beta_i=1$ and $b=1$) the number of trials where dengue is extant at 10 years increases to the high fifties out of 100 (see Figure 7.13E and Figure 7.17). This excludes many of the trials for this trend of epidemics becoming endemic from the more conservative samples of parameter space for the proportion of infections developing symptoms (p) and level of transmission from the asymptomatic class (c), but not all.

The fourth of the trends in parameter space where dengue becomes endemic occurs for proportions of infection becoming symptomatic (p) that are lower than 1, combined with higher lengths of time spent in the asymptomatic class than in the symptomatic class ($d>1$), when the transmission from asymptomatic and symptomatic infections are the same ($c=1$), at the high transmission setting ($\beta_i=1$ and $b=1$) (see Figure 7.14E and Figure 7.18). As both the length of time spent in the asymptomatic class increases to double of that spent in the symptomatic class ($d=2$) and the proportion

of infections developing symptoms (p) decreases to 0, at this high transmission setting (β_i=1 and b=1) the number of trials where dengue is extant at 10 years increases to 100 out of 100 (see Figure 7.14E and Figure 7.18). This excludes many of the trials for this trend of epidemics becoming endemic from the more conservative samples of parameter space for the proportion of infections developing symptoms (p) and duration of transmission from the asymptomatic class (d), but not all. However this exclusion from the more conservative samples of parameter space for trials were dengue becomes endemic is to a lesser extent than seen in the third trend (compare Figure 7.14E to Figure 7.13E).

It should be noted that the third and fourth trends in regions of parameter space where dengue becomes endemic were observed in the deterministic version of model A in Chapter 6. This would suggest that these two regions are “true” regions of endemic disease. The other trends in parameter regions where the dengue virus becomes endemic are regions where this has occurred due to the role of stochasticity.

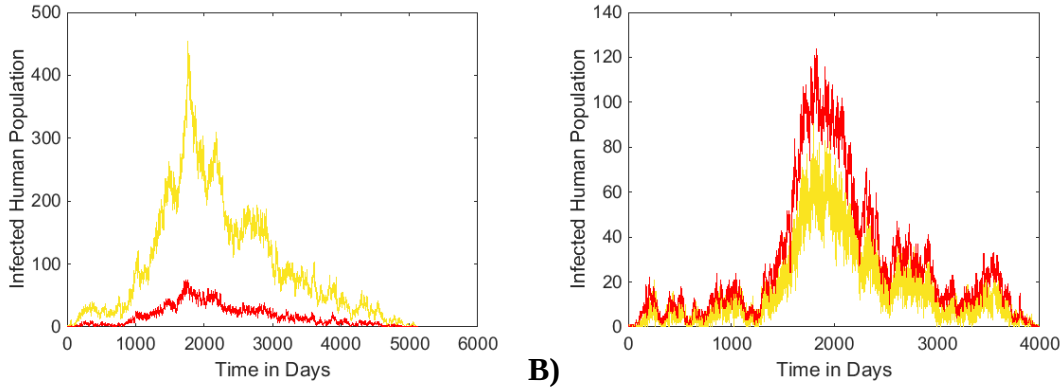


Figure 7.15: Example trials where dengue viruses extant are extant after 10 years but do eventually die out, in the mosquito dependent version of Model A. Asymptomatic human infections in yellow and symptomatic human infections in red. A) At low-level transmission (β_i=0.5 and b=0.3), when transmission asymptomatic infections is 20% of symptomatic infection c=0.2, the duration of asymptomatic and symptomatic infection are the same (d=1) and the proportion of infections that develop symptoms is 15% (p=0.15). B) At low-level transmission (β_i=0.5 and b=0.3), when transmission from symptomatic and asymptomatic infections is the same (c=1), the proportional duration of asymptomatic infection is 15% that of symptomatic infection (d=0.15) and 20% of infections develop symptoms p=0.2.

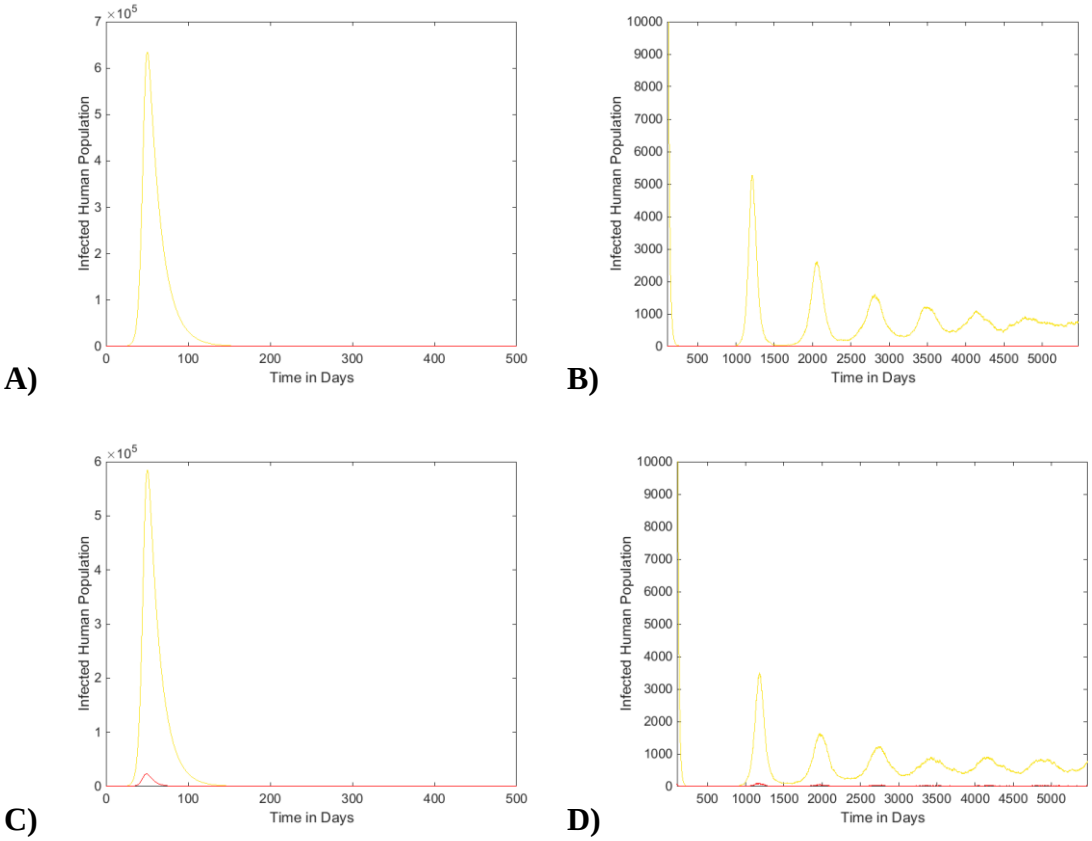


Figure 7.16: Example trials where dengue viruses are extant after 10 years through becoming endemic, in the mosquito dependent version of Model A, at mid-level transmission (β_i=0.75 and b=0.65). Asymptomatic human infections in yellow and symptomatic human infections in red. A) When transmission from asymptomatic and symptomatic infections is the same (c=1), the duration of asymptomatic infections is twice that of symptomatic infections (d=2) and no infections develop symptoms (p=0), from 0-500 days. B) Same trial from 100 days – 15 years. C) When transmission from asymptomatic and symptomatic infections is the same (c=1), the duration of infections for asymptomatic infections is 185% that of symptomatic infections (d=1.85) and 5% of infections develop symptoms (p=0.05), from 0-500 days. D) Same trial from 100 days – 15 years.

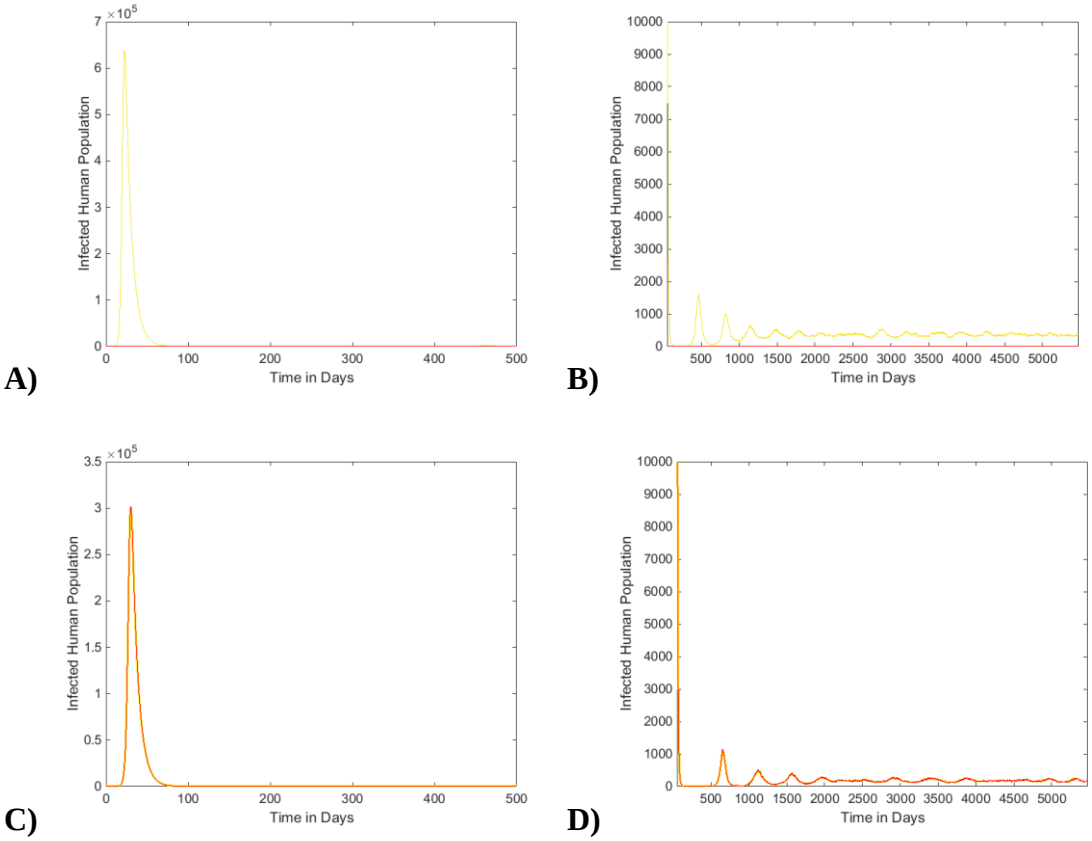


Figure 7.17: Example trials where dengue viruses are extant after 10 years through becoming endemic, in the mosquito dependent version of Model A, at high-level transmission (β_i=1 and b=1). Asymptomatic human infections in yellow and symptomatic human infections in red. A) When transmission from asymptomatic infections is twice that of symptomatic infections (c=2), the duration of asymptomatic and symptomatic infections if the same (d=1) and no infections develop symptoms (p=0), from 0-500 days. B) Same trial from 50 days – 15 years. C) When transmission from asymptomatic infections is 145% that of symptomatic infections (c=1.45), durations of asymptomatic and symptomatic infections is the same (d=1) and half of infections develop symptoms (p=0.5), from 0-500 days. D) Same trial from 50 days – 15 years.

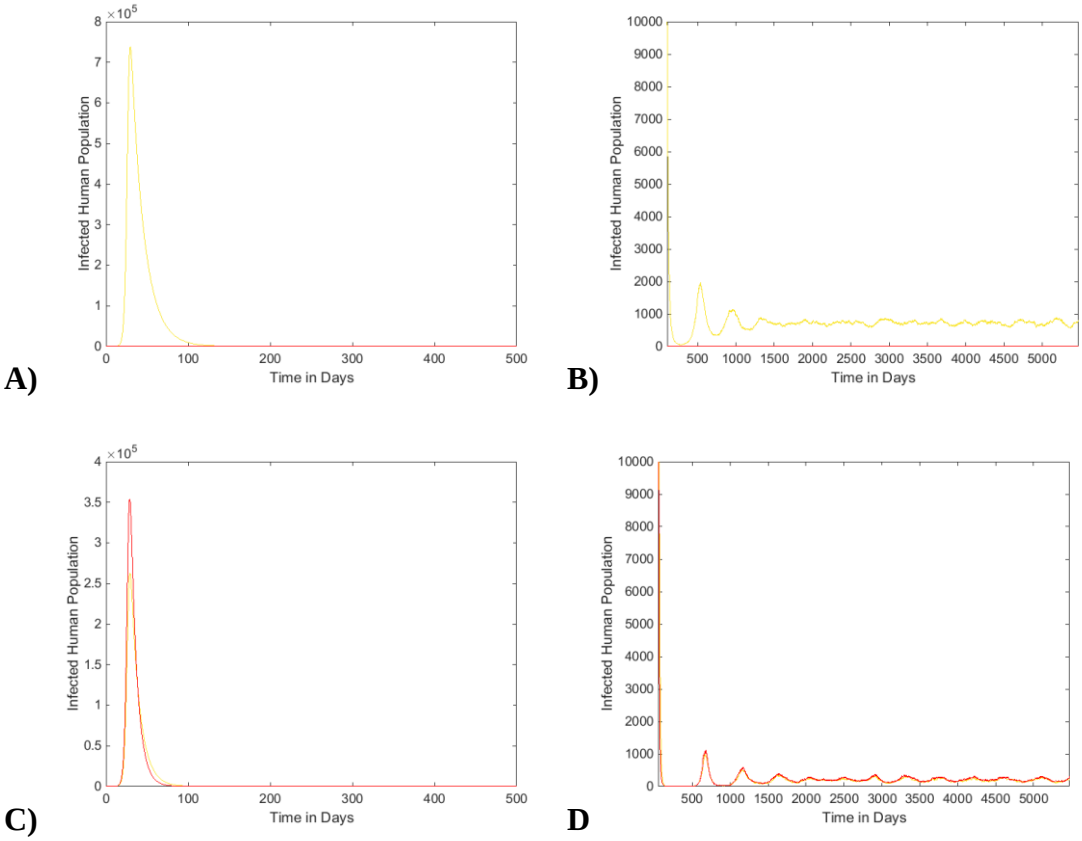


Figure 7.18: Example trials where dengue viruses are extant after 10 years through becoming endemic, in the mosquito dependent version of Model A, at high-level transmission β_i=1 and b=1. Asymptomatic human infections in yellow and symptomatic human infections in red. A) When transmission from asymptomatic and symptomatic infections is the same (c=1), durations of asymptomatic infections are twice that of symptomatic infections (d=2) and no infections develop symptoms (p=0), from 0-500 days. B) Same trial from 100 days – 15 years. C) When transmission from asymptomatic and symptomatic infections is the same (c=1), durations of infection in asymptomatic infections are 140% that of symptomatic infections (d=1.4) and half of infections develop symptoms (p=0.5), from 0-500 days. D) Same trial from 50 days – 15 years.

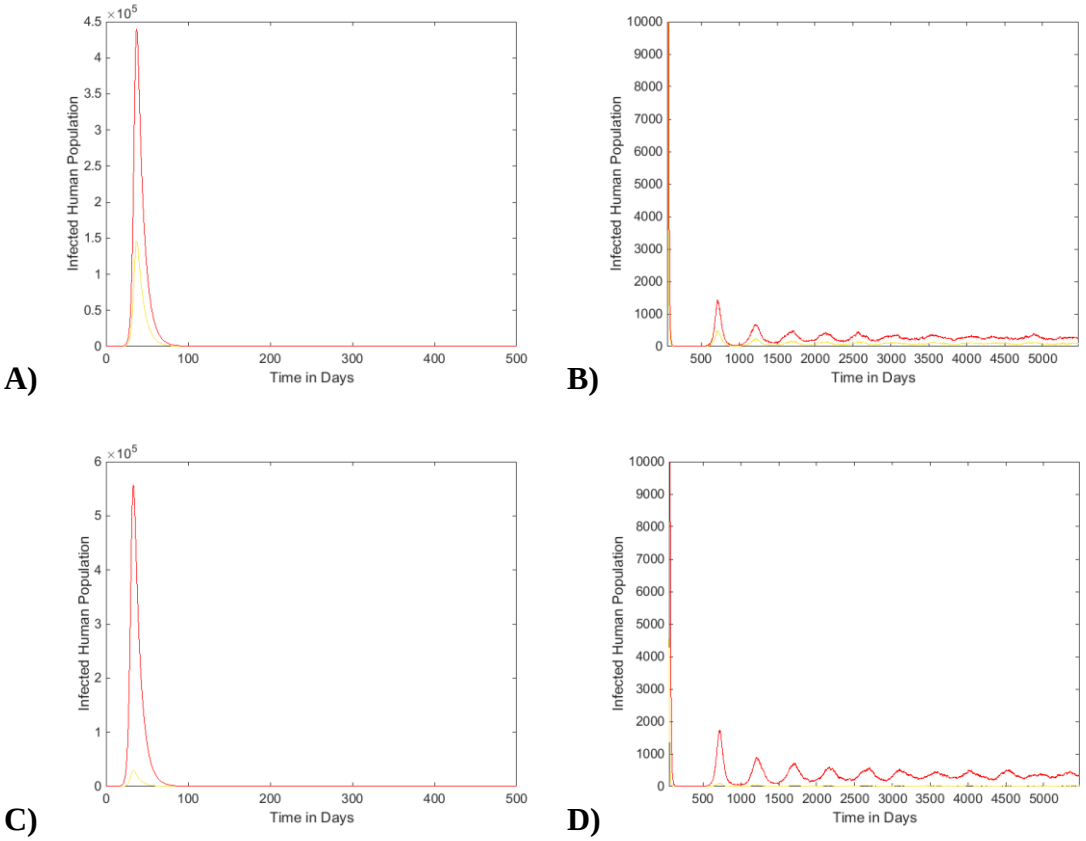


Figure 7.19: Example trials where dengue viruses are extant after 10 years through becoming endemic, in the mosquito dependent version of Model A, at high-level transmission (β_i=1 and b=1). Asymptomatic human infections in yellow and symptomatic human infections in red. A) When transmission from asymptomatic infections is 95% that of symptomatic infections (c=0.95), durations of asymptomatic and symptomatic infection are the same (d=1) and three quarters of infections develop symptoms (p=0.75), from 0-500 days. B) Same trial from 100 days – 15 years. C) When transmission from asymptomatic infections is 85% that of symptomatic infections (c=0.85), durations of asymptomatic and symptomatic infection are the same (d=1) and 95% of infections develop symptoms (p=0.95), from 0-500 days. D) Same trial from 50 days – 15 years.

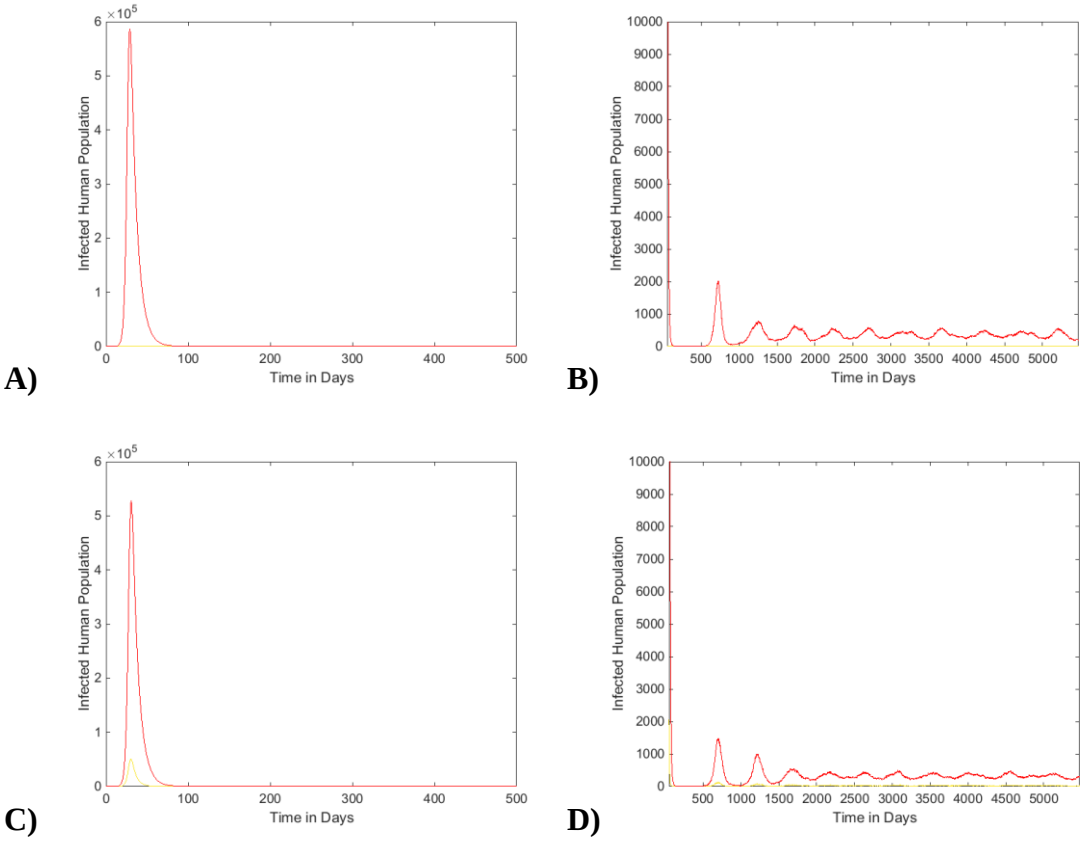


Figure 7.20: Example trials where dengue viruses are extant after 10 years through becoming endemic, in the mosquito dependent version of Model A, at high-level transmission (β_i=1 and b=1). Asymptomatic human infections in yellow and symptomatic human infections in red. A) When transmission from asymptomatic and symptomatic infections is the same (c=1), durations of asymptomatic infection are 80% that of symptomatic infections (d=0.8) and all infections develop symptoms (p=1), from 0-500 days. B) Same trial from 50 days – 15 years. C) When transmission from asymptomatic and symptomatic infections is the same (c=1), durations of asymptomatic infection are 70% that of symptomatic infections (d=0.7) and 90% of infections develop symptoms (p=0.9), from 0-500 days. D) Same trial from 50 days – 15 years.

7.3.4 Population left immune to the invading dengue serotype and thereby at risk of DHF

For the stochastic form of modelling both mosquito dependent and frequency dependent versions of Model A, provided epidemics occur, the proportion of population

that becomes resistant to the invading serotype and thereby at risk of DHF, remains similar to the deterministic form of modelling (see Chapter 5 and Chapter 6). For the most part on average over 80-90% of the population is in this at risk group, however this becomes lower for lower transmission settings (β_I, β_i and b), proportions of infections that lead to symptoms (p), levels and durations of transmission in asymptomatic class (c and d respectively) (see Figure 7.21-22). Within the more conservative samples of parameter space for levels and durations of transmission in asymptomatic class (c and d respectively) on average epidemics lead to from 60% to near 100% of the population being resistant to dengue virus (see Figure 7.21-22). This is also the case for the more conservative samples of parameter space for proportions of infections that lead to symptoms (p) at mid and high level transmission setting (see Sub-figures C-F of Figure 7.21-22). However, at the lower transmission setting the conservative samples of proportions of infections that lead to symptoms (p), combined with lower levels and durations of transmission in asymptomatic class (c and d respectively) can lead to epidemics with a mean percentage of population resistant as low as a single digit (see Sub-figures A-B of Figure 7.21-22). Where an invading dengue viral serotype becomes endemic on average above 90% of the population is in this at risk group (see Figure 7.23).

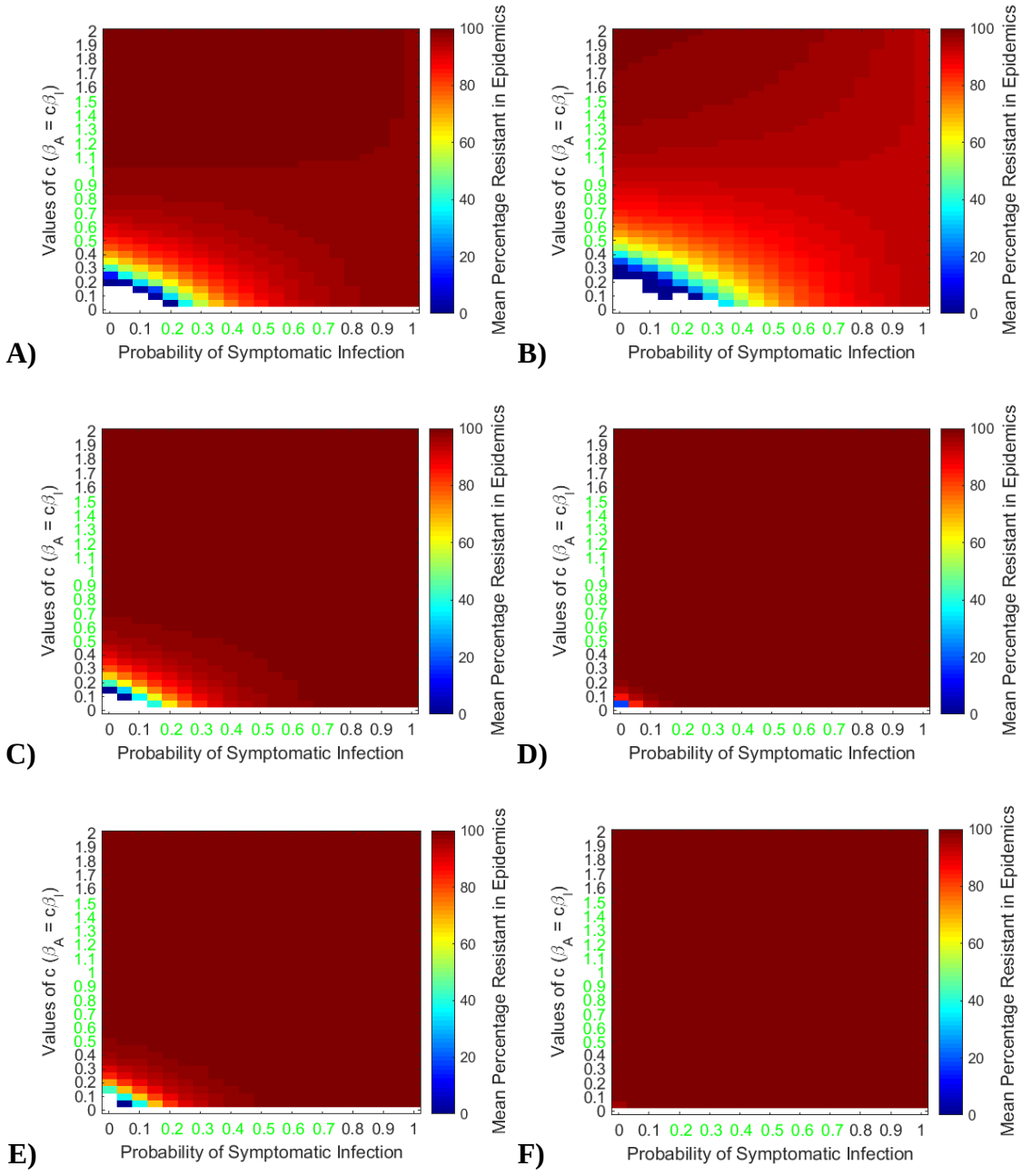


Figure 7.21: Mean percentage of the population resistant at the end of an epidemic, against different levels of asymptomatic infection (c) and proportions of infections developing symptoms (p), when the durations of asymptomatic and symptomatic infections are the same d=1. A) Model A under frequency dependent transmission, at low level transmission (β_I=200/365). B) Model A under mosquito dependent transmission, at low level transmission (β_i=0.5 and b=0.3). C) Model A under frequency dependent transmission, at mid-level transmission (β_I=300/365). D) Model A under mosquito dependent transmission at mid-level transmission (β_i=0.75 and b=0.65). E) Model A under frequency dependent transmission, at high-level transmission (β_I=400/365). F) Model A under mosquito dependent transmission, at (β_i=1 and b=1). For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

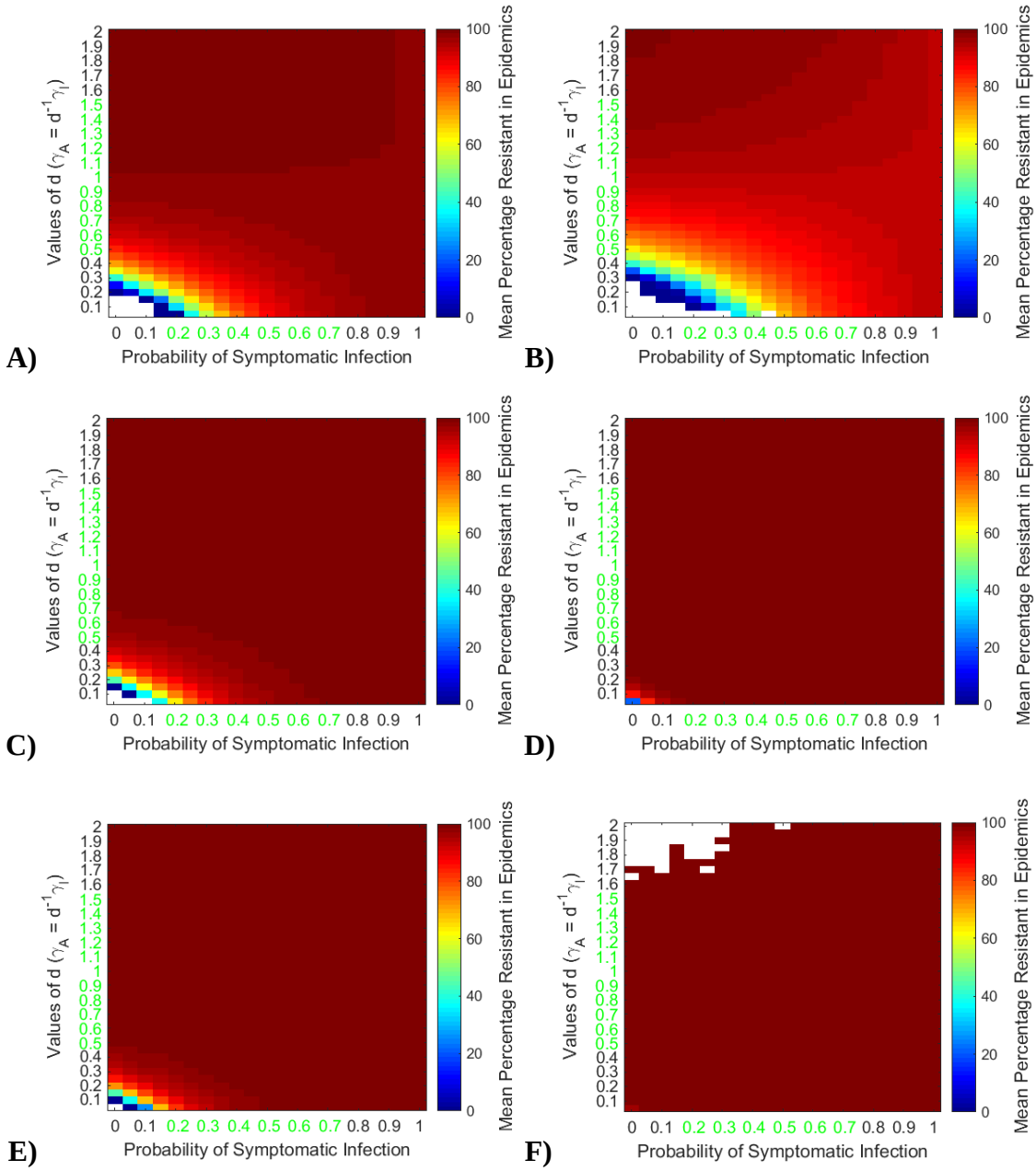


Figure 7.22: Mean percentage of the population resistant at the end of an epidemic, against duration of asymptomatic infections (d) and proportions of infections developing symptoms (p), when transmission from asymptomatic and symptomatic infections is the same (c=1). A) Model A under frequency dependent transmission, at low level transmission (β_I=200/365). B) Model A under mosquito dependent transmission, at low level transmission (β_i=0.5 and b=0.3). C) Model A under frequency dependent transmission, at mid-level transmission (β_I=300/365). D) Model A under mosquito dependent transmission at mid-level transmission (β_i=0.75 and b=0.65). E) Model A under frequency dependent transmission, at high-level transmission (β_I=400/365). F) Model A under mosquito dependent transmission, at (β_i=1 and b=1). For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

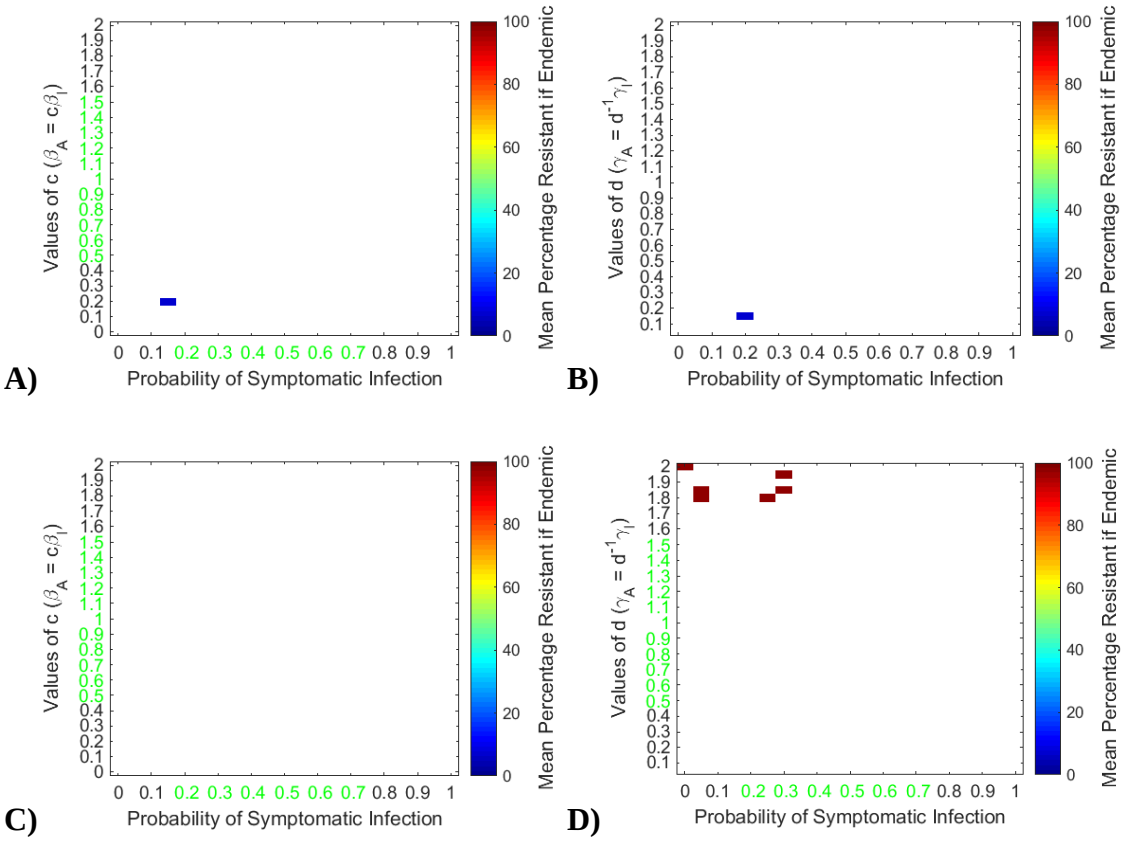


Figure 7.23: Mean percentage of the population resistant after 10 years if dengue is still within the population, in Model A under mosquito dependent transmission. A) Against asymptomatic transmission (c) and proportion of symptomatic infections (p), duration asymptomatic and symptomatic infections being the same (d=1), at low-level transmission (β_i=0.5 and b=0.3). B) Against durations of asymptomatic infection (d) and proportion of symptomatic infections (p), asymptomatic and symptomatic transmission being the same (c=1), at low-level transmission (β_i=0.5 and b=0.3). C) Against asymptomatic transmission (c) and proportion of symptomatic infections (p), duration of asymptomatic and symptomatic infections being the same (d=1), at mid-level transmission (β_i=0.75 and b=0.65). D) Against durations of asymptomatic infection (d) and proportion of symptomatic infections (p), when asymptomatic and symptomatic transmission is the same (c=1), at mid-level transmission (β_i=0.75 and b=0.65). For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

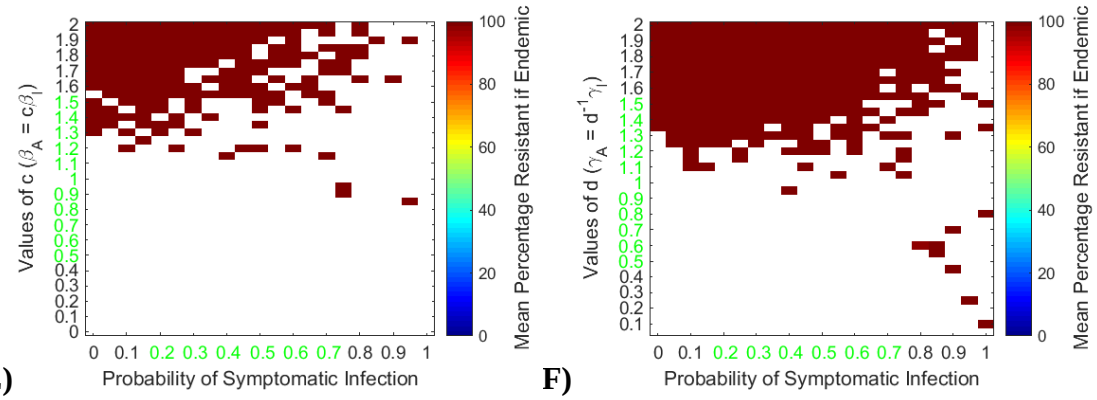


Figure 7.23 continued: E) Against asymptomatic transmission (c) and proportion of symptomatic infections (p), duration asymptomatic and symptomatic infections being the same (d=1), at high-level transmission (β_i=1 and b=1). F) Against duration of asymptomatic infection (d) and proportion of symptomatic infections (p), when asymptomatic and symptomatic transmission is the same (c=1), at high-level transmission (β_i=1 and b=1). For all sub-figures the more conservative region of parameter space is displayed through the axis labelled values being green.

7.4 Discussion

The switching from deterministic to a stochastic version of the mosquito dependent Model A caused several changes to epidemiological outcomes. Firstly in the higher transmission setting, when not all infections led to symptoms (p<1), combined with a higher duration or level of transmission in the asymptomatic class (d>1 and c>1, respectively), there was no longer a guarantee of epidemics becoming endemic. As values of the length of time before immunity takes hold in the asymptomatic class increased (d) and the proportion of infections that developed symptoms decreased more of the trials had epidemics becoming endemic. For increased transmission from the asymptomatic class (c) combined with a decreased proportion of infections developing symptoms this effect exists to a lesser extent (compare subfigure E of Figure 7.13-14). Meaning that more of these trials were dengue virus becomes endemic were excluded from the more conservative sample of parameter space for the level transmission from

the asymptomatic class (c), than for the duration transmission from the asymptomatic class (d). Similarly the occasional epidemic becomes endemic in the medium transmission setting (β_i=0.75 and b=0.65) for longer lengths of time until immunity is acquired for asymptomatic infections (d) combined with lower proportions of infections becoming symptomatic (p) (see Figure 7.12E and Figure 7.16). This did not occur in the deterministic models for the mosquito dependent versions of Model A. This would suggest that when combined with stochasticity, a greater duration of asymptomatic dengue virus infection than symptomatic dengue virus infection (d) is more likely to lead to dengue becoming endemic than a similar increase in transmission from asymptomatic dengue virus infection (c). To this author's knowledge and that of several reviewers on the different epidemiological roles of asymptomatic dengue infection (Chastel 2012; Carrington & Simmons 2014; Grange et al. 2014), there have been no studies quantifying the duration of viremia of asymptomatic dengue virus infections; let alone comparing it with the duration of viremia seen in symptomatic dengue virus infections.

This is not to suggest that the level of transmission from asymptomatic dengue virus infections (c) is not important to dengue's epidemiology. At lower transmission settings (β_I=200/365 or β_i=0.5 and b=0.3), low proportions of infections becoming symptomatic (p) combined with both lower levels and durations of transmission in the asymptomatic class (c and d respectively) in the stochastically modelled versions of Model A led to a greater chance of a dengue epidemic not occurring (compare subfigures A and C of Figure 7.3-13). This occurred either towards the low end of or excluded from the more conservative samples of parameter space for the proportion of infections developing symptoms (p) and level or duration of transmission from the asymptomatic class (c and d respectively). These regions of parameter space also

coincided with R_0 values just above 1. Therefore, validating the suggestion made in Chapter 5 and Chapter 6 that sets of parameters, which led to epidemics persisting more than two years without becoming endemic, would in a stochastic framework lead to a higher chance of those epidemics not occurring in the first place. As at values of R_0 being just above 1 there was a higher chance of an infected individual either becoming immune or dying before infecting someone else then at much higher values of R_0 (Anderson & May 1991a; Keeling & Rohani 2008a).

The lower transmission settings that had the highest number of trials not leading to an epidemic could be seen as representing Italy, several other European countries, 29 states of the USA and the parts of the Australasian continent, that have all seen the introduction of *Ae. albopictus* (M. Service 2012b; WHO 2015c). As discussed in the previous chapter *Ae. albopictus* feeds much more readily on other animals and is less suited to the urban environment then *Ae. aegypti* (Lambrechts et al. 2010). As such the low transmission setting for the mosquito dependent transmission version of Model A (β_i=0.5 and b=0.3), with a low biting rate is probably indicative of the areas where *Ae. albopictus* has recently been introduced. The change from modelling Model A in a deterministic fashion to stochastic fashion would modify the suggestion made in the previous chapter that from looking at Model A in the low transmission setting, that an epidemic may well occur in an area where *Ae. albopictus* has been introduced but it would not become endemic. To add the prevision that if the duration or level of asymptomatic transmission is low and there is a high rate of asymptomatic infection, then stochasticity would make such dengue epidemics less likely. This would also seem to validate the point made in Chapter 5 that considering if Quam et al. (2015) approximation of 572 apparent and 1747 inapparent dengue infections being imported through Rome's airport in a year is reliable, then it may be the case that stochasticity

plays a role in preventing imported dengue from causing regular epidemics from taking place in Italy or other countries where *Ae. albopictus* has recently become established.

As discussed in Chapter 5 and Chapter 6 the level of transmission from asymptomatic dengue infected individuals will be related to two features of asymptomatic dengue infection. The first is the level of viremia in asymptomatic dengue virus infections. Nguyet et al. (2013) reported that symptomatic dengue virus infections that where ambulatory had an infectious viremia, but it was lower than dengue virus infections in hospitalised patients. This work would suggest that asymptomatic dengue virus infections may have a lower viremia leading them to being less infectious to blood feeding mosquitoes (Nguyet et al. 2013). However there have been very few studies that have detected viremia in asymptomatic dengue virus infections (Beckett et al. 2005; Reyes et al. 2010; Duong et al. 2011; Chastel 2012; Carrington & Simmons 2014). Duong et al. (2011) being the only study to quantify the level of viremia in asymptomatic dengue virus infections, found no significant difference in the levels of viremia in asymptomatic and symptomatic dengue virus infections. It should be pointed out that Duong et al. (2011) had a small sample size and to date there has been no study testing whether mosquitoes feeding on asymptomatically dengue infected humans can become infected (Carrington & Simmons 2014).

It should also be noted that moving to a stochastic modelling approach from a deterministic one, allowed the occasional simulated epidemic of dengue virus to become endemic, when those same parameter settings did not lead to the dengue virus becoming endemic for the deterministic modelling approach seen Chapter 6. This happened for the mosquito dependent transmission version of Model A at a high transmission setting (β_i=1 and b=1), when Model A resembled an SIR type mode in one of two ways. Firstly through infections being symptomatic (p=1) or Model A closely resembling an SIR

model, due to nearly all infections developing symptoms (p is close to 1) (see Figure 7.13E, Figure 7.14E and Figure 7.19-20). This region of parameter space is excluded from the more conservative sample of parameter space for the proportion of infections developing symptoms (p), as $p>0.7$. Secondly the level and duration of transmission from the asymptomatic class being similar to the symptomatic class (c and d are 1 or close to it) (see Figure 7.13E, Figure 7.14E and Figure 7.19-20). This would suggest that not only that parameter settings that allow a disease to become endemic when modelled deterministically, do not necessarily lead to a disease becoming endemic when modelled stochastically, but parameter settings that do not allow a disease to become endemic when modelled deterministically can lead to a disease becoming endemic when modelled stochastically. This would effectively happen through a run of "bad luck" where repeatedly a number of individuals remain in a disease transmitting state for much longer than average, or more mosquitoes are infected from biting dengue virus infected humans than average.

The incorporation of stochasticity in to frequency dependent transmission and mosquito dependent transmission versions of Model A led to a similar pattern in the proportion of population resistant to the invading dengue serotype, provided an epidemic occurred. This is also true for the more conservative samples of parameter space for the proportion of infections developing symptoms (p), level and duration of transmission from the asymptomatic class (c and d respectively). Therefore there is no change to the two suggestions made in Chapter 5 and Chapter 6 regarding the proportion of population resistant to the invading dengue serotype. Firstly that should there be a second epidemic caused by a different dengue virus serotype a larger proportion of the population may be at risk of DHF then would be suggested from the number of reported DF cases in the first epidemic. Secondly that if in the first epidemic

dengue virus were to become endemic this at risk population would remain at a high proportion of the population through time.

7.5 Conclusion

The conclusions in Chapter 5 and Chapter 6 pointed to the need for a greater knowledge of the mosquito biting rate experienced by asymptomatic dengue virus infections, the level and duration of transmission from these infections, as well as the proportion of dengue virus infections that develop symptoms. In this chapter moving from a deterministic process to a stochastic process for frequency dependent transmission and mosquito dependent transmission versions of Model A, not only adds credence to these points but led to key additional insights being made. Where combinations of parameters led to R_0 being only just over 1 epidemics often did not occur due to stochasticity. Such parameter settings occurred at a lower transmission level, with low rates of symptomatic dengue virus infection combined with lower transmission or durations of transmission in asymptomatic dengue virus infections. This would suggest that in countries where *Ae. albopictus* has recently become established, if it is the case that most dengue virus infections are asymptomatic and such infections have either a lower level or duration of transmission, then stochasticity could prevent many epidemics from occurring. Therefore the proportion of dengue virus infections that are symptomatic needs to be verified. As discussed in Chapter 5, Grange et al. (2014) review found a large degree of variation in the measure of the proportion of dengue virus infections that are symptomatic, especially between estimate using index cluster methods and cohort studies. Therefore the findings of this chapter along with Chapter 5 and Grange et al. (2014) highlight the need for comparative research between cohort studies and index cluster studies in order to assess which is the most accurate, followed by standardisation of methodologies in future studies assessing this.

In the higher transmission setting where the lower rates of progression to symptomatic dengue virus infection combined with higher durations or levels of asymptomatic dengue virus infection transmission led to dengue becoming endemic, the inclusion of stochasticity made this endemicity no longer guaranteed. Higher durations of asymptomatic dengue virus infection where less affected by this, suggesting that the duration of asymptomatic dengue virus infection may be more of a key determinant for a dengue epidemic becoming endemic than the transmission from such infections. As discussed in Chapter 5 and 6 direct research via screening mosquitoes fed on asymptomatic dengue virus infected humans for dengue virus, in order to ascertain the duration of transmission, has so far been completely lacking (possibly due to feasibility). There has been some related research on the viremia of asymptomatic dengue virus infected humans but this so far has been limited (Beckett et al. 2005; Reyes et al. 2010; Duong et al. 2011; Chastel 2012; Carrington & Simmons 2014), none of it looking into the duration of viremia. Such research could be furthered by studying how the level and duration viremia in asymptomatically dengue virus infected humans compares to symptomatically dengue virus infected humans. Hopefully such research would lead to a greater understanding of the transmission from asymptomatically dengue virus infected humans through the lifetime of infection.

Chapter 8: Discussion

8.1 Summary of thesis findings

The molecular analyses of some of the samples in Chapter 2 revealed a higher infection rate for *Plodia interpunctella* larvae raised on low quality food. This suggests that decreased food resource level, reduced the second generation of *P. interpunctella's* ability to suppress or ultimately clear PiGV infection. Further experimentation would be needed to untie the effect of food resource on the processes involved in the infection of baculoviruses. Injection of PiGV into *P. interpunctella* may provide a possible solution, as the infection route would not be affected by any changes in *P. interpunctella* caused by food resource level. Within Chapter 2 none of the 995 larvae from PiGV treatments raised on low quality food showed overt signs of PiGV infection, suggesting that the lower quality food does not stress the larvae enough to cause the activation of PiGV. Then again neither did any of the1774 larval offspring from parents infected with PiGV. This would suggest therefore that a greater range of food resources (including starvation), differing nutrient ratios and unusual food resources need to be studied to ascertain whether or not food resource can affect the activation and vertical transmission of covert baculoviruses, not only in the offspring generation but the parental generation, as well. There is also a lack of research surrounding the effects of viral dose and parental host instar at the point of infection, on the vertical transmission of covert baculoviruses. The role of other infectious agents in causing activation of covert baculovirus infections also needs to be further explored.

Chapter 3 found that inbreeding had no significant effect on the vertical transmission of PiGV in *P. interpunctella*. This could be due to the low rate of vertical transmission leading to a low sample in terms of PiGV infected larvae. Further study of

other insect baculovirus systems with much higher vertical transmission rates may be needed to verify the effect of inbreeding on the vertical transmission of baculoviruses. Other studies have found differing results regarding the effect of inbreeding on pathogen susceptibility and immunity. Therefore it may be the case that the effects of inbreeding on an insect's immunity and susceptibility to a pathogen may be specific to that insect, its ecological niche or the pathogen.

Chapter 4 overall concludes that vertical transmission of dengue virus is of little importance to the epidemiology and persistence of dengue virus; pointing to the low rates of vertical transmission seen in the field, the sheer sampling effort in obtaining such results, as well as the many field studies, which found no evidence of vertical transmission of dengue virus. A combination of asymptomatic dengue virus infection in humans and movement of dengue virus infected humans may well be more important. That being said vertical transmission may possibly lead to persistence of dengue viruses if combined with two other processes. The possibility of vertical transmission of dengue virus to larvae leading to the horizontal transmission of dengue virus between larvae via cannibalism needs to be further explored as a possible contributing factor in the persistence of dengue virus. Furthermore, there is a lack of work on vertical transmission of dengue virus into diapausing or desiccated eggs and the impact of dengue virus infection on survivorship. However, work on these two areas may be hampered logistically due to the low rate of vertical transmission and sampling effort required.

Whilst Chapter 5 found that in two frequency dependent models asymptomatic infections did not lead to dengue becoming endemic. However, Chapter 5 demonstrated that asymptomatic dengue virus infections could lead to dengue virus persisting for several years in lower transmission settings with either a low level or duration of

transmission from asymptomatic dengue virus infections. Furthermore, Chapter 5 reveals that a larger proportion of the population could also be left at risk of DHF than necessarily suggested by dengue fever cases (symptomatic dengue virus infections). This is despite one of the models making unrealistic assumptions about the length of time an infection progresses from an asymptomatic state to a symptomatic state.

Lacking from Chapter 5's two models was the explicit inclusion of mosquitoes in the transmission dynamics of dengue viruses; Chapter 6 included mosquitoes within these two models and broadly found the same results. Except for finding that the dengue virus could become endemic, provided the transmission setting was high and the probability of developing symptoms was less than 1 and there was either a high rate or duration of transmission in asymptomatic infections.

With the inclusion of stochasticity into one of the models of asymptomatic dengue virus infections, Chapter 7 caused key additional insights to be made. Where combinations of parameters led to R_0 being only just over 1, epidemics often did not occur due to stochasticity. Such parameter settings occurred at a lower transmission level with low rates of symptomatic dengue virus infection combined with lower transmission or durations of transmission in asymptomatic dengue virus infections. This would suggest that in countries where *Ae. albopictus* has recently become established, if it is the case that most dengue virus infections are asymptomatic and such infections have either a lower level or duration of transmission, then stochasticity could prevent many epidemics from occurring. In the higher transmission setting were the lower rates of progression to symptoms combined with higher durations or levels of transmission from asymptomatic dengue virus infections led to dengue becoming endemic in Chapter 6. The inclusion of stochasticity in Chapter 7 meant that the dengue virus did not always become endemic under these circumstances. Higher durations of asymptomatic dengue

virus infection were less affected by this, suggesting that the duration of asymptomatic dengue virus infection may be more of a key determinant for a dengue epidemic becoming endemic than the transmission from such infections.

Chapter 5, 6 and 7 point to the need for a greater knowledge of the rate at which mosquitos bite asymptomatically dengue virus infected people, as well as the level and duration of viremia in asymptomatically dengue virus infected people.

8.2 Future directions for studying vertical transmission in insect virus systems

Recapping back to Chapter 2, the first repeat of the experiment that was analysed molecularly found a vertical infection rate from a parent infected with PiGV of 2.5%-5.6%. An even lower vertical infection rate of 0.35-0.48% was obtained for PiGV in Chapter 4. This unfortunately suggests PiGV's vertical infection rate is rather low. This means that further experimentation using the *P. interpunctella* PiGV model system to explore the ecology and evolution of vertical transmission or covert baculoviruses may be logistically unfeasible. Logistically, therefore, it may be better to use an insect baculovirus system with a higher rate of vertical infection.

One such area of research is the circumstances that select for vertical transmission of viruses. Fuxa & Richter (1991) were able to select for an increased rate of vertical transmission of SfNPV through isolation SfNPV in the host pupae of *Spodoptera frugiperda* produced by parents who had survived SfNPV infection. Of interest regarding this PhD thesis is that both the wild type and selected strains of SfNPV produced overt and covert infections within the offspring of orally infected *S. frugiperda* (Fuxa & Richter 1991). It is of note that further experimentation by Fuxa & Richter (1992) followed transgenerational mortality of SfNPV overt infections up to the F5 and F7 generations for the wild-type and selected strains of SfNPV. This demonstrates that the vertical transmission of baculoviruses can be selected for. The

circumstances under which a covert vertically transmitted baculovirus would be selected for in nature were hypothesised by Sorrell et al. (2009). Through mathematical simulations Sorrell et al. (2009) suggested that vertically transmitted covert viral infection would be promoted in highly fecund hosts that go through fluctuating population densities, as found in many insect baculovirus systems. Therefore, the use of another insect baculovirus system with a higher vertical transmission rate could be used to test the suggestion made by Sorrell et al. (2009). An experimental setup within a microcosm where population densities of an insect host alternate across many generations with the baculovirus present in the host population would be one way to test this suggestion.

Chapter 2 concluded that a greater range of food resources, different protein:carbohydrate levels and unusual food resources need to be studied to ascertain whether or not food resource can affect the activation of covert baculovirus infections. Furthermore, that the role of other infectious agents in causing activation of covert baculovirus infections also needs to be further explored. As suggested in Chapter 2, regarding food quality, if the possible causes of covert baculovirus activation were to be further explored, it may be logistically wiser to use an insect baculovirus system with a higher rate of vertical transmission than that found in PiGV infections. It was also suggested in Chapter 3 that the use of an insect baculovirus system with a higher rate of vertical transmission may be necessary to further explore the effect of inbreeding on vertically transmitted covert viruses in insects.

8.3 Vertical transmission in other mosquito borne viral infections

Vertical transmission has been demonstrated within many other mosquito borne viral pathogens (Rosen et al. 1978; Diallo et al. 2000; Nelms et al. 2013; Agarwal et al. 2014; Lequime & Lambrechts 2014). There has been a recent systematic review by

Lequime & Lambrechts (2014) of studies researching vertical transmission of mosquito borne viral pathogens. Unfortunately this systematic review does not focus on the role of vertical transmission in the ecological dynamics of these diseases, but simply focuses on the trends of such studies, such as increases in there occurrence after major disease outbreaks and the development of different screening techniques. That being said Lequime & Lambrechts (2014) in their conclusion point out that in the field vertical transmission's prevalence was typically <0.1%. Lequime & Lambrechts (2014) point out that vertical transmission's prevalence in laboratory experiments may be 10 to 10,000 fold higher due to 100% of the parental generation being infected. This leads on to Lequime & Lambrechts (2014) suggesting that even with an efficient form of vertical transmission, it is unlikely that vertical transmission can explain the persistence of arboviruses, through periods lacking in horizontal transmission (i.e. low numbers of or none existence of vectors).

With many of the arbovirus pathogens of medical importance humans are not the main host. The yellow fever virus for instance is maintained within populations of monkeys and there is the occasional isolated case or spill-over outbreak in humans (M. Service 2012b; WHO 2014b). Similarly with both West Nile virus and Japanese encephalitis virus humans are dead end hosts, only being able to become infected but not developing a high enough viremia to be infectious to a biting mosquito (WHO 2011; M. Service 2012b; WHO 2014a). In the case of West Nile virus populations of multiple bird species (mostly corvids) maintain the virus (WHO 2011; M. Service 2012b). Likewise, for Japanese encephalitis virus, multiple water based bird species and pigs act as transmitting hosts (M. Service 2012b; WHO 2014a). Where there are multiple host species, transmission dynamics between these host species may be a more likely candidate for the persistence of an arbovirus through seasons of low vector abundance

than vertical transmission of the arbovirus. It may be the case that vertical transmission combined with transmission from an alternate host could lead to persistence of arbovirus where horizontal transmission between mosquitos and the main host would not allow an arbovirus to persist through seasons of low vector abundance. A recent theoretical study by Manore & Beechler (2015) on the persistence of Rift Valley fever virus demonstrates this. Rift Valley fever virus is a much more significant veterinary problem than a medical one, causing unexpected abortions in livestock and large mortalities in lambs (WHO 2010). Manore & Beechler (2015) modelled the zoological transmission cycle of Rift Valley fever virus with buffalo as the main host. Manore & Beechler (2015) found that one of the most realistic scenarios for the persistence of Rift Valley fever virus through seasons of low vector abundance was a combination of vertical transmission with in mosquito vectors (at levels seen in previous studies) and the involvement of an alternate mammalian host (possessing a lifespan of 7 years as opposed to a buffalo's 15 years).

Therefore Lequime & Lambrechts (2014) suggestion that, even with an efficient form of vertical transmission, it is unlikely that vertical transmission can explain the persistence of arboviruses through periods lacking in horizontal transmission and may be too broad a generalisation. Considering that most arboviruses have multiple hosts and that the review in Chapter 4 highlighted the many problems with research on vertical transmission of dengue viruses and pointed to the few possibilities where vertical transmission could cause the persistence of dengue viruses. Therefore I would hope that the large database of studies researching vertical transmission mosquito borne viral pathogens collected for Lequime & Lambrechts (2014) systematic review is used to conduct further reviews, looking at specific arboviruses and the role that vertical transmission plays in the ecological dynamics of that specific arbovirus. In order to

assess any problems with the research on vertical transmission of that specific arbovirus and point to the possible way in which vertical transmission could cause the persistence of that specific arbovirus.

8.4 Possible future avenues for modelling work on the role of asymptomatic dengue infections

The work on modelling asymptomatic dengue infections in this PhD is by its nature simplistic, but considering the paucity of models of dengue epidemiology that include asymptomatic dengue infections, this could be a vital first step in our understanding of asymptomatic dengue infections in dengue's epidemiology. Future models on asymptomatic dengue virus infections, looking at how such infections interact with other aspects of dengue virus ecology, could reveal the extent to which transmission from asymptomatic dengue virus infections drive dengue virus epidemiology.

For example work by Getis et al. (2003) showed that adult *Ae. aegypti* clustered within houses and weakly up to 30 meters beyond houses, suggesting that adult *Ae. aegypti* populations are heterogeneously spaced. Furthermore Boyer et al. (2014) found heterogeneous spacing of immature forms of *Ae. ablopictus*, suggesting that adult *Ae. ablopictus* may also be heterogeneously spaced. If both of these key vectors are dispersed in clusters it could be the case that asymptomatic dengue infections could cause greater transmission through their greater movement between these different clusters. Luz et al. (2003) modelled the effect of human movement between two patches of differing mosquito density, likewise Adams & Kapan (2009) modelled people regularly commuting through and between different patches of mosquito densities. Luz et al. (2003) found that human movement could lead to an increase chance of epidemics taking hold in a population and Adams & Kapan (2009) found that high degrees of

human movement to patches of high mosquito densities could lead to dengue becoming endemic. Incorporation of an ambulatory asymptomatic dengue virus infected class into either of these models could reveal a significant role for asymptomatic infection in spreading dengue virus.

Getis et al. (2003) work on clustering of *Ae. aegypti* suggested that adult *Ae. aegypti* in urban settings do not fly far from the container where they developed as larvae and pupae. Whilst the maximum flight range of *Ae. aegypti* can be quite large and varies from less than 40m to greater than 1.2 km (Silver 2008), it has been argued that more epidemiologically relevant is the mean mosquito flight range, especially after the first blood meal or oviposition (Rodhain & Rosen 1997). Harrington et al. (2005) found a range of 28-199m mean *Ae. aegypti* dispersal across several different sites in Thailand and Puerto Rico. The key finding was that the majority of mosquitos were found in the house in which they were released or in an adjacent house (Harrington et al. 2005). Maciel-de-Freitas et al. (2010) found that most *Ae. aegypti* did not move much after the first 1-2 days after release. This suggests the mosquitoes did not move much after locating there first blood meal. Both the work by Harrington et al.(2005) and Maciel-de-Freitas et al. (2010) would suggest *Ae. aegypti* move little. Stoddard et al. (2013) analysed dengue infection in Iquitos, Peru, finding that the risk of infection was characterised by visits to places where contact with infected mosquitoes was most likely and independent of proximity to the homes of other infected people. Considering the lack of movement of *Ae. aegypti* (Rodhain & Rosen 1997; Harrington et al. 2005; Maciel-de-Freitas et al. 2010), Stoddard et al. (2013) and Reiner et al. (2014) point to human social movement as the main driver dengue of transmission. As with models that take into account mosquito spatial heterogeneity (Luz et al. 2003; Adams & Kapan 2009), incorporation of an ambulatory asymptomatic infected class into models of

dengue transmission where mosquitoes move little, could reveal that through its greater host movement asymptomatic infection drives the spread of dengue.

Reiner et al. (2014) through models of human social movement between groups was able to recreate the dengue infection dynamics seen by Stoddard et al. (2013). Asymptomatic infections do not lead to the decreased social movement seen in symptomatic infections. Therefore the consideration of asymptomatic dengue virus infections in such a social structured agent-based models as Reiner et al. (2014), could also cast further light on the importance of asymptomatic infections in dengue virus transmission dynamics.

8.5 Inapparent and vertically transmitted infections in two host-virus systems: the wider ecological context.

The main aim of studying inapparent and vertically transmitted infections in these two host-virus systems was to determine the role of these two processes in the ecological persistence of pathogens. Inapparent infections whether in the form of covert or asymptomatic infections could allow a pathogen to persist by providing a reservoir of infected hosts, whose role would be at first sight hidden from researchers, medics, vets and conservationists. Similarly vertical transmission could provide a pathogen with a reservoir of infection that persists across generations.

The introductory chapter (see Section 1.4) mentioned that several viruses are thought to persist in their host population with the majority of infections being inapparant infected in the form of asymptomatic infections. For example cowpox within wood mice and bank voles (Telfer et al. 2005) and squirrel parapoxvirus causing no signs of infection for the vast majority of grey squirrels (Sainsbury et al. 2000). Furthermore the polio virus being thought to persist, in the few countries where it is endemic, through the large proportion of asymptomatic infections (72%) acting as a

reservoir (Hamborsky et al. 2015). As discussed further research may be needed to prove that asymptomatic infections definitively can cause dengue viruses to persist for longer or indefinitely (see Section 5.4-5, 6.4-5, 7.4-5 and 8.4). However, the models in Chapter 5-7 demonstrate that inapparent (asymptomatic) infections can theoretically cause a pathogen to persist for longer or indefinitely under certain combinations of overall level of transmission, proportions of asymptomatic infection, levels and durations of transmission from asymptomatic infections. Taken all together this would suggest that under certain ecological conditions, asymptomatic infections could lead to a persisting reservoir of infection. Where asymptomatic infections are suspected to be a means of pathogen persistence, further research on the proportion of asymptomatic infections, the level and duration of transmission from such infections would verify such ecological conditions.

As mentioned in section 1.5 of the introductory chapter, Sorrell et al. (2009) predicted that highly fecund hosts that go through fluctuating population densities would select for an infectious agent using a covert strategy for persistence. Sorrell et al. (2009) pointed to a few studies which found insects covertly infected with baculoviruses as examples, of which there are many dating back to the 1950s (Steinhaus 1958; Steinhaus & Dineen 1960; Jaques 1962; Longworth & Cunningham 1968; Etzel 1976; Biever & Wilkinson 1978; Jurkovíčová 1979; Fuxa & Richter 1992; Hughes et al. 1997; Fuxa et al. 1999; Cooper et al. 2003; Burden et al. 2003; Burden et al. 2006; Vilaplana et al. 2008; Vilaplana et al. 2010; Murillo et al. 2011). A key feature of covert infections is that at some point the infection may switch to an overt form (activation), leading to the horizontal transmission of the infectious agent (Sorrell et al. 2009). The covert inapparent form of PiGV infection was not demonstrated to be activated into an overt form by low quality food (see Chapter 2), neither was this activation seen to be

affected by host inbreeding (see Chapter 3). Chapter 2 pointed to the many studies definitely showing that covert baculoviruses could be activated by infection from other baculoviruses (Longworth & Cunningham 1968; Jurkovíčová 1979; Hughes et al. 1993; Cooper et al. 2003; Burden et al. 2003). Whilst there is some evidence hinting at lack of food (Jaques 1962; Myers et al. 2011) and unusual diet (David & Gardiner 1965; Ilyinykh et al. 2013) as means of activation of overt infection Chapter 2 highlights the further research needed in these areas. Sorrell et al. (2009) models also found that such covert infection would be selected for in long-lived slow reproducing hosts when there was even a small variation in transmission. Viruses of the family Herpesviridae, such as varicella-zoster virus, herpes simplex 1 and 2 (Goering et al. 2008d) (see section 1.5) do persist by activating from a covert form to an overt form within long lived hosts. Within humans many covert infections as these have been demonstrated to be activated by old age, pregnancy and other infections leading to host immunosuppression (Goering et al. 2008d). Therefore there is empirical support for Sorrell et al. (2009) suggestion that covert infections could provide a pathogen with a persisting reservoir of infection provided there is variation in horizontal transmission in a long lived slow reproducing host, or fluctuating host density in a short lived highly fecund host. However particularly in the case of covert infections of highly fecund hosts with fluctuating population densities there is need for further research into the means of activation to overt forms of infection.

Chapters 2 and 3 found extremely low rates of vertical transmission for PiGV suggesting that vertical transmission was not a means of persistence for strain of PiGV available. Likewise, Chapter 4 concluded that due to the low rates of vertical transmission seen in the field and the many field studies which found no evidence of vertical transmission of dengue virus, it is unlikely that vertical transmission of dengue

virus is of any importance to the persistence of dengue virus. However, there are many pathogens which are thought to persist through vertical transmission. As mentioned in the introductory chapter (see Section 1.3) Human T-cell Lymphotropic Virus type 1 (HTLV-1) is thought to persist through vertical transmission being transmitted through breast milk with some horizontal transmission through sexual intercourse (Goering et al. 2008g; Goering et al. 2008c). The protozoan pathogen *Ophryocystis elektroscirrha* persist through being vertically transmitted. Vertical transmission occurring when adult monarch butterflies *Danaus plexippus* scatter *O. elektroscirrha* spores over their eggs and surrounding milkweed foliage, these spores are then eaten by host larvae shortly after hatching (McLaughlin & Myers 1970; Lefèvre et al. 2012). Vilaplana et al. (2010) found extremely high rates of covert infection of African armyworm's (*Spodoptera exemta*) NPV in the field and nearly 100% of *S. exemta* were covertly infected within a lab colony. Due to the very rare occurrence of overt infection over the 5 years that this lab colony was maintained Vilaplana et al. (2010) point to these findings as evidence that *S. exemta's* NPV was persisting through vertical transmission in the lab and in the field. Lipsitch et al. (1996) used mathematical models to explore the circumstances which led to vertical and horizontal transmission being evolutionary stable strategies (ESSs), under the constraining influence of virulence on these transmission routes. Lipsitch et al. (1996) found that low virulence selected for high rates of vertical transmission and lower rates of horizontal transmission, but horizontally transmitting strains would outcompete vertically transmitting strains under high virulence. This first scenario does as such have vertical transmission providing a pathogen with a persisting reservoir of infection. However, Lipsitch et al. (1996) warns evolutionary trajectories depend on the constraints in the associations of vertical and horizontal transmission to virulence that are caused by the biology of host and pathogen. Therefore vertical

transmissions can provide a pathogen with a persisting reservoir of infection and Lipsitch et al. (1996) research provides a theoretical framework for understanding the circumstance for its evolution. However, further research needs to add to Lipsitch et al. (1996) findings and demonstrate more of the ecological conditions under which vertical transmission provides a pathogen with a persisting reservoir of infection.

Over all inapparent and vertically transmitted infections may provide pathogens with a method of persistence in host populations. However, in the research outlined in Chapters 2-4 inapparent or vertically transmitted infections do not provide pathogens with a method of persistence. It is therefore difficult to see if there are broad trends or parallels in pathogens using inapparent and vertically transmitted infections as means of persistence, from looking at the findings of Chapters 2-4. There are other host pathogen systems in nature where pathogens do persist through inapparent, vertically transmitted infections or a combination of both. Chapters 5-7 firstly demonstrate that dengue viruses could be persisting through inapparent (asymptomatic) infections. Secondly Chapters 5-7 demonstrate that in theory that inapparent (asymptomatic) infections can provide a pathogen a persisting reservoir of infection under certain combinations of overall level of transmission, proportions of asymptomatic infection, level and durations of transmission from asymptomatic infections. Similarly Sorrell et al. (2009) theoretical work demonstrated that the ESS for covert infections was produced in short lived, fecund hosts with fluctuating population densities or long lived, slow reproducing hosts with variable horizontal transmission rates. Likewise, Lipsitch et al. (1996) found that under low virulence the ESS was for high rates of vertical transmission and lower rates of horizontal transmission, but under high virulence horizontally transmitting strains would outcompete vertically transmitting strains. The work in Chapter 5-7, Sorrell et al. (2009) and Lipsitch et al. (1996) are all theoretical; as such further investigations are

needed either to clarify these studies or provide evidence from the field to confirm their findings. Further investigation into asymptomatic dengue virus infections may shed further light on the role of inapparant (asymptomatic) infections in the persistence of pathogens. However, for vertically transmitted or inapparant (covert) infections investigation of other pathogen host systems where these process occur at higher rates may be more likely to demonstrate their effect on pathogen persistence.

Bibliography

Adams, B. & Boots, M., 2010. How important is vertical transmission in mosquitoes for the persistence of dengue? Insights from a mathematical model. *Epidemics*, 2(1), pp.1–10.

Adams, B. & Kapan, D.D., 2009. Man bites mosquito: understanding the contribution of human movement to vector-borne disease dynamics. *PloS one*, 4(8), p.e6763.

Agarwal, A. et al., 2014. Evidence of experimental vertical transmission of emerging novel ECSA genotype of Chikungunya virus in *Aedes aegypti*. *PLoS neglected tropical diseases*, 8(7), p.e2990.

Ahmad, R. et al., 1997. Detection of dengue virus from field *Aedes aegypti* and *Aedes albopictus* adults and larvae. *The Southeast Asian journal of tropical medicine and public health*, 28(1), pp.138–42.

Akbar, M.R. et al., 2008. PCR detection of dengue transovarial transmissibility in *Aedes aegypti* in Bandung, Indonesia. *Proceedings of The ASEAN Congress of Tropical Medicine and Parasitology*, 3, pp.84–89.

Amaku, M. et al., 2014. A comparative analysis of the relative efficacy of vector-control strategies against dengue fever. *Bulletin of Mathematical Biology*, 76, pp.697–717.

Anderson, R.M. & May, R.M., 1991a. Basic reproductive rate of a parasite. In *Infectious Diseases of Humans: Dynamics and Control*. Oxford Science Publications, pp. 17–19.

Anderson, R.M. & May, R.M., 1991b. Threshold host densities. In *Infectious Diseases of Humans: Dynamics and Control*. Oxford University Press, pp. 19–21.

Andraud, M. et al., 2012. Dynamic epidemiological models for dengue transmission: a systematic review of structural approaches. *PloS one*, 7(11), p.e49085.

Angel, B. & Joshi, V., 2008. Distribution and seasonality of vertically transmitted dengue viruses in Aedes mosquitoes in arid and semi-arid areas of Rajasthan, India. *Journal Of Vector Borne Diseases*, 45(1), pp.56–59.

Angel, B., Sharma, K. & Joshi, V., 2008. Association of ovarian proteins with transovarial transmission of dengue viruses by Aedes mosquitoes in Rajasthan , India. *Indian Journal Of Medical Research*, (September), pp.320–323.

Arguello, D.F. et al., 2015. Incidence of dengue virus infection in school-aged children in Puerto Rico: A prospective seroepidemiologic study. *American Journal of Tropical Medicine and Hygiene*, 92(3), pp.486–491.

Arunachalam, N. et al., 2008. Natural vertical transmission of dengue viruses by A*edes aegypti* in Chennai, Tamil Nadu, India. *The Indian journal of medical research*, 127(4), pp.395–7.

Bara, J.J., Clark, T.M. & Remold, S.K., 2013. Susceptibility of larval *Aedes aegypti* and *Aedes albopictus* (Diptera : Culicidae) to dengue virus. *Journal of medical entomology*, 50(1), pp.179–184.

Bartlett, M.S., 1960. The critical community size for measles in the U.S. *J R Stat Soc A*, 123(for 1940), pp.37–44.

Beckett, C.G. et al., 2005. Early detection of dengue infections using cluster sampling

around index cases. *The American journal of tropical medicine and hygiene*, 72(6), pp.777–82.

Begon, M., Sait, S.M. & Thompson, D.J., 1996. Predator-prey cycles with period shifts between two and three-species systems. *Nature*, 381, pp.311–315.

Bhatt, S. et al., 2013. The global distribution and burden of dengue. *Nature*, 496(7446), pp.504–7.

Biever, K. & Wilkinson, J., 1978. A stress-induced granulosis virus of *Pieris rapae*. *Environmental Entomology*, 7, pp.572–573.

Das Bina, P. et al., 2008. Natural vertical transmission of dengue virus in peak summer collections of *Aedes aegypti* (Diptera : Culicidae) from urban areas of Jaipur (Rajasthan) and Delhi. *Journal of Communicable Disease*, 40(2), pp.155–157.

Bjornstad, O.N. et al., 1998. Population dynamics of the Indian meal moth: demographic stochasticity and delayed regulatory mechanisms. *Journal of Animal Ecology*, 67(1), pp.110–126.

Bonsall, M.B., Sait, S.M. & Hails, R.S., 2005. Invasion and dynamics of covert infection strategies in structured insect-pathogen populations. *Journal of Animal Ecology*, 74(3), pp.464–474.

Boots, M., 2000. Density-independent resource limitation and the transmission of an insect pathogen. *Oecologia*, 124(2), pp.172–175.

Boots, M. et al., 2003. The population dynamical implications of covert infections in host – microparasite interactions. *Journal of Animal Ecology*, 72, pp.1064 – 1072.

Boots, M. & Begon, M., 1994. Resource limitation and the lethal and sublethal effects of a viral pathogen in the Indian meal moth , *PIodia interpunctella*. *Ecological Entomology*, 19, pp.319–326.

Boots, M. & Begon, M., 1993. Trade-offs with resistance to a granulosis virus in the Indian meal moth, examined by a laboratory evolution experiment. *Functional Ecology*, 7(5), pp.528–534.

Boots, M. & Mealor, M., 2007. Local interactions select for lower pathogen infectivity. *Science*, 315(5816), pp.1284–6.

Boots, M. & Roberts, K.E., 2012. Maternal effects in disease resistance: poor maternal environment increases offspring resistance to an insect virus. *Proceedings. Biological sciences / The Royal Society*, 279(1744), pp.4009–14.

Bosio, C.F. et al., 1992. Variation in the efficiency of vertical transmission of dengue-l virus by strain s of *Aedes albopictus* (Diptera : Culicidae). *Journal of medical entomology*, 29(6), pp.985–989.

Boyer, S., Foray, C. & Dehecq, J.S., 2014. Spatial and temporal heterogeneities of *Aedes albopictus* density in La Reunion Island: Rise and weakness of entomological indices. *PLoS ONE*, 9(3), pp.1–12.

Brady, O.J. et al., 2013. Modelling adult *Aedes aegypti* and *Aedes albopictus* survival at different temperatures in laboratory and field settings. *Parasites & vectors*, 6, p.351.

Buckner, E.A., Alto, B.W. & Lounibos, L.P., 2013. Vertical transmission of Key West dengue-1 virus by *Aedes aegypti* and *Aedes albopictus* (Diptera : Culicidae) Mosquitoes from Florida. *Journal of medical entomology*, 50(6), pp.1291–1297.

Burden, J. et al., 2002. Vertical transmission of sublethal granulovirus infection in the Indian meal moth, *Plodia interpunctella*. *Molecular Ecology*, 11(3), pp.547–555.

Burden, J.P. et al., 2003. Covert infections as a mechanism for long-term persistence of baculoviruses. *Ecology Letters*, 6(6), pp.524–531.

Burden, J.P. et al., 2006. Phenotypic and genotypic characterisation of persistent baculovirus infections in populations of the cabbage moth (*Mamestra brassicae*) within the British Isles. *Archives of virology*, 151(4), pp.635–49.

Calleri, D. V et al., 2006. Inbreeding and disease resistance in a social insect: effects of heterozygosity on immunocompetence in the termite *Zootermopsis angusticollis*. *Proceedings. Biological sciences / The Royal Society*, 273, pp.2633–2640.

Campbell, A., 2003. The future of bacteriophage biology. *Nature reviews. Genetics*, 4(6), pp.471–477.

Campbell-lendrum, D. et al., 2015. Climate change and vector-borne diseases: what are the implications for public health research and policy? *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 370(1665).

Carrington, L.B. et al., 2015. Naturally-acquired dengue virus infections do not reduce short-term survival of infected *Aedes aegypti* from Ho Chi Minh City, Vietnam. *American Journal of Tropical Medicine and Hygiene*, 92(3), pp.492–496.

Carrington, L.B. & Simmons, C.P., 2014. Human to mosquito transmission of dengue viruses. *Frontiers in Immunology*, 5(JUN), pp.1–8.

de Castro, M.G. et al., 2004. Dengue virus detection by using reverse transcription-polymerase chain reaction in saliva and progeny of experimentally infected *Aedes albopictus* from Brazil. *Memórias do Instituto Oswaldo Cruz*, 99(8), pp.809–14.

Cecílio, A.B. et al., 2009. Natural vertical transmission by *Stegomyia albopicta* as dengue vector in Brazil. *Brazilian journal of biology*, 69(1), pp.123–7.

Charlesworth, D., 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics*, 18(1), pp.237–268.

Charron, M.V.P. et al., 2013. How much can Diptera-borne viruses persist over unfavourable seasons? *PloS one*, 8(9), p.e74213.

Chastel, C., 2012. Eventual role of asymptomatic cases of dengue for the introduction and spread of dengue viruses in non-endemic regions. *Frontiers in physiology*, 3(March), p.70.

Chen, C.-F. et al., 2010. Screening of dengue virus in field-caught *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) by one-step SYBR green-based reverse transcriptase-polymerase chain reaction assay during 2004-2007 in Southern Taiwan. *Vector borne and zoonotic diseases (Larchmont, N.Y.)*, 10(10), pp.1017–25.

Chen, W. et al., 1993. Vector competence of *Aedes albopictus* and *Ae. aegypti* (Diptera: Culicidae) to dengue I virus on Taiwan: development of the virus in orally and parenterally infected mosquitoes. *Journal of Medical Entomology*, 30(3), pp.524–530.

Chen, W.J., Wu, H.R. & Chiou, S.S., 2003. E/NS1 modifications of dengue 2 virus after serial passages in mammalian and/or mosquito cells. *Intervirology*, 46(5), pp.289–295.

Chiang, C.L. & Reeves, W.C., 1962. Statistical estimation of virus infection rates in mosquito vector populations. *American journal of hygiene*, 75, pp.377–391.

Chow, V.T. et al., 1998. Monitoring of dengue viruses in field-caught *Aedes aegypti* and *Aedes albopictus* mosquitoes by a type-specific polymerase chain reaction and cycle sequencing. *The American journal of tropical medicine and hygiene*, 58(5), pp.578–86.

Choy, M.M. et al., 2013. Short report: comparison of the mosquito inoculation technique and quantitative real time polymerase chain reaction to measure dengue virus concentration. *American Journal of Tropical Medicine and Hygiene*, 89(5), pp.1001–1005.

Coltman, D. et al., 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution*, 53(4), pp.1259–1267.

Cooper, D. et al., 2003. Nucleopolyhedroviruses of forest and western tent caterpillars: cross-infectivity and evidence for activation of latent virus in high-density field populations. *Ecological Entomology*, 28(1), pp.41–50.

Cory, J.S. & Myers, J.H., 2003. The ecology and evolution of insect baculoviruses. *Annual Review of Ecology, Evolution, and Systematics*, 34(1), pp.239–272.

Coutinho, F.A.B. et al., 2006. Threshold conditions for a non-autonomous epidemic system describing the population dynamics of dengue. *Bulletin of mathematical biology*, 68(8), pp.2263–82.

Cummings, D. et al., 2004. Travelling waves in the occurrence of dengue haemorrhagic fever in Thailand. *Nature*, 427(January), pp.344–347.

Das, B. et al., 2013. Molecular investigations of dengue virus during outbreaks in Orissa state, Eastern India from 2010 to 2011. *Infection, genetics and evolution*, 16, pp.401–10.

David, W. a & Taylor, C.E., 1977. The effect of sucrose content of diets on susceptibility to granulosis virus disease in *Pieris brassicae*. *Journal of invertebrate pathology*, 30(1), pp.117–8.

David, W.A.L. & Gardiner, B.O.C., 1965. The incidence of granulosis deaths in susceptible and resistant *Pieris brassicae* (Linnaeus) larvae following changes of population density, food and temperature. *Journal of Invertebrate Pathology*, 7, pp.347–355.

Davies, S.C., 2011. *Infections and the Rise of Antimicrobial Resistance*,

DEFRA TB Programme, 2013. *Request for Information: Various Bovine TB Costs (2008-2013*,

DeGrandi-Hoffman, G. et al., 2010. The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (*Apis mellifera* L.). *Journal of insect physiology*, 56(9), pp.1184–91.

Diallo, M., Thonnon, J. & Fontenille, D., 2000. Vertical transmission of the yellow fever virus by *Aedes aegypti* (Diptera, Culicidae): dynamics of infection in F1 adult progeny of orally infected females. *The American journal of tropical medicine and hygiene*, 62(1), pp.151–6.

Diamond, J., 1997. Lethal gift of livestock. In *Guns, Germs and Steel*. Vintage, pp. 195–214.

Diekmann, O., Heesterbeek, J. a P. & Roberts, M.G., 2010. The construction of next-generation matrices for compartmental epidemic models. *Journal of the Royal Society, Interface / the Royal Society*, 7, pp.873–885.

Drayton, J.M. & Jennions, M.D., 2011. Inbreeding and measures of immune function in the cricket *Teleogryllus commodus*. *Behavioural Ecology*, 22, pp.486–492.

Duong, V. et al., 2011. Clinical and virological factors influencing the performance of a ns1 antigen-capture assay and potential use as a marker of dengue disease severity. *PLoS Neglected Tropical Diseases*, 5(7).

Emery, V.J., Landry, J.-F. & Eckert, C.G., 2009. Combining DNA barcoding and morphological analysis to identify specialist floral parasites (Lepidoptera: Coleophoridae: Momphinae: Mompha). *Molecular ecology resources*, 9 Suppl s1, pp.217–23.

Endy, T.P., 2002a. Epidemiology of Inapparent and Symptomatic Acute Dengue Virus Infection: A Prospective Study of Primary School Children in Kamphaeng Phet, Thailand. *American Journal of Epidemiology*, 156(1), pp.40–51.

Endy, T.P., 2002b. Spatial and temporal circulation of dengue virus serotypes: a prospective study of primary school children in Kamphaeng Phet, Thailand. *American Journal of Epidemiology*, 156(1), pp.52–59.

Espinosa, M. et al., 2014. Vertical transmission of dengue virus in *Aedes aegypti* collected in Puerto Iguazú, Misiones, Argentina. *Revista do Instituto de Medicina Tropical de São Paulo*, 56(2), pp.165–7.

Esteva, L. & Vargas, C., 2000. Influence of vertical and mechanical transmission on the dynamics of dengue disease. *Mathematical biosciences*, 167(1), pp.51–64.

Etzel, L.K., 1976. Studies of transovum of a granulosis and transstadial transmission Virus of the Codling Moth. *Journal of invertebrate pathology*, 27, pp.13–26.

Fan, J. et al., 2014. A systematic review and meta-analysis of dengue risk with temperature change. *International Journal of Environmental Research and Public Health*, 12(1), pp.1–15.

Fasulo, T.R., 2008. History and insects. In J. L. Capinera, ed. *Encyclopaedia of Entomology*. Springer Science+Buisness Media, pp. 1810–1826.

de Figueiredo, M.L.G. et al., 2010. Mosquitoes infected with dengue viruses in Brazil. *Virology journal*, 7(Table 1), p.152.

Fouque, F., Garinci, R. & Gaborit, P., 2004. Epidemiological and entomological surveillance of the co-circulation of DEN-1, DEN-2 and DEN-4 viruses in French Guiana. *Tropical medicine & international health : TM & IH*, 9(1), pp.41–6.

Freedman, D.O. et al., 2010. Spectrum of disease and relation to place of exposure amoung ill returned travelers. *The New England Journal of Medicine*, 354(2), pp.119–130.

Freier, J.E. & Rosen, L., 1988. Vertical transmission of dengue viruses by *Aedes mediovittatus*. *The American journal of tropical medicine and hygiene*, 39(2), pp.218–222.

Freier, J.E. & Rosen, L., 1987. Vertical transmission of dengue viruses by mosquitoes of the *Aedes scutelaris* group. *The American journal of tropical medicine and hygiene*, 37(3), pp.640–647.

Fuxa, J. & Richter, A., 1991. Selection for an increased rate of vertical transmission of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) nuclear polyhedrosis virus. *Environmental Entomology*, 06, pp.603–609.

Fuxa, J.R. et al., 1999. Stressors and rearing diseases of *Trichoplusia ni*: evidence of vertical transmission of NPV and CPV. *Journal of invertebrate pathology*, 74(2), pp.149–55.

Fuxa, J.R. & Richter, A.R., 1992. Virulence and multigeneration passage of a nuclear polyhedrosis virus selected for an increased rate of vertical transmission. *Biological Control*, 2(3), pp.171–175.

Fuxa, J.R., Weidner, E.H. & Richter, a. R., 1992. Polyhedra without virions in a vertically transmitted nuclear polyhedrosis virus. *Journal of Invertebrate Pathology*, 60(1), pp.53–58.

Gerloff, C.U., Ottmer, B.K. & Schmid-Hempel, P., 2003. Effects of inbreeding on immune response and body size in a social insect, *Bombus terrestris*. *Functional Ecology*, 17(5), pp.582–589.

Getis, a et al., 2003. Characteristics of the spatial pattern of the dengue vector, *Aedes aegypti*, in Iquitos, Peru. *American Journal of Tropical Medicine and Hygiene*, 69(5), pp.494–505.

Goering, R. V et al., 2008a. Causes of infectious disease. In *Mims' Medical Microbiology*. Elsevier, pp. 125–157.

Goering, R. V et al., 2008b. Enteric fevers: typhoid and paratyphoid. In *Mims' Medical Microbiology*. Elsevier, pp. 311–313.

Goering, R. V et al., 2008c. HTLV-1 infection. In 4th, ed. *Mims' Medical Microbiology*. Elsevier, pp. 389–390.

Goering, R. V et al., 2008d. Persistent infections. In *Mims' Medical Microbiology*. Elsevier, pp. 185–189.

Goering, R. V et al., 2008e. Plague. In *Mims' Medical Microbiology*. Elsevier, pp. 413–415.

Goering, R. V et al., 2008f. Trail of illness from a slippery cook. In *Mims' Medical Microbiology*. Elsevier, p. 177.

Goering, R. V et al., 2008g. Vertical and horizontal transmission. In *Mims' Medical Microbiology*. Elsevier, p. 143.

Le Goff, G. et al., 2011. Natural vertical transmission of dengue viruses by *Aedes aegypti* in Bolivia. *Parasite*, 18, pp.277–280.

Gokhale, M.D. et al., 2001. Vertical transmission of dengue-2 virus through *Aedes albopictus* mosquitoes. *Journal of Communicable Disease*, 33(3), pp.212–215.

Grange, L. et al., 2014. Epidemiological risk factors associated with high global frequency of inapparent dengue virus infections. *Frontiers in Immunology*, 5(JUN), pp.1–10.

Grenfell, B., 1997. (Meta)population dynamics of infectious diseases. *Trends in Ecology & Evolution*, 12(10), pp.395–399.

Grzywacz, D. et al., 2013. The use of indigenous ecological resources for pest control in Africa. *Food Security*, 6(1), pp.71–86.

Gu, W., Lampman, R. & Novak, R.J., 2004. Assessment of arbovirus vector infection rates using variable size pooling. *Medical and veterinary entomology*, 18(2), pp.200–4.

Gu, W., Lampman, R. & Novak, R.J., 2003. Problems in estimating mosquito infection rates using minimum infection rate. *Journal of medical entomology*, 40(5), pp.595–6.

Gu, W. & Novak, R.J., 2004. Detection probability of arbovirus infection in mosquito populations. *The American journal of tropical medicine and hygiene*, 71(5), pp.636–8.

Gubler, D.J. et al., 1985. *Aedes* (Gymnometopa) *mediovittatus* (Diptera: Culicidae) a potential maintenance vector of dengue viruses in Puerto Rico. *Journal of medical entomology*, 22(5), pp.469–475.

Gubler, D.J., 2004. Cities spawn epidemic dengue viruses. *Nature Medicine*, 10(2), pp.129–130.

Guedes, D.R.D. et al., 2010. Patient-based dengue virus surveillance in *Aedes aegypti* from Recife, Brazil. *Journal of vector borne diseases*, 47(2), pp.67–75.

Günther, J. et al., 2007. Evidence of vertical transmission of dengue virus in two endemic localities in the state of Oaxaca, Mexico. *Intervirology*, 50(5), pp.347–52.

Guo, X. et al., 2007. Survival and replication of dengue-2 virus in diapausing eggs of *Aedes albopictus* (Diptera : Culicidae). *Journal of medical entomology*, 44(3), pp.492–497.

Guzman, M.G. et al., 2010. Dengue: a continuing global threat. *Nature reviews. Microbiology*, 8(12 Supplementary), pp.S7–S16.

Guzman, M.G. et al., 2007. Neutralizing antibodies after infection with dengue 1 virus. *Emerging Infectious Diseases*, 13(2), pp.282–286.

Guzmán, M.G. et al., 2000. Dr . Guzmán et al . Respond to Dr . Vaughn. *American Journal of Epidemiology*, 152(9), p.804.

Guzmán, M.G. et al., 2002. Enhanced severity of secondary dengue-2 infections: death rates in 1981 and 1997 Cuban outbreaks. *Revista panamericana de salud publica = Pan American journal of public health*, 11(4), pp.223–227.

Guzmán, M.G., 2000. Epidemiological studies on dengue in Santiago de Cuba, 1997. *American Journal of Epidemiology*, 152(9), pp.793–799.

Haag-Liautard, C. et al., 2009. Fitness and the level of homozygosity in a social insect. *Journal of Evolutionary Biology*, 22(1), pp.134–142.

Haig, D., 2012. Retroviruses and the placenta. *Current Biology*, 22(15), pp.R609–R613.

Hamborsky, J., Kroger, A. & Wolfe, C., 2015. Poliomyelitis. In *Epidemiology and Prevention of Vaccine-Preventable Diseases*. Centres for Disease Control and Prevention, pp. 297–310.

Harrington, L.C. et al., 2005. Dispersal of the dengue vector *Aedes aegypti* within and between rural communities. *The American journal of tropical medicine and hygiene*, 72(2), pp.209–20.

Haverkroft, A.J. et al., 2008. Societal costs of late blight in potato and prospects of durable resistance through cisgenic modification. *Potato Research*, 51(1), pp.47–57.

Hughes, D.S., Possee, R.D. & King, L. a, 1993. Activation and detection of a latent baculovirus resembling *Mamestra brassicae* nuclear polyhedrosis virus in *M. brassicae* insects. *Virology*, 194(2), pp.608–15.

Hughes, D.S., Possee, R.D. & King, L. a, 1997. Evidence for the presence of a low-level, persistent baculovirus infection of *Mamestra brassicae* insects. *The Journal of general virology*, 78 (Pt 7), pp.1801–5.

Hull, B. et al., 1984. Natural transovarial transmission of dengue 4 virus in *Aedes aegypti* in Trinidad. *The American journal of tropical medicine and hygiene*, 33(6), pp.1248–1250.

Hutamai, S. et al., 2007. A survey of dengue viral infection in *Aedes aegypti* and *Aedes albopictus* from re-epidemic areas in the north of Thailand using nucleic acid sequence based amplification assay. *The Southeast Asian journal of tropical medicine and public health*, 38(3), pp.448–454.

Ibáñez-Bernal, S. et al., 1997. First record in America of *Aedes albopictus* naturally infected with dengue virus during the 1995 outbreak at Reynosa, Mexico. *Medical and veterinary entomology*, 11(4), pp.305–9.

Ilkal, M. a et al., 1991. Entomological investigations during outbreaks of dengue fever in certain villages in Maharashtra state. *The Indian journal of medical research*, 93(MaY), pp.174–8.

Ilmonen, P. et al., 2008. Experimental infection magnifies inbreeding depression in house mice. *Journal of evolutionary biology*, 21(3), pp.834–41.

Ilyinykh, A. et al., 2013. Sensitivity of gypsy moth *Lymantria dispar* (L ., 1758) larvae from geographically removed populations to nucleopolyhedrovirus (Lepidoptera : Erebidae , Lymantriinae). *Shilap Revista de Lepidopterologia*, 41(163), pp.349–356.

Jaques, R.P., 1962. Stress and Nuclear Polyhedrosis in Crowded Populations of Trihoplusia ni (Hubner). *Journal of Insect Pathology*, 4, pp.1–22.

Jelinek, T., 2000. Dengue fever in international travellers. *Clinical infectious disease*, 31, pp.144–147.

Johansson, M. a., Hombach, J. & Cummings, D. a T., 2011. Models of the impact of dengue vaccines: A review of current research and potential approaches. *Vaccine*, 29(35), pp.5860–5868.

Joshi, V. et al., 2006. Importance of socioeconomic status and tree holes in distribution of Aedes mosquitoes (Diptera : Culicidae) in Jodhpur, Rajasthan , India. *Journal of medical entomology*, 43(2), pp.330–336.

Joshi, V., Mourya, D.T. & Sharma, R.C., 2002. Persistence of dengue-3 virus through transovarial transmission passage in successive generations of *Aedes aegypti* mosquitoes. *The American journal of tropical medicine and hygiene*, 67(2), pp.158–61.

Joshi, V. & Sharma, R.C., 2001. Impact of vertically-transmitted dengue virus on viability of eggs of virus-inoculated *Aedes aegypti*. *Dengue Bulletin*, 25, pp.103–106.

Joshi, V., Singhi, M. & Chaudhary, R.C., 1996. Transovarial transmission of dengue 3 virus by *Aedes aegypti*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 90, pp.643–644.

Jousset, F.-X., 1981. Geographic *Aedes aegypti* strains and dengue-2 virus: susceptibility, ability to transmit to vertebrate and transovarial transmission. *Annals de virologie*, 132, pp.357–370.

Jurkovíčová, M., 1979. Activation of latent virus infections in larvae of *Adoxophyes orana* (Lepidoptera: Tortricidae) and *Barathra brassicae* (Lepidoptera: Noctuidae) by foreign polyhedra. *Journal of Invertebrate Pathology*, 34, pp.213–223.

Katholi, C.R. & Unnasch, T.R., 2006. Important experimental parameters for determining infection rates in arthropod vectors using pool screening approaches. *The American journal of tropical medicine and hygiene*, 74(5), pp.779–85.

Keeling, M.J. & Rohani, P., 2008a. Introduction to simple epidemic models. In *Modelling Infectious Diseases in Humans and Animals*. pp. 15–53.

Keeling, M.J. & Rohani, P., 2008b. Stochastic dynamics: event-driven approaches. In *Modelling Infectious Diseases in Humans and Animals*. pp. 200–219.

Keeling, M.J. & Rohani, P., 2008c. Stochastic extinctions and the critical community size. In *Modelling Infectious Diseases in Humans and Animals*. Princeton University Press, pp. 205–209.

Keller, L.F. & Waller, D.M., 2002. Inbreeding effects in wild populations. *Trends in Ecology and Evolution*, 17(5), pp.19–23.

Khin, M.M. & Than, K.A., 1983. Transovarial transmission of dengue-2 virus by *Aedes aegypti* in nature. *The American journal of tropical medicine and hygiene*, 32(3), pp.590–594.

Kourí, G. et al., 1998. Re-emergence of dengue in Cuba: A 1997 epidemic in Santiago de Cuba. *Emerging Infectious Diseases*, 4(1), pp.89–92.

Kow, C.Y., Koon, L.L. & Yin, P.F., 2001. Detection of dengue viruses in field caught male *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Singapore by type-specific PCR. *Journal of medical entomology*, 38(4), pp.475–9.

Kuan, M.-M. et al., 2010. Epidemiological trends and the effect of airport fever screening on prevention of domestic dengue fever outbreaks in Taiwan, 1998-2007. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases*, 14(8), pp.e693–7.

Kuan, M.-M. & Chang, F.-Y., 2012. Airport sentinel surveillance and entry quarantine for dengue infections following a fever screening program in Taiwan. *BMC infectious diseases*, 12(1), p.182.

Kuberski, T., 1979. Fluorescent antibody studies on the development of dengue-2 virus in *Aedes Albopictus* (Diptera: Culicidae). *Journal of medical entomology*, 16(4), pp.343–349.

Kuberski, T.T. & Rosen, L., 1977. A simple technique for the detection of dengue antigen in mosquitoes by immunofluorescence. *The American journal of tropical medicine and hygiene*, 26(3), pp.533–7.

Lambrechts, L., Scott, T.W. & Gubler, D.J., 2010. Consequences of the expanding global distribution of *Aedes albopictus* for dengue virus transmission. *PLoS Neglected Tropical Diseases*, 4(5).

Le, C.T., 1981. A new estimator for infection rates using pools of variable size. *The American Journal of Epidemiology*, 114(1), pp.132–136.

Lee, G.M., Brown, M.J.F. & Oldroyd, B.P., 2012. Inbred and outbred honey bees (*Apis mellifera*) have similar innate immune responses. *Insects Sociaux*, 60, pp.97–102.

Lee, H.L., Mustafakamal, I. & Rohani, A., 1997. Does transovarial transmission of dengue virus occur in Malaysian *Aedes aegypti* and *Aedes albopictus*? *The Southeast Asian journal of tropical medicine and public health*, 28(1), pp.230–232.

Lee, H.L. & Rohani, A., 2005. Transovarial transmission of dengue virus in *Aedes aegypti* and *Aedes albopictus* in relation to dengue outbreak in an urban area in Malaysia. *Dengue Bulletin*, 29, pp.106–111.

Lee, K.P. et al., 2006. Flexible diet choice offsets protein costs of pathogen resistance in a caterpillar. *Proceedings. Biological sciences / The Royal Society*, 273(1588), pp.823–9.

Lefèvre, T. et al., 2012. Behavioural resistance against a protozoan parasite in the monarch butterfly. *Journal of Animal Ecology*, 81(1), pp.70–79.

Lequime, S. & Lambrechts, L., 2014. Vertical transmission of arboviruses in mosquitoes: a historical perspective. *Infection, Genetics and Evolution*, 28, pp.681–690.

Linnen, J.M. et al., 2008. Dengue viremia in blood donors from Honduras, Brazil, and Australia. *Transfusion*, 48(7), pp.1355–1362.

Lipsitch, M., Siller, S. & Nowak, M., 1996. The evolution of virulence in pathogens with vertical and horizontal transmission. *Evolution*, 50(5), pp.1729–1741.

Longworth, J.F. & Cunningham, J.C., 1968. The activation of occult nuclear-polyhedrosis viruses by foreign nuclear polyhedra. *Journal of invertebrate pathology*, 10, pp.361–367.

Luz, P.M. et al., 2003. Uncertainties regarding dengue modelling in Rio de Janeiro, Brazil. *Memórias do Instituto Oswaldo Cruz*, 98(7), pp.871–8.

Maciel-de-Freitas, R. et al., 2010. Influence of the spatial distribution of human hosts and large size containers on the dispersal of the mosquito *Aedes aegypti* within the first gonotrophic cycle. *Medical and Veterinary Entomology*, 24(1), pp.74–82.

Maciel-de-Freitas, R., Koella, J.C. & Lourenço-de-Oliveira, R., 2011. Lower survival rate, longevity and fecundity of *Aedes aegypti* (Diptera: Culicidae) females orally challenged with dengue virus serotype 2. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 105(8), pp.452–8.

Madigan, M.T., Martinko, J.M. & Parker, J., 2003. Temperate bacteriophage lambda. In *Brock: Biology of Microorganisms: 10th edition*. pp. 250–255.

Manley, R., Boots, M. & Wilfert, L., 2015. Emerging viral disease risk to pollinating insects: ecological, evolutionary and anthropogenic factors. *Journal of Applied Ecology*, 52(2), pp.331–340.

Manore, C. a. & Beechler, B.R., 2015. Inter-epidemic and between-season resistance of Rift Valley fever: vertical transmission or cryptic cycling? *Transboundary and Emerging Diseases*, 62(1), pp.13–23.

Martínez, N.E. et al., 2014. Natural vertical transmission of dengue-1 virus in *Aedes aegypti* populations in Acapulco , Mexico. *Journal of American Mosquito Control Association*, 30(2), pp.143–146.

Martins, V.E.P. et al., 2012. Occurrence of natural vertical transmission of dengue-2

and dengue-3 viruses in *Aedes aegypti* and *Aedes albopictus* in Fortaleza, Ceará, Brazil. *PloS one*, 7(7), p.e41386.

Mattey, S.N., Strutt, L. & Smiseth, P.T., 2013. Intergenerational effects of inbreeding in *Nicrophorus vespilloides*: offspring suffer fitness costs when either they or their parents are inbred. *Journal of evolutionary biology*, 26(4), pp.843–53.

McLaughlin, R.E. & Myers, J.H., 1970. *Ophryocystis elektroscirrha* sp. n., a neogregarine pathogen of the Monarch butterfly *Danaus plexippus* (L.) and the Florida Queen butterfly *D. gilippus berenice* Cramer. *Journal of Protozoology*, 17, pp.300–305.

McVean, R.I.. et al., 2002. Dietary stress reduces the susceptibility of *Plodia interpunctella* to infection by a granulovirus. *Biological Control*, 25(1), pp.81–84.

Mitchell, C.J. & Miller, B.R., 1990. Vertical transmission of dengue viruses by strains of *Aedes albopictus* recently introduced into Brazil. *Journal of the American Mosquito Control Association*, 6(2), pp.251–253.

Mohammed, H. et al., 2008. Dengue virus in blood donations, Puerto Rico, 2005. *Transfusion*, 48(7), pp.1348–1354.

Mohandass, S. et al., 2007. Biology and management of *Plodia interpunctella* (Lepidoptera: Pyralidae) in stored products. *Journal of Stored Products Research*, 43(3), pp.302–311.

Mourya, D.T. et al., 2001. Horizontal and vertical transmission of dengue virus type 2 in highly and lowly susceptible strains of *Aedes aegypti* mosquitoes. *Acta virologica*, 45(2), pp.67–72.

Mulyatno, K.C. et al., 2012. Vertical transmission of dengue virus in *Aedes aegypti* collected in Surabaya, Indonesia, during 2008-2011. *Japanese journal of infectious diseases*, 65(3), pp.274–6.

Muñoz-jordán, J.L. et al., 2013. Genetic relatedness of dengue viruses in Key West, Florida, USA, 2009-2010. *Emerging infectious diseases*, 19(4), pp.652–654.

Murillo, R., Hussey, M.S. & Possee, R.D., 2011. Evidence for covert baculovirus infections in a *Spodoptera exigua* laboratory culture. *The Journal of general virology*, 92(Pt 5), pp.1061–70.

Myers, J.H. et al., 2011. The effect of food limitation on immunity factors and disease resistance in the western tent caterpillar. *Oecologia*, 167(3), pp.647–55.

Nathan, M.B., Dayal-Drager, R. & Guzman, M., 2009. *Dengue Guidelines for Diagnosis Treatment, Prevention and Control*,

National Audit Office, 2002. *The 2001 Outbreak of Foot and Mouth Disease.*,

Neiman, M. & Koskella, B., 2009. Sex and the red queen. In I. Schön, K. Martens, & P. Dijk, eds. *Lost Sex: The Evolutionary Biology of Parthenogenesis*. Springer Science+Business Media, pp. 133–159.

Nelms, B.M. et al., 2013. Experimental and natural vertical transmission of West Nile virus by California Culex (Diptera : Culicidae) mosquitoes. *Journal of Medical Entomology*, 50(2), pp.371–378.

Nguyet, M.N. et al., 2013. Host and viral features of human dengue cases shape the population of infected and infectious *Aedes aegypti* mosquitoes. *Proceedings of the National Academy of Sciences of the United States of America*, 110(22), pp.9072–

7.

Olson, D.H. et al., 2013. Mapping the global emergence of *Batrachochytrium dendrobatidis*, the Amphibian Chytrid fungus. *PLoS ONE*, 8(2).

Pinheiro, V.C.S. et al., 2005. Detection of dengue virus serotype 3 by reverse transcription-polymerase chain reaction in *Aedes aegypti* (Diptera, Culicidae) captured in Manaus, Amazonas. *Memórias do Instituto Oswaldo Cruz*, 100(8), pp.833–9.

Povey, S. et al., 2013. Dynamics of macronutrient self-medication and illness-induced anorexia in virally infected insects. *The Journal of animal ecology*, 83(1), pp.245–55.

Quam, M.B. et al., 2015. Estimating air travel-associated importations of dengue virus into Italy. *Journal of Travel Medicine*, 22(3),pp.186-93

Radke, E.G. et al., 2012. Dengue outbreak in Key West, Florida, USA, 2009. *Emerging infectious diseases*, 18(1), pp.135–137.

Ramalingam, S. et al., 1986. Does transovarial transmission of dengue viruses occur in Malaysia? *Tropical Biomedicine*, 3, pp.87–88.

Rantala, M.J. & Roff, D. a, 2007. Inbreeding and extreme outbreeding cause sex differences in immune defence and life history traits in *Epirrita autumnata*. *Heredity*, 98(5), pp.329–336.

Reich, N.G. et al., 2013. Interactions between serotypes of dengue highlight epidemiological impact of cross-immunity. *Journal of the Royal Society, Interface / the Royal Society*, 10, p.20130414.

Reiner, R.C., Stoddard, S.T. & Scott, T.W., 2014. Socially structured human movement shapes dengue transmission despite the diffusive effect of mosquito dispersal. *Epidemics*, 6, pp.30–36.

Reyes, M. et al., 2010. Index cluster study of dengue virus infection in Nicaragua. *American Journal of Tropical Medicine and Hygiene*, 83(3), pp.683–689.

Roberts, L.S. & Janovy, J.J., 2006. Genus Trypanosoma. In *Foundation Parasitology*. McGraw Hill, pp. 64–65.

Robinson, M. & Stilianakis, N.I., 2013. A model for the emergence of drug resistance in the presence of asymptomatic infections. *Mathematical biosciences*, 243(2), pp.163–77.

Rodenhuis-Zybert, I. a., Wilschut, J. & Smit, J.M., 2010. Dengue virus life cycle: viral and host factors modulating infectivity. *Cellular and Molecular Life Sciences*, 67(16), pp.2773–2786.

Rodhain, F. & Rosen, L., 1997. Mosquito vectors and dengue virus-vector relationships. In *Dengue and dengue haemorrhagic fever*. pp. 45–60.

Roff, D. a & DeRose, M. a, 2001. The evolution of trade-offs: effects of inbreeding on fecundity relationships in the cricket *Gryllus firmus*. *Evolution; international journal of organic evolution*, 55(1), pp.111–21.

Roff, D.A., 1998. Effects of inbreeding on morphological and life history traits of the sand cricket , *Gryllus firmus*. *Heredity*, 81(January), pp.28–37.

Rohani, A. et al., 2007. Detection of transovarial dengue virus from field-caught *Aedes aegypti* and *Ae* . *albopictus* larvae using C6 / 36 cell culture and reverse

transcriptase-polymerase chain reaction (RT-PCR) techniques. *Dengue Bulletin*, 31, pp.47–57.

Rohani, A. et al., 2008. Persistency of transovarial dengue virus in *Aedes aegypti* (Linn.). *The Southeast Asian journal of tropical medicine and public health*, 39(5), pp.813–816.

Romero-Vivas, C.M., Leake, C.J. & Falconar, a K., 1998. Determination of dengue virus serotypes in individual *Aedes aegypti* mosquitoes in Colombia. *Medical and veterinary entomology*, 12(3), pp.284–8.

Rosen, L., 1988. Further Observations on the mechanism of vertical transmission of Flaviviruses by Aedes mosquitoes. *The American Journal Of Tropical Medicine And Hygiene*, 39(1), pp.123–126.

Rosen, L., 1987a. On the mechanism of vertical transmission of dengue virus in mosquitoes. *C. R. Acad. Sc. Paris, t.*, 304(13), pp.347–350.

Rosen, L., 1987b. Sexual transmission of dengue viruses by *Aedes Albopictus*. *The American Journal Of Tropical Medicine And Hygiene*, 37(2), pp.398–402.

Rosen, L. et al., 1983. Transovarial transmission of dengue viruses by mosqutios: *Aedes albopictus* and *Aedes aegypti*. *The American Journal Of Tropical Medicine And Hygiene*, 32(5), pp.1108–1119.

Rosen, L. et al., 1978. Transovarial transmission of Japanese encephalitis virus by mosquitoes. *Science*, 199(4331), pp.909–911.

Sainsbury, A.W. et al., 2000. Grey squirrels have high seroprevalence to a parapoxvirus associated with deaths in red squirrels. *Animal Conservation*, 3(3), pp.229–233.

Sait, S., Begon, M. & Thompson, D., 1994. The influence of larval age on the response of *Plodia interpunctella* to a granulosis virus. *Journal of Invertebrate …*, 63, pp.107–110.

Sait, S.M., Begon, M. & Thompson, D.J., 1994a. Long-term population dynamics of the Indian meal moth *Plodia interpunctella* and its granulosis virus. *Journal of Animal Ecology*, 63(4), pp.861–870.

Sait, S.M., Begon, M. & Thompson, D.J., 1994b. The effects of a sublethal baculovirus infection in the Indian meal moth , *Plodia interpunctella*. *Journal of Animal Ecology*, 63(3), pp.541–550.

Salunkhe, R.C., Narkhede, K.P. & Shouche, Y.S., 2014. Distribution and evolutionary impact of Wolbachia on butterfly hosts. *Indian Journal of Microbiology*, 54(3), pp.249–254.

Sanchez-Rodríguez, A.O.S. et al., 2014. Natural transmission of dengue virus by *Aedes albopictus* at Monterrey , Northeastern Mexico. *Southwestern Entomologist*, 39(3), pp.459–468.

Serufo, J.C. et al., 1993. Isolation of dengue virus type 1 from larvae of *Aedes albopictus* in Campos Altos City, State of Minas Gerais, Brazil. *Memórias do Instituto Oswaldo Cruz*, 88(3), pp.503–4.

Service, M., 2012a. Anopheline mosquitoes (Anophelinae). In *Medical Entomology for Students*. Cambridge University Press, pp. 34–53.

Service, M., 2012b. Culcine mosquitoes (Culicinae). In *Medical Entomology for Student (Fifth Edition)*. Cambridge University Press, pp. 54–84.

Service, M.W., 2012. Introduction to mosquitoes (Culididae). In *Medical Entomology for Student (Fifth Edition)*. pp. 1–31.

Shaw, a. P.M. et al., 2014. Mapping the economic benefits to livestock keepers from intervening against bovine trypanosomiasis in Eastern Africa. *Preventive Veterinary Medicine*, 113(2), pp.197–210.

Shroyer, D.A., 1990. Vertical maintenance of dengue-1 virus in sequential generations of *Aedes albopictus*. *Journal of the American Mosquito Control Association*, 6(2), pp.312–314.

Siler, J.F., Hall, M.W. & Hitchens, A.P., 1926. Possibility of the hereditary transmission of the virus of dengue in the mosquito. *Philippine Journal of Science*, 29, pp.109–114.

Silver, J.B., 2008. Measuring adult dispersal. In *Mosquito Ecology, Field Sampling Methods, Third Edition*. pp. 1377–1424.

Simmons, J.F., St John, J.H. & Reynolds, F.H.K., 1931. Observations on the possibility of hereditary transmission of dengue from infected female *Aedes aegypti* through the egg to the offspring. *Philippine Journal of Science*, 44, pp.57–58.

Singh, J. et al., 2000. Silent spread of dengue and dengue haemorrhagic fever to Coimbatore and Erode districts in Tamil Nadu, India, 1998: need for effective surveillance to monitor and control disease. *Epidemiology and Infection*, 125(1), pp.195–200.

Smith, K.M. & Rivers, C.F., 1956. Some viruses affecting insects of economic importance. *Parasitology*, 46(1-2), pp.235–42.

Sorrell, I. et al., 2009. The evolution of covert, silent infection as a parasite strategy. *Proceedings. Biological sciences / The Royal Society*, 276(1665), pp.2217–26.

de Souza, M. & Freier, J.E., 1991. Vertical transmission of dengue 1 virus by *Haemagogus equinus* mosquitoes. *Journal of the American Mosquito Control Association*, 7(1), pp.118–120.

Steinhaus, E., 1958. Crowding as a possible stress factor in insect disease. *Ecology*, 39(3), pp.503–514.

Steinhaus, E.A. & Dineen, J.P., 1960. Observations on the role of stress in a granulosis of the Yariegated cutworm. *Journal of Insect Pathology*, 2, pp.55–65.

Stevens, L., Yan, G. & Pray, L.A., 1997. Consequences of inbreeding on invertebrate host susceptibility to parasitic infection. *Evolution*, 51(6), pp.2032–2039.

Stoddard, S.T. et al., 2013. House-to-house human movement drives dengue virus transmission. *Proceedings of the National Academy of Sciences of the United States of America*, 110(3), pp.994–9.

Stoddard, S.T. et al., 2014. Long-term and seasonal dynamics of dengue in Iquitos, Peru. *PLoS Neglected Tropical Diseases*, 8(7), pp.19–21.

Stuart, S.N. et al., 2004. Status and trends of amphibian declines and extinctions worldwide. *Science (New York, N.Y.)*, 306(5702), pp.1783–1786.

Sylvestre, G., Gandini, M. & Maciel-de-Freitas, R., 2013. Age-dependent effects of oral infection with dengue virus on *Aedes aegypti* (Diptera: Culicidae) feeding behavior, survival, oviposition success and fecundity. *PloS one*, 8(3), p.e59933.

Telfer, S. et al., 2005. Infection with cowpox virus decreases female maturation rates in

wild populations of woodland rodents. *Oikos*, 109(2), pp.317–322.

Telfer, S. et al., 2002. The effects of cowpox virus on survival in natural rodent populations : increases and decreases. *Journal of Animal Ecology*, 71, pp.558–568.

Tennakone, K., 2014. Analysis and mathematical modelling of possible inter-larval spread of the dengue virus. *Journal of the National Science Foundation of Sri Lanka*, 42(3), pp.273–277.

Teo, D., Ng, L. & Lam, S., 2009. Is dengue a threat to the blood supply? *Transfusion Medicine*, 19, pp.66–77.

Thavara, U. et al., 2006. Double infection of heteroserotypes of dengue viruses in field populations of *Aedes aegypti* and *Aedes albopictus* (Diptera: culcidae) and serological features of dengue viruses found in patients in southern Thailand. *The Southeast Asian journal of tropical medicine and public health*, 37(3), pp.468–476.

Thenmozhi, V. et al., 2000. Natural vertical dengue viruses transmission of in *Aedes aegypti* in southern India. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 94, p.507.

Thenmozhi, V. et al., 2007. Natural vertical transmission of dengue virus in *Aedes albopictus* (Diptera: Culicidae) in Kerala, a southern Indian state. *Japanese journal of infectious diseases*, 60(5), pp.245–9.

Thongrungkiat, S. et al., 2011. Prospective field study of transovarial dengue-virus transmission by two different forms of *Aedes aegypti* in an urban area of Bangkok, Thailand. *Journal of Vector Ecology*, 36(1), pp.147–52.

Triggs, A. & Knell, R.J., 2012. Interactions between environmental variables determine immunity in the Indian meal moth *Plodia interpunctella*. *The Journal of animal ecology*, 81(2), pp.386–94.

Trout, A. et al., 2010. Locally acquired dengue — Key West, Florida, 2009–2010 Dengue. , 59(19), pp.577–581.

Tu, W.-C., Chen, C.-C. & Hou, R.F., 1998. Ultrastructural studies on the reproductive system of male *Aedes aegypti* (Diptera: Culicidae) infected with dengue 2 virus. *Journal of medical entomology*, 35(1), pp.71–76.

Turner, S.R., 2005. After the famine: Plant pathology, *Phytophthora infestans*, and the late blight of potatoes, 1845—1960. *Historical Studies in Biological and Physical Sciences*, 35(2), pp.341–370.

Vaughn, D.W. et al., 2000. Invited commentary: dengue lessons from Cuba. *American Journal of Epidemiology*, 152(9), pp.800–804.

Vilaplana, L. et al., 2008. Density-related variation in vertical transmission of a virus in the African armyworm. *Oecologia*, 155(2), pp.237–46.

Vilaplana, L. et al., 2010. Pathogen persistence in migratory insects: high levels of vertically-transmitted virus infection in field populations of the African armyworm. *Evolutionary Ecology*, 24, pp.147–160.

Vilela, A.P.P. et al., 2010. Dengue virus 3 genotype I in *Aedes aegypti* mosquitoes and eggs, Brazil, 2005-2006. *Emerging Infectious Diseases*, 16(6), pp.3–6.

Vitikainen, E. & Sundström, L., 2010. Inbreeding and caste-specific variation in immune defence in the ant *Formica exsecta*. *Behavioural Ecology and Sociobiology*, 65, pp.899–907.

Wake, D.B. & Vredenburg, V.T., 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of the United States of America*, 105, pp.11466–11473.

Walsh, F., 2013. Antibiotics resistance "as big a risk as terrorism" - medical chief. *BBC NEWS: Health*. Available at: http://www.bbc.co.uk/news/health-21737844 [Accessed September 11, 2015].

Walter, S.D., Hildreth, S.W. & Beaty, B.J., 1980. Estimation of infection rates in populations of organisms using pools of variable size. *American journal of Epidemiology*, 112(1), pp.124–128.

Wang, T. et al., 2015. Evaluation of inapparent dengue infections during an outbreak in southern china. *PLOS Neglected Tropical Diseases*, 9(3), p.e0003677.

Wasinpiyamongkol, L. et al., 2003. Susceptibility and transovarial transmission of dengue virus in *Aedes aegypti*: a preliminary study of morphological variations. *The Southeast Asian journal of tropical medicine and public health*, 34 (Supplementary 2), pp.131–5.

Watts, D.M. et al., 1985. Failure to detect natural transovarial transmission of dengue viruses by *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). *Journal of medical entomology*, 22(3), pp.261–5.

Whitehorn, P.R. et al., 2011. Genetic diversity, parasite prevalence and immunity in wild bumblebees. *Proceedings. Biological sciences / The Royal Society*, 278, pp.1195–1202.

WHO, 2015a. Chikungunya. *WHO Media Centre*, (May), p. Fact sheet N°327. Available at: http://www.who.int/mediacentre/factsheets/fs327/en/# [Accessed September 23, 2015].

WHO, 2015b. Dengue and severe dengue. *WHO Media Centre*, (May). Available at: http://www.who.int/mediacentre/factsheets/fs117/en/# [Accessed September 23, 2015].

WHO, 2015c. Dengue and severe dengue. , (May).

WHO, 2015d. Dengue control: the mosquito. Available at: http://www.who.int/denguecontrol/mosquito/en/# [Accessed September 3, 2015].

WHO, 2014a. Japanese encephalitis. *WHO Media Centre*. Available at: http://www.who.int/mediacentre/factsheets/fs386/en/ [Accessed October 9, 2015].

WHO, 2015e. Metrics: disability-adjusted life year (DALY). *Health statistics and information systems*. Available at: http://www.who.int/healthinfo/global_burden_disease/metrics_daly/en/.

WHO, 2010. Rift Valley fever. *WHO Media Centre*. Available at: http://www.who.int/mediacentre/factsheets/fs207/en/ [Accessed October 9, 2015].

WHO, 2012a. The top 10 causes of death. *Media Centre*. Available at: http://www.who.int/mediacentre/factsheets/fs310/en/ [Accessed September 11, 2015].

WHO, 2011. West Nile virus. *WHO Media Centre*. Available at: http://www.who.int/mediacentre/factsheets/fs354/en/ [Accessed October 9, 2015].

WHO, 2012b. World disability adjusted life years by cause. *Global Health Observatory Data Repository*. Available at:

http://apps.who.int/gho/data/node.main.DALYNUMWORLD?lang=en [Accessed September 11, 2015].

WHO, 2014b. Yellow fever. *WHO Media Centre*. Available at: http://www.who.int/mediacentre/factsheets/fs100/en/ [Accessed October 9, 2015].

Wichmann, O. et al., 2005. Dengue antibody prevalence in German travellers. *Emerging Infectious Diseases*, 11(5), pp.762–765.

Wilson, K. et al., 2013. Pest control: biopesticides' potential. *Science*, 342(November), p.799.

Yang, F. et al., 2014. Molecular identification of the first local dengue fever outbreak in Shenzhen city, China: a potential imported vertical transmission from Southeast Asia? *Epidemiology and infection*, 142, pp.225–33.

Yoon, I.-K. et al., 2012. Under recognized mildly symptomatic viremic dengue virus infections in rural Thai schools and villages. *The Journal of infectious diseases*, 206(3), pp.389–98.

Zeidler, J.D. et al., 2008. Dengue virus in *Aedes aegypti* larvae and infestation dynamics in Roraima, Brazil. *Revista de saúde pública*, 42(6), pp.986–91.

Zhang, M. et al., 2010. Quantitative analysis of replication and tropisms of dengue virus type 2 in *Aedes albopictus*. *The American journal of tropical medicine and hygiene*, 83(3), pp.700–7.