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Modelling the relationship between antibody-dependent enhancement and immunological distance with application to dengue

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Abstract

When antibodies raised in response to a particular pathogen bind with immunologically similar pathogens it may facilitate infection through a phenomenon known as antibody-dependent enhancement (ADE). This process occurs between the four serotypes of dengue virus and, furthermore, secondary infection is a major risk factor in dengue hemorrhagic fever (DHF). Theory has suggested that ADE may be responsible for the large immunological distance between dengue serotypes. We investigate this hypothesis using an epidemic model for dengue in which immunological distance and the strength of immune cross-reaction are expressed separately. Cross-enhancement is considered in three alternative forms acting on susceptibility, transmission and mortality. Previous models have shown that transmission and mortality enhancement can lead to periodicity or chaos. We confirm this result for reasonable levels of susceptibility and transmission enhancement but not for mortality enhancement. We also show that when the two strains have identical basic reproductive numbers no form of enhancement leads to competitive exclusion. When the two strains have different basic reproductive numbers susceptibility or transmission enhancement allow strains with greater immunological similarity to stably coexist but mortality enhancement forces strains to be more distinct. All three forms of enhancement can be associated with DHF and we conclude that mortality enhancement must be dominant if ADE really is responsible for the immunological distance between dengue serotypes.

Keywords: Antibody-dependent enhancement; Dengue; Serological distance; Mathematical model

1. Introduction

Understanding the epidemiological processes that underpin the evolutionary history and therefore phylodynamics (Grenfell et al., 2004) of disease is an important challenge to evolutionary theory. The considerable variation in the evolutionary history of pathogens is revealed in their molecular phylogenies and particular characteristics of disease interactions may cause the patterns that we see. One such process is antibody-dependent enhancement (ADE). Antibodies produced in response to infection by a particular pathogen often offer protection against similar pathogens. However, in some cases ADE (or cross-enhancement) occurs and antibodies have the converse effect, actually facilitating the invasion of similar pathogens. One of the pathogens for which this has been investigated most extensively is the dengue virus (DEN) and ADE has been linked to severe infection and increased mortality. It has also been suggested that ADE may be a factor determining the immunological distance between DEN strains (Holmes, 2004; Twiddy et al., 2002). Previous mathematical models have incorporated cross-enhancement but employ either a framework without a clear definition of immunological distance (Ferguson et al., 1999a; Cummings et al., 2005) or use characteristics not applicable to dengue (Kawaguchi et al., 2003). In this paper, we will address these issues and present a unified model framework to investigate the impact of different forms of cross-enhancement on the immunological distance of dengue strains. Our aim is to clarify theoretically the role that different characterizations of ADE may play in the epidemiological and evolutionary dynamics of dengue.

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DEN is a mosquito-borne arbovirus of humans. There are four distinct DEN serotypes in circulation throughout tropical and subtropical regions and all generally cause dengue fever (DF), a mild febrile illness without lasting complications. However, they can also cause dengue hemorrhagic fever (DHF) a serious condition characterized by high fever and vascular permeability. With modern medical treatment DHF mortality rates are around 1 percent, without it they may be as high as 20 percent (WHO, 2002). DHF has been associated with ADE resulting from secondary infection with a distinct DEN serotype (Halstead, 1997; McBride and Bielefelt-Ohmann, 2000; Nisalak et al., 2003) although the absence of DHF in some regions experiencing consecutive or simultaneous circulation of more than one DEN serotype (Messer et al., 2003; Travassos da Rosa et al., 2000; Watts et al., 1999) has led to speculation that genetic differences within a serotype may also be important (Foster et al., 2004; Halstead, 1997; Messer et al., 2003).

Antibodies contribute to the immune response by neutralization, opsonization and complement activation (Janeway et al., 1999). Neutralization occurs when antibodies bind to the pathogen and inactivate it by blocking access to receptors on the potential host cell. Opsonization occurs when the antibodies coating the pathogen facilitate recognition and destruction by macrophages. Complement activation has a similar effect although in this case the antibodies coating the pathogen facilitate binding with complement proteins that promote uptake by macrophages. Cross-immunity occurs when two non-identical pathogens have sufficiently similar immunological structures that antibodies to one are able to bind with, and neutralize or opsonize, the other. Cross-enhancement occurs when the antibodies bind to the second pathogen but fail to neutralize it (Halstead, 1997). Receptors on the antibody part of the resulting antibody–pathogen complex then facilitate binding with macrophages, either directly or through the complement (Takada and Kawaoka, 2003) but opsonization does not occur and the pathogen is more easily able to enter the macrophage and use it for replication (Rothman and Ennis, 1999).

Phylogenetic analysis indicates that the four dengue serotypes probably evolved in independent sylvatic cycles before jumping to humans (Holmes, 2004; Holmes and Twiddy, 2003) but it has also been suggested that the serological distinction could have arisen through the evolutionary pressure of ADE (Holmes, 2004; Holmes and Twiddy, 2003; Twiddy et al., 2002). A mathematical model based on the assumption that cross-enhancement acts by increasing the transmission rate of secondary infections (Ferguson et al., 1999a) is generally cited in relation to this hypothesis. That study actually shows that ADE can result in periodic or chaotic coexistence of two pathogen strains. But, because immunity and enhancement are represented by the same parameter it does not, and cannot, make any claim connecting ADE and immunological distance. An alternative model (Kawaguchi et al., 2003), based on the assumption that cross-enhancement acts by increasing the mortality rate associated with secondary infections, shows that ADE can lead to a greater immunological distance between strains and, under certain circumstances, periodic coexistence. However, closer inspection of this model reveals that it employs inappropriate parameter values for dengue. The aim of this paper is to clarify the situation by considering both of the above assumptions about ADE, together with the assumption that it increases susceptibility, in the context of a model that maintains a clear concept of immunological distance and uses reasonable parameter values for dengue.

2. Model description

2.1. General SIR framework

The model used in this study is a standard SIR formulation (Anderson and May, 1991) with two pathogen strains. To maintain a straightforward correspondence to previous work (Ferguson et al., 1999a; Kawaguchi et al., 2003) and focus on the immune dynamics in the host population, the vector population is not explicitly modeled. In order to incorporate cross-immunity and cross-enhancement the immune history of the host population must be recorded. Thus compartments are used corresponding to the host population susceptible to both pathogen strains (SS), susceptible to strain 1 and infected/infectious with strain 2 (SI), susceptible to strain 1 and recovered from strain 2 (SR) and similarly for IS, IR, RS, RI and RR. For a disease such as dengue with an infectious period generally less than a week (Kuno, 1997) the incidence of multiple simultaneous infection is likely to be low and so, for simplicity, this is not permitted in the model and the II compartment is omitted. Base transmission rates are β_1 and β_2 for strains 1 and 2, respectively. As discussed in detail below, these may be modified by cross-immunity $f(\sigma)$, where σ is the immunological distance between strains, cross-enhancement of susceptibility η_i and cross-enhancement of transmission ϕ_i. Infected individuals recover at rate γ. Since DF is not usually fatal no additional deaths are associated with infection except when mortality enhancement is being considered in which case secondary infections are subject to addition deaths at rates ζ_i (see below). For convenience the equilibrium size of the disease-free population is assumed to be 1. For a population of arbitrary size N the number of individuals in, for instance, the SS compartment is simply $N \cdot SS$.

In each compartment deaths occur at a constant rate so the total number of deaths in time Δt is $\Delta t(SS+SI+\cdots+RR)$. For simplicity the birth rate is also constant but independent of the population size. All births are considered susceptible to both strains and so the SS compartment increases at rate μ. In the absence of disease-induced deaths this means that the rate of change of the total population size $\mathrm{d}N/\mathrm{d}t = \mu - \mu N$ is 0 if $N = 1$. Hence the total population size is constant and the absolute size of

a compartment, for example SS, is the same as the proportion of the total population in this state SS/N. When disease-induced death is non-zero $dN/dt = \mu - \zeta_1 IR - \zeta_2 RI - \mu N$ and the equilibrium population size is $N^* = (\mu - \zeta_1 IR^* - \zeta_2 RI^*)/\mu$. Since this is less than 1 the absolute and proportional compartment sizes are different. The absolute size is used throughout this paper although the proportional size is similar since IR^* and RI^* are of order 10^{-5} while μ is of order 10^{-2} and N^* remains fairly close to 1 even for relatively large ζ_i.. The system is described by Eqs. (1)–(8):

$$\frac{dSS}{dt} = \mu - \beta_1 SS(IS + \phi_1 IR) - \beta_2 SS(SI + \phi_2 RI) - \mu SS, \tag{1}$$

$$\frac{dSI}{dt} = \beta_2 SS(SI + \phi_2 RI) - (\gamma + \mu)SI, \tag{2}$$

$$\frac{dSR}{dt} = \gamma SI - f(\sigma)\eta_1\beta_1 SR(IS + \phi_1 IR) - \mu SR, \tag{3}$$

$$\frac{dIS}{dt} = \beta_1 SS(IS + \phi_1 IR) - (\gamma + \mu)IS, \tag{4}$$

$$\frac{dIR}{dt} = f(\sigma)\eta_1\beta_1 SR(IS + \phi_1 IR) - (\gamma + \mu + \zeta_1)IR, \tag{5}$$

$$\frac{dRS}{dt} = \gamma IS - f(\sigma)\eta_2\beta_2 RS(SI + \phi_2 RI) - \mu RS, \tag{6}$$

$$\frac{dRI}{dt} = f(\sigma)\eta_2\beta_2 RS(SI + \phi_2 RI) - (\gamma + \mu + \zeta_2)RI, \tag{7}$$

$$\frac{dRR}{dt} = \gamma(IR + RI) - \mu RR. \tag{8}$$

2.2. Cross-immunity and cross-enhancement

The model requires a concept of immunological distance between strains and this is denoted by the parameter σ. When $\sigma = 0$ the two strains are immunologically identical. When $\sigma = 1$ they are entirely distinct. Cross-immunity can be represented as either a reduction in susceptibility (the probability of contracting a secondary infection is lower but if it does occur it is indistinguishable from a primary infection) or a reduction in transmission (the probability of infection is the same for primary and secondary cases but secondary cases are milder, viremia is lower and so the probability of transmission is lower). In this model cross-immunity is assumed to act on susceptibility although there is little qualitative difference if it is assumed to act on transmission. Hence, cross-immunity is a function of immunological distance $f(\sigma)$ which reduces the probability of contracting a secondary infection. The most straightforward approach is to assume that cross-immunity and immunological distance are equivalent and so σ can be used interchangeably for both. Thus when $\sigma = 0, f(\sigma) = 0$ and there is perfect cross-immunity and primary infection with one strain prevents secondary infection with the other. When $\sigma = 1, f(\sigma) = 1$ and there is no cross-immunity and primary infection with one strain does not affect the probability of secondary infection with the other. Intermediate values of σ result in a proportional reduction in the probability of secondary infection. Note that cross-immunity is assumed to prevent infection and so the proportion $1 - f(\sigma)$ that is challenged but not infected remains susceptible. Homotypic immunity is considered to be complete and reinfection with the same strain is not permitted.

Three alternative representations of cross-enhancement will be considered. Susceptibility enhancement means that primary infection increases the probability of contracting a secondary infection. This corresponds to the observation that heterotypic antibodies enhance infection by forming non-neutralizing antibody–pathogen complexes expressing $Fc\gamma R$ that focuses them at the host cell surface (Mady et al., 1991). Enhancement is thus represented by parameters η_1 and η_2 which increase the probability of contracting a secondary infection (Eqs. (3) and (6)). Enhancement need not be symmetric and η_1 represents the increase in susceptibility to strain 1 following infection with strain 2. η_2 is similarly defined. In both cases $\eta_i = 1$ means that no enhancement occurs and is taken as the default value. Enhancement occurs if $\eta_i > 1$ with $\eta_i = 2$ representing a doubling in the probability of infection. Note that values of η_i significantly greater than 1 may be unreasonable as there must be some maximum level of susceptibility in the host and increasing it beyond this level implies that a primary infection in some way increases exposure to secondary infection.

Transmission enhancement corresponds to the observation that DHF patients show peak viremia levels 100–1000 times higher than DF patients (Vaughn et al., 2000). This may arise because more cells are infected or pathogen replication is higher in each cell (Cologna and Rico-Hesse, 2003). The former case can be attributed to the facilitation of cell entry by non-neutralizing antibodies but the latter case does not correspond to any ADE mechanism observed so far and may be better considered as a symptom of ADE occurring earlier in the infection process. In the model transmission enhancement is represented by the parameters ϕ_1 and ϕ_2 which increase the transmission rate of hosts suffering a secondary infection (Eqs. (1), (3) and (6)). ϕ_1 represents the increase in transmission rate of hosts infected by strain 1 following a previous infection with strain 2. ϕ_2 is similarly defined. Enhancement occurs if $\phi_i > 1$ with $\phi_i = 2$ representing a doubling in the transmission rate. As with susceptibility enhancement described above, there must be some maximum level of transmission in a host and values of ϕ_i significantly greater than 1 may imply that secondary infections somehow increase exposure to either the susceptible or implicit vector populations.

Mortality enhancement is associated with the observation that DF rarely leads to death but case fatality rates for DHF can exceed 20 percent if intensive medical support is

not available (WHO, 2002). The severe complications indicative of DHF are believed to result from a pathological response of monocytes and T-cells leading to very high production of inflammatory cytokines (Diamond et al., 2000; Cologna and Rico-Hesse, 2003). As with transmission enhancement this does not directly correspond to the known mechanisms of ADE of cell invasion (although it has been suggested that the increase in cytoxic factors upregulates $Fc\gamma R$ expression and so establishes a positive feedback system that augments DHF (Kuno, 1997; Mady et al., 1991)) and increased mortality may be best thought of as a symptom of ADE. Mortality associated with DHF is represented in the model by increasing mortality rates for hosts suffering secondary infections (Eqs. (5) and (7)). ζ_1 represents the mortality rate associated with a secondary strain 1 infection, ζ_2 a secondary strain 2 infection. $\zeta_i = 0$ means that no enhancement occurs (there is no additional mortality). Values of $\zeta_i > 0$ result in increased mortality. If $\zeta_i = 2$ and time is measured in years then the life expectancy of a host with secondary infection is 1/2 a year and the daily mortality rate is $200/365 = 0.54$ percent. Given an average duration of infection of 7 days, the case fatality rate associated with $\zeta_i = 2$ is 3.8 percent. There is no theoretical maximum to ζ_i as very high values will simply lead to rapid death of all hosts with secondary infections.

2.3. Parameterization

The model is parameterized with reasonable values for dengue. Time is specified in years. The natural host death rate $\mu = 0.0167$ corresponds to an average life expectancy of 60 years. The recovery rate from infection $\gamma = 52$ corresponds to an infectious period of 7 days and is the same for both strains. Two versions of the model are considered, one in which the two strains are identical, the other in which they are differentiated through the base transmission rate. In the first case $\beta_1 = \beta_2 = 104$. In the latter case $\beta_1 = 104$ and $\beta_2 = 78$. These values were calculated to give reasonable basic reproductive numbers (R_0) for dengue, actual estimates for which range from 1.33 to 2.5 (Kuno, 1997) and 1.38 to 7.86 (Ferguson et al., 1999b). For the model described here the basic reproductive number is given by $\beta_i/(\gamma + \mu)$ from which $R_{01} = 2$ and $R_{02} = 1.5$. A summary of parameterization is given in Table 1.

2.4. Relationship to previous models

Although this model is similar to the two other models of ADE in dengue previously mentioned there are several important differences. The model described by Ferguson et al. (1999a) uses a similar structure but immunity and enhancement are both considered to act on transmission through the same parameter. In the model presented here this is equivalent to fixing $\sigma = \eta_1 = \eta_2 = 1$ and $\zeta_1 = \zeta_2 = 0$ but allowing ϕ_1 and ϕ_2 to vary between 0 and 3 with values between 0 and 1 representing cross-immunity and values greater than 1 representing cross-enhancement. This formulation is not unreasonable but no connection can be made between ϕ_i and immunological distance because ϕ_1 and ϕ_2 are not symmetrically related and the only possible interpretation for values of ϕ_i greater than 1 would be complete immunological distinction. The model described by Kawaguchi et al. (2003) is similar to the model described here with $\eta_1 = \eta_2 = \phi_1 = \phi_2 = 1$ and $\zeta_1 = \zeta_2$ varying between 0 and 40. It uses a logistic, rather than constant, growth term for the host population and retains a compartment representing simultaneous infection with both strains. It is also parameterized with infection durations (γ) of 1.1 or 1.4 years (compared with a life expectancy of 100 years) and base transmission rates (β) resulting in R_0 values of 26.9 and 67.8.

Table 1
Parameter values used throughout this paper unless otherwise specified

μ	Host natural death rate	0.0167
γ	Recovery rate from infection	52
β_1	Base transmission rate of strain 1	104
β_2	Base transmission rate of strain 2	104 or 78
σ	Immunological distance/degree of cross-immunity	0–1
η_1	Enhancement of susceptibility to secondary strain 1 infections	1–8 (default 1)
η_2	Enhancement of susceptibility to secondary strain 2 infections	1–8 (default 1)
ϕ_1	Enhancement of transmission of secondary strain 1 infections	1–8 (default 1)
ϕ_2	Enhancement of transmission of secondary strain 2 infections	1–8 (default 1)
ζ_1	Mortality rate of secondary strain 1 infections	0–20 (default 0)
ζ_2	Mortality rate of secondary strain 2 infections	0–20 (default 0)

3. Model results

Numerical methods (using the xppaut differential equation tool, Bard Ermentrout, University of Pittsburgh, 2000) were used to investigate the impact of cross-immunity and the three different forms of cross-enhancement on the coexistence of the two strains. In each case the enhancement parameters were assigned fixed values and a stable equilibrium solution was determined for $\sigma = 0$. This was then used as a starting point for the calculation of subsequent equilibrium solutions for $0 < \sigma < 1$, essentially creating a bifurcation diagram with σ as the bifurcation parameter. Two strain parameterizations were considered. In the first both strains were identical. This is similar to Ferguson et al. (1999a) and means that coexistence at identical infected populations sizes is expected to occur unless cross-enhancement (or in principle cross-immunity) is asymmetric. In the second strain 1 had a higher base transmission rate and hence a higher basic reproductive number. This is similar to Kawaguchi et al. (2003) and

means that strain 1 will exclude strain 2 unless cross-immunity is sufficiently weak to allow strain 2 to be semi-independent of strain 1.

3.1. Susceptibility enhancement

The impact of susceptibility enhancement was studied by fixing $\eta_i > 1$ but holding $\phi_i = 1$ and $\zeta_i = 0$, so there is no transmission or mortality enhancement. When the two strains are identical and there is no enhancement of any kind they always coexist at identical levels that increase as σ increases and competition is reduced (Fig. 1a and b). If η_1 is fixed at a value greater than 1 the number of strain 1 infections increases due to cross-enhancement but strain 2 decreases due to cross-immunity except when σ is close to 1 and competition is weak. Susceptibility enhancement at the levels investigated never leads to competitive exclusion although in a small region delimited by Hopf bifurcations there are no stable solutions and the system displays periodic, aperiodic or chaotic behavior. This is described in detail in Ferguson et al. (1999a) and will not be considered further here. Fig. 2a shows the location of the Hopf bifurcation points and the resultant region of oscillations in σ–η_1 parameter space.

When the two strains are distinct and there is no enhancement, strain 2 is excluded by strain 1 until cross-immunity is sufficiently weak to allow coexistence (Fig. 1c and d). Once coexistence is possible strain 2 always increases with σ. In response the size of the population infected with strain 1 initially declines due to competition but recovers as the effect of cross-immunity weakens. When η_2 is fixed greater than 1 (and strain 1 enhances strain 2) coexistence becomes stable at lower vales of σ, i.e. when there is a higher degree immunological similarity. Because strain 2 is enhanced the infected population is increased and pressure from cross-immunity increases the magnitude of the decline in strain 1. Hopf bifurcations may occur when η_2 is large enough, delimiting a transition into

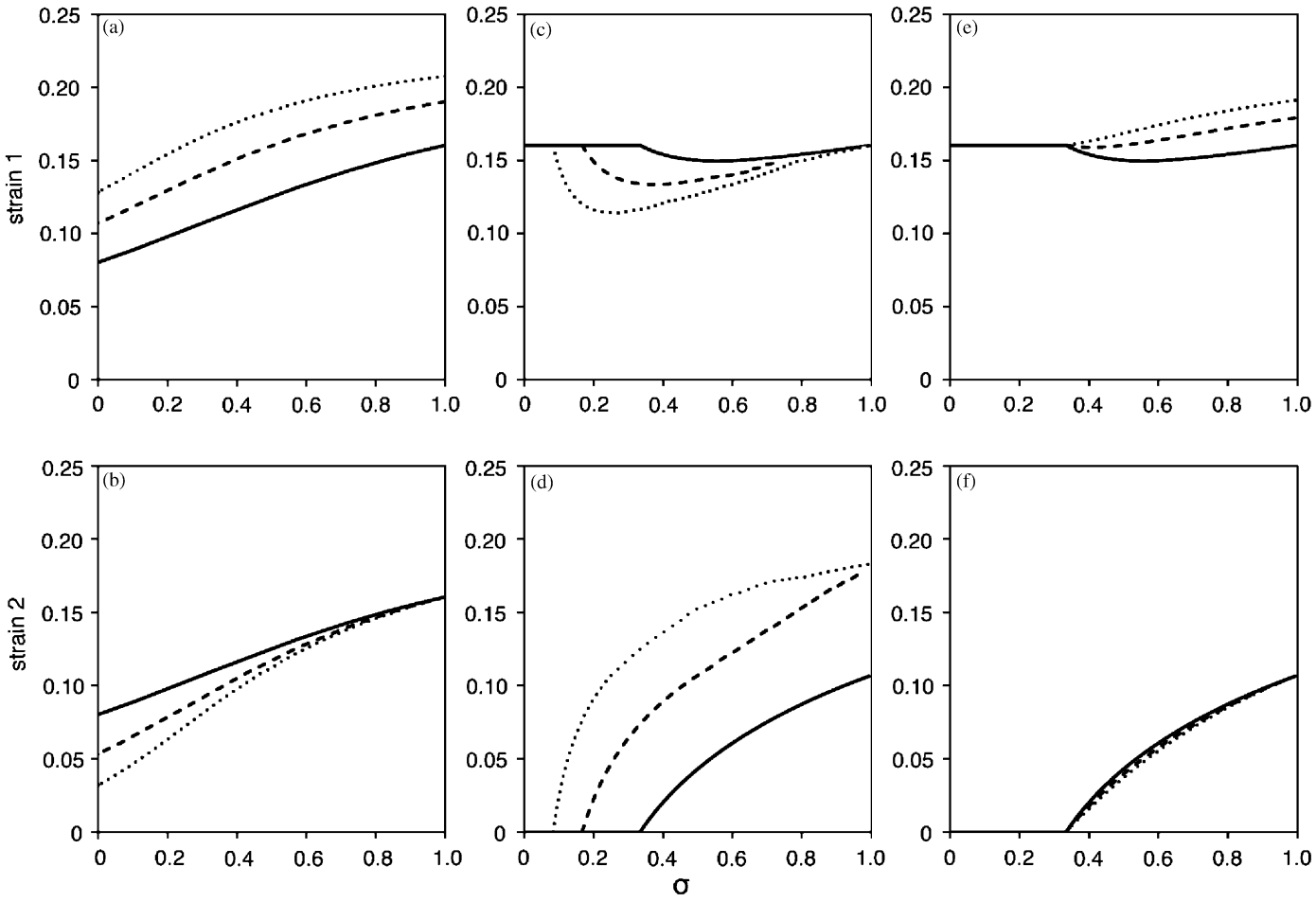

Fig. 1. Equilibrium population infected with strain 1 ($IS+IR$) and strain 2 ($SI+RI$) when σ is varied and enhancement acts on susceptibility through η_1 and η_2. In all frames: $\mu = 0.0167, \gamma = 52, \phi_1 = \phi_2 = 1, \zeta_1 = \zeta_2 = 0$. Frames a and b: identical strains, $\beta_1 = \beta_2 = 104, \eta_2 = 0$. (—) $\eta_1 = 1$ (no enhancement), (- - -) $\eta_1 = 2$, (···) $\eta_1 = 4$ (strain 2 enhances susceptibility to strain 1). Frames c and d: distinct strains, stronger strain enhances weaker, $\beta_1 = 104$, $\beta_2 = 78$, $\eta_1 = 0$. (—) $\eta_2 = 1$ (no enhancement), (- - -) $\eta_2 = 2$, (···) $\eta_2 = 4$ (strain 1 enhances susceptibility to strain 2). Frames e and f: distinct strains, weaker strain enhancing, $\beta_1 = 104$, $\beta_2 = 78$, $\eta_2 = 0$. (—) $\eta_1 = 1$ (no enhancement), (- - -) $\eta_1 = 2$, (···) $\eta_1 = 4$ (strain 2 enhances susceptibility to strain 1). Black lines indicate the stable equilibrium. Gray lines indicate the unstable coexistence equilibrium. In this region there are no steady-state solutions and oscillating solutions occur.

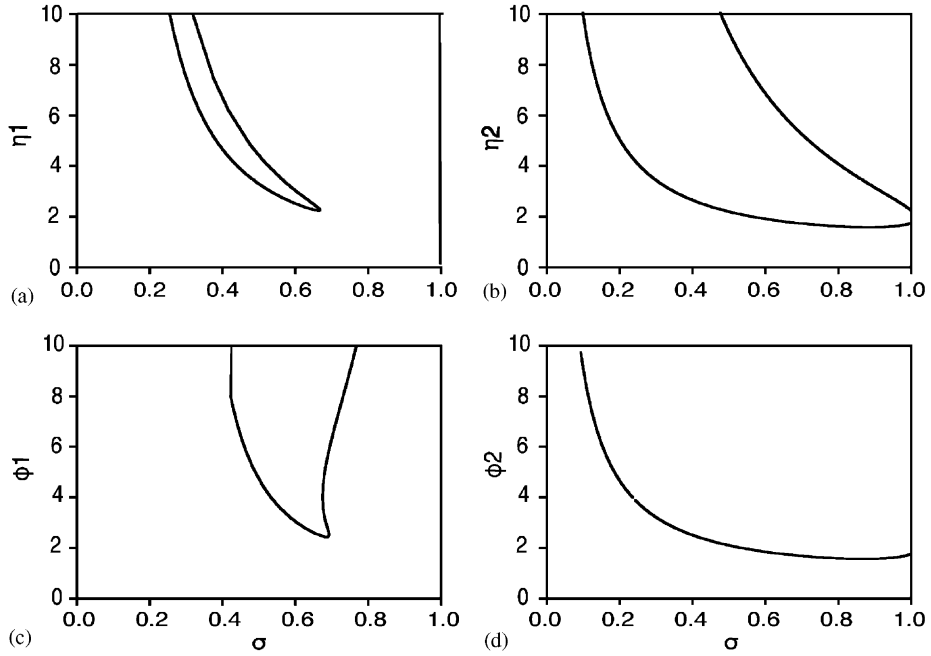

Fig. 2. Regions (C), delimited by Hopf bifurcations, in which there are no stable solutions and the system show shows complex oscillations. In all frames: $\mu = 0.0167, \gamma = 52$. Frame a: identical strains, enhancement of susceptibility, $\beta_1 = \beta_2 = 104, \eta_2 = 0, \phi_1 = \phi_2 = 1, \zeta_1 = \zeta_2 = 0$. Frame b: distinct strains, enhancement of susceptibility, $\beta_1 = 104, \beta_2 = 78, \eta_1 = 0, \phi_1 = \phi_2 = 1, \zeta_1 = \zeta_2 = 0$. Frame c: identical strains, enhancement of transmission, $\beta_1 = \beta_2 = 104, \phi_2 = 0, \eta_1 = \eta_2 = 1, \zeta_1 = \zeta_2 = 0$. Frame d: distinct strains enhancement of transmission, $\beta_1 = 104, \beta_2 = 78, \phi_1 = 0, \eta_1 = \eta_2 = 1, \zeta_1 = \zeta_2 = 0$.

complex oscillations. The location of these points in σ–η_2 parameter space is shown in Fig. 2b.

When η_1 is fixed greater than 1 (and strain 2 enhances strain 1) strain 2 is always excluded until cross-immunity drops below a certain threshold, which is the same for all values of η_1, after which coexistence is always stable. There was no evidence of Hopf bifurcations (Fig. 1e and f). The number of strain 1 infections does not decrease in response to the presence of strain 2 and is higher than in the absence of enhancement. Despite considerable cross-immunity the number of strain 2 infections is only slightly lower.

3.2. Transmission enhancement

The impact of transmission enhancement was studied by fixing $\phi_i > 1$ but holding $\eta_i = 1$ and $\zeta_i = 0$, so there is no susceptibility or mortality enhancement. As may be expected from the model equations, the impact of transmission enhancement is very similar to that of susceptibility enhancement. When both strains are identical, fixing $\phi_1 > 1$ (strain 2 enhances transmission of strain 1) increases the population infected with strain 1 and decreases the populaion infected with strain 2 (Fig. 3a and b). Transmission enhancement never results in the exclusion of either strain but Hopf bifurcations delimiting regions of complex oscillations occur at certain values of σ when ϕ_1 is large enough (Fig. 2c).

When the two strains are distinct and ϕ_2 is fixed greater than 1 (strain 1 enhances transmission of strain 2) stable coexistence occurs when cross-immunity is stronger (i.e. σ is lower, Fig. 3c and d). As before, further increases in σ lead to an increase in strain 2 and decline in strain 1 although this recovers as σ approaches 1 and competition becomes weaker. For large enough ϕ_1 a Hopf bifurcation occurs at a certain values of σ, there are no stable solutions, and the system shows complex oscillations. In contrast to previous cases, further increases in σ do not lead to a return to stable coexistence (Fig. 2d).

If ϕ_1 is fixed greater than 1 and infection with strain 2 enhances transmission of strain 1 then coexistence is always stable when the strength of cross-immunity falls below a certain threshold which is the same for all values of ϕ_1 (Fig. 3e and f). However, the presence of strain 2 increases the population infected with strain 1. Higher values of ϕ_1 lead to a larger population infected with strain 1 but have little impact on the size of the population infected with strain 2.

3.3. Mortality enhancement

The impact of mortality enhancement was studied by fixing $\zeta_i > 0$ but holding $\eta_i = 1$ and $\phi_i = 1$, so there is no susceptibility or transmission enhancement.

Mortality enhancement has only a small impact on the equilibrium solutions of the system. When the strains are identical and $\zeta_1 > 0$ (strain 2 enhances mortality from strain 1) there is a small decrease in the population infected with strain 1 due to higher mortality and a small increase in the population infected with strain 2 due to decreased

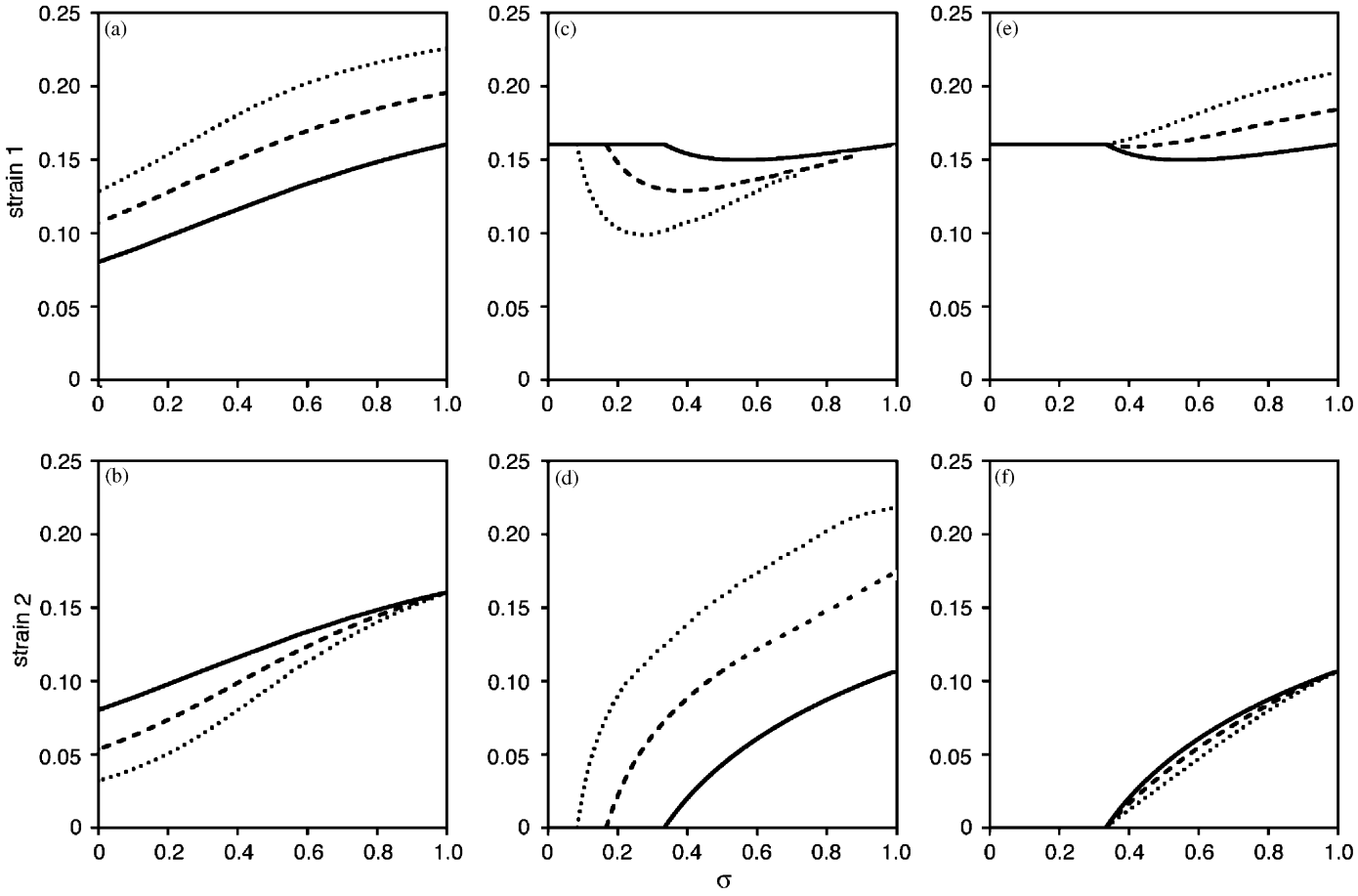

Fig. 3. Equilibrium population infected with strain 1 ($IS + IR$) and strain 2 ($SI + RI$) when σ is varied and enhancement acts on transmission through ϕ_1 and ϕ_2. In all frames: $\mu = 0.0167, \gamma = 52, \eta_1 = \eta_2 = 1, \zeta_1 = \zeta_2 = 0$. Frames a and b: identical strains, $\beta_1 = \beta_2 = 104, \phi_2 = 0$. (—) $\phi_1 = 1$ (no enhancement), (- - -) $\phi_1 = 2$, (···) $\phi_1 = 4$ (strain 2 enhances transmission of strain 1). Frames c and d: distinct strains, stronger strain enhancing, $\beta_1 = 104, \beta_2 = 78, \phi_1 = 0$. (—) $\phi_2 = 1$ (no enhancement), (- - -) $\phi_2 = 2$, (···) $\phi_2 = 4$ (strain 1 enhances transmission of strain 2). Frames e and f: distinct strains, weaker strain enhancing, $\beta_1 = 104, \beta_2 = 78, \phi_2 = 0$. (—) $\phi_1 = 1$ (no enhancement), (- - -) $\phi_1 = 2$, (···) $\phi_1 = 4$ (strain 2 enhances transmission of strain 1). Black lines indicate the stable equilibrium. Gray lines indicate the unstable coexistence equilibrium. In this region there are no steady-state solutions and oscillating solutions occur.

competition (Fig. 4a and b). As σ is increased both infected populations increases due to weaker competition and the higher number of strain 2 infections amplifies the impact of mortality enhancement on strain 1.

When the two strains are distinct, fixing $\zeta_2 > 0$ (strain 1 enhances mortality from strain 2) has no qualitative impact on the equilibrium solutions (Fig. 4c and d). There is a barely perceptible increase in the population infected with strain 1 and a small decrease in the population infected with strain 2. However, the increased mortality associated with strain 2 means that stable coexistence can only occur when the strains are more distinct and competition (cross-immunity) is weaker. Fixing $\zeta_1 > 0$ has no significant impact on strain 2 and there is just a slight decrease in strain 1 infections (Fig. 4e and f). For all values of ζ_1 stable coexistence occurs when the strength of cross-immunity falls below the same threshold. There was no indication that any form of mortality enhancement investigated led to Hopf bifurcations or oscillations.

4. Discussion

The model presented in this article shows that cross-enhancement of susceptibility or transmission can allow two pathogen strains with non-identical transmission rates to stably coexist at higher levels of cross-immunity, i.e. when they have a higher degree of immunological similarity. It also confirms the results of Kawaguchi et al. (2003), showing that cross-enhancement of mortality has the converse effect and forces the immunological distance to be greater before stable coexistence can occur. When the two pathogen strains have identical transmission rates cross-enhancement of susceptibility, transmission or mortality does not lead to the exclusion of either strain. However, for both identical and non-identical strains, enhancement of susceptibility or transmission can lead to complex oscillations (confirming the results of Ferguson et al. (1999a)) when the immunological distance is within certain bounds. This was not found to occur with mortality

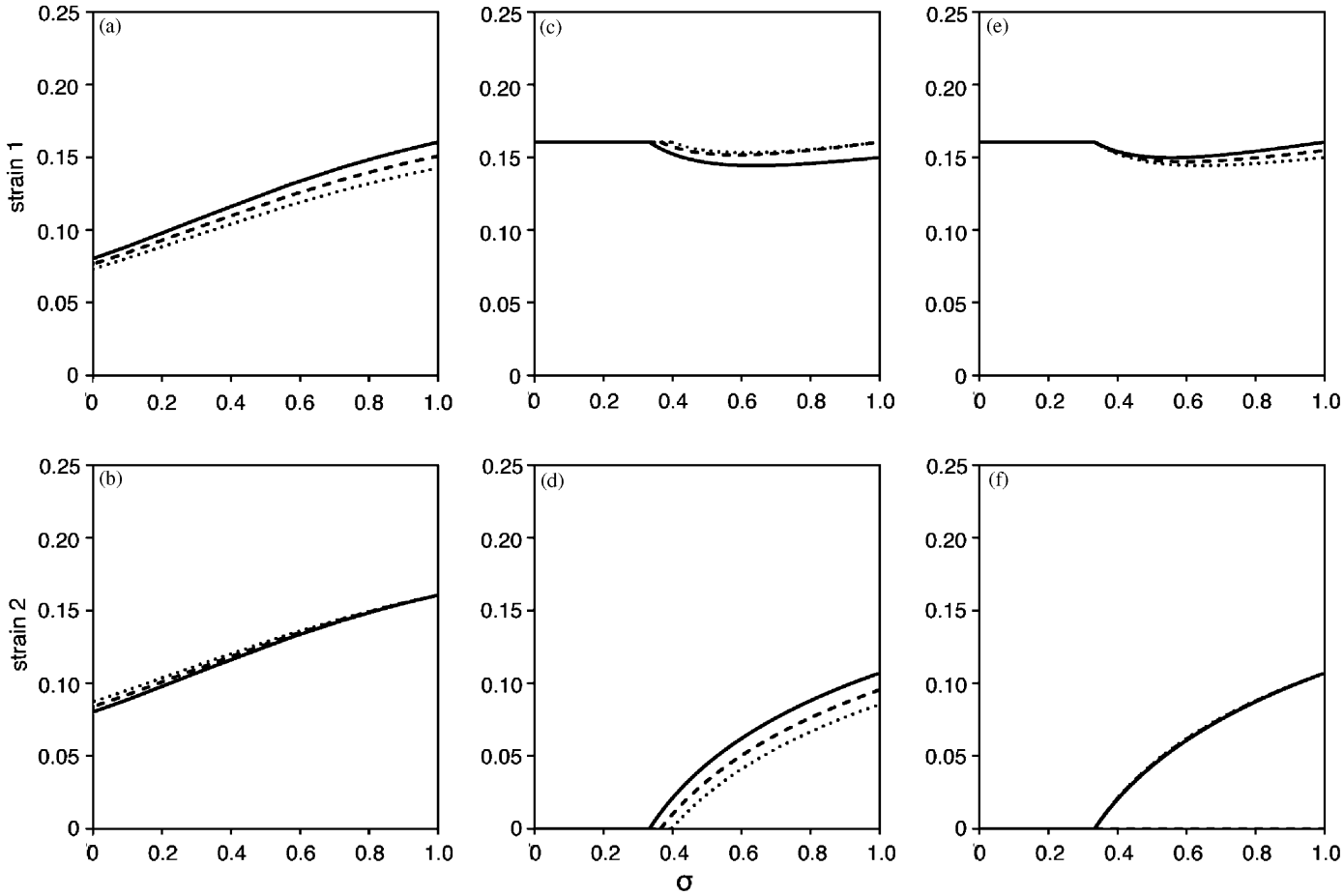

Fig. 4. Stable equilibrium population infected with strain 1 ($IS + IR$) and strain 2 ($SI + RI$) when σ is varied and enhancement acts on mortality through ζ_1 and ζ_2. In all frames: $\mu = 0.0167$, $\gamma = 52$, $\eta_1 = \eta_2 = 1, \phi_1 = \phi_2 = 1$. Frames a and b: identical strains, $\beta_1 = \beta_2 = 104, \zeta_2 = 0$. (—) $\zeta_1 = 0$ (no enhancement), (- - -) $\zeta_1 = 5$, (···) $\zeta_1 = 10$ (strain 2 enhances mortality from strain 1). Frames c and d: distinct strains, stronger strain enhancing, $\beta_1 = 104, \beta_2 = 78, \zeta_1 = 0$. (—) $\zeta_2 = 0$ (no enhancement), (- - -) $\zeta_2 = 5$, (···) $\zeta_2 = 10$ (strain 1 enhances mortality from strain 2). Frames e and f: distinct strains, weaker strain enhancing, $\beta_1 = 104, \beta_2 = 78, \zeta_2 = 0$. (—) $\zeta_1 = 0$ (no enhancement), (- - -) $\zeta_1 = 5$, (···) $\zeta_1 = 10$ (strain 2 enhances mortality strain 1).

enhancement. Numerical experiments not shown here indicate that the periodicity reported by Kawaguchi et al. (2003) depends on logistic growth and simultaneous coinfection in the host population and a very long infectious period. It does not persist if any of these are omitted.

That susceptibility or transmission enhancement should reduce the immunological distance required for stable coexistence can be recognized in the model equations. This is clearest for susceptibility enhancement (Eqs. (3) and (6)). The enhancement parameter η_i directly modifies the cross-immunity parameter σ to produce a composite parameter $\eta_1\sigma$ which is always greater than or equal to σ and so has an effect equivalent to weaker cross-immunity. Transmission enhancement is similar but only increases the effective immunological distance of part of the infectious population, viz., IR and RI. However, these terms are relevant to the infection of double susceptibles (SS, Eq. (1)), where susceptibility enhancement does not act, and the net result is that the impact of transmission enhancement is slightly stronger than that of susceptibility enhancement. In contrast to this, mortality enhancement does not directly interact with cross-immunity. However, the higher mortality rate reduces the number of infected hosts, effectively decreasing the transmission rate, reducing the basic reproductive number and making it harder for the enhanced strain to compete with the enhancing strain.

The fact that no form of enhancement can lead to competitive exclusion when the strains are identical can be understood as follows: Suppose strain 2 enhances strain 1 and the increased competition leads to the exclusion of strain 2. Then the system will tend towards the strain 1 only equilibrium. However this is an unstable state since both strains have the same basic reproductive number and, since coexistence is also unstable, the system would oscillate. Similar reasoning applies to the regions of unstable coexistence arising from susceptibility and transmission enhancement at moderate levels of cross-immunity. According to Ferguson et al. (1999a) endemic coexistence is unstable because cross-enhancement causes the enhanced strain to overshoot and temporarily exhaust the pool of susceptibles. However, in these parameter regions

cross-immunity is always sufficiently weak for the strain with the lower basic reproductive number to invade and so the single-strain equilibria are not stable either.

As far as the emergence of four immunologically distant DEN serotypes is concerned this model suggests that, if ADE can be implicated at all, it is only through mortality enhancement, which increases the immunological distance required for two strains to coexist. Susceptibility or transmission enhancement have the opposite affect, allowing more similar strains to co-exist. Although Kawaguchi et al. (2003) demonstrate a fairly strong impact from mortality enhancement their model uses an infectious period of order 1 year and a life expectancy associated with secondary infection of order 1 month (i.e. a diseased-induced death rate of order 10). In contrast, the model presented here shows increases in immunological distance resulting from mortality enhancement that are too small to entirely support a hypothesis of immunological distinction. However, even if the four DEN serotypes evolved independently and ADE arose by chance (Twiddy et al., 2002) the widespread co-circulation of multiple DEN serotypes observed in the last 30 years means that understanding the future evolution of the virus is as important as understanding the past evolution. This model provides a framework in which to study the impact of different forms of ADE and how they may influence the evolutionary pathway. It also highlights a number of practical and theoretical issues that need to be addressed by future work.

The results shown in this paper consider three forms of enhancement separately. However, experimental and epidemiological observations suggest that susceptibility, transmission and mortality enhancement are all components of the same phenomenon although for dengue the actual contribution of each factor and the resultant net impact is currently difficult to assess. Further numerical experiments with two or three forms of enhancement acting at the same time indicate that the impact of susceptibility and transmission enhancement is cumulative, allowing coexistence of increasingly similar strains. This is counteracted by mortality enhancement although the impact is weak and the system tends to be dominated by the susceptibility and transmission enhancement. The model also assumes that cross-immunity and cross-enhancement can occur in response to the same antibodies. This is reasonable on the basis that immune responses can be varied and may lead to the simultaneous production of neutralizing, null and enhancing antibodies in variable concentrations (Takada and Kawaoka, 2003). It is also supported by the observation that dengue virus specific antibodies can both neutralize and enhance infection (Stephens et al., 2002) and differences within the host may determine which of these effects is dominant (Rothman, 2004). However, epidemiological observations suggest that heterotypic immunity in dengue only lasts for a few months and turns to cross-enhancement as antibody concentrations drop (Kliks et al., 1988). This, and the hypothesis that antibodies are homogenous and either only enhancing or only protective, makes the definition of 'immunologically distinct' problematic where ADE is concerned. On the one hand, there must be a certain degree of immunological similarity for the enhancing antibodies to bind with the antigen. On the other hand, the fact that this confers no benefit to the host effectively implies complete immunological distinction.

Assuming that antibodies can be both enhancing and protective, it has been suggested that in the presence of ADE natural selection may favor a certain level of immunological dissimilarity. Cross-protective antibodies would neutralize more similar strains whereas more divergent strains would not stimulate enhancement (Grenfell et al., 2004). The model presented here cannot comment on this since it assumes that enhancement is a constant function of immunological distance and so is either enabled or disabled but has no gradient of influence. Future work will address this by using alternative functional forms to relate the degree of enhancement to the immunological distance.

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