TITLE: Pathogen Interactions, Population Cycles and Phase Shifts.

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ABSTRACT

Interspecific pathogen interactions can profoundly affect pathogen population dynamics and the efficacy of control strategies. However, many pathogens exhibit cyclic abundance patterns (e.g. seasonality) and temporal asynchrony between interacting pathogens has the potential to reduce the impact of those interactions. Here we use an extension of our previously published model to investigate the effects of cyclic abundance patterns on pathogen interaction. We demonstrate that for interactions mediated through host immunity, immune memory can maintain the impact of an interaction even when the effector pathogen abundance is low or the pathogen is absent. Paradoxically, immune memory can result in pathogens interacting more strongly when temporally out of phase. We find that interactions between species can not only alter pathogen abundance but can also result in changes to the temporal pattern of the affected species. We further demonstrate that this phenomenon may be observed in a natural host / pathogen data set. Given that there is both a continuing debate as to the relevance of pathogen interactions in natural systems and increasing concern regarding treatment of coinfections of veterinary and medical importance, both the discovery of this measurable shift in cycle in the empirical data and the mechanism by which we identified the shift are important. Finally, as the model structure used here is analogous to simple predator-prey system models we also consider the consequences of these findings in the context of that system.

INTRODUCTION

Interspecific interactions of all forms (e.g. predator-prey relationships [1], host-pathogen relationships [2], resource competition [3]) have the capacity to alter the population dynamics of the interacting species. Further, there is growing interest in the dynamical consequences that co-infecting pathogen species have on each other [4]. Such interspecific pathogen interactions can crucially alter pathogen dynamics, host health and the success of control strategies [5]. Nevertheless, while most forms of interspecific interaction are well documented, unequivocal evidence of the existence of interspecific pathogen interactions under field conditions is rare. This has led to suggestions that interspecific interactions are of little importance in shaping pathogen communities under natural conditions [6-16]**.** This debate continues despite the fact that the importance of pathogen interactions is becoming increasingly obvious in clinical settings [17-21]. One possible explanation for the apparent lack of interactions between pathogen species in wild host systems is that the pathogens may be temporally asynchronous within their hosts, resulting in a form of niche segregation and reducing the likelihood of direct interaction [12,22].

In earlier work we were able to detect evidence of a network of interspecific parasite interactions among the gut helminth community of the wild rabbit (*Oryctolagus* cuniculus) [4]. However, in all the statistical analyses of these helminths, month emerged as a strongly significant term [4]. Therefore, the interplay between seasonal dynamics and the interspecific pathogen interactions may be important. If two pathogen species are temporally separated because of differing cyclic (e.g. seasonal) abundance, it is feasible that any potential interaction between them might be nullified. However, we hypothesise

that if an interspecific pathogen interaction is mediated by the host's immune system, via some level of cross-immunity between the pathogens, interaction may still occur due to the "ghost of infection past" acting through immune memory. Under such conditions the longevity of the immune memory mediating the interaction will be critical in determining the overall net strength of the interaction and its impact on the dynamics of the component species.

Using a theoretical framework (see Methods), we examine the relationship between immune mediated interspecific interactions and seasonal patterns of pathogen abundance. Specifically, we address four key questions: 1) Do seasonality alter host immune-mediated interactions between pathogens? 2) How does temporal asynchrony between pathogens affect the impact of their interaction? 3) How does immune memory alter the interaction between species? 4) Can immune-mediated interactions alter the cyclic dynamics of a pathogen? We then analyse a natural pathogen system and ask whether such shifts in dynamics may be observed in the seasonal abundance patterns of a pathogen species for which interaction has already been suggested by other evidence.

It is worth noting that, due to the simplicity of its construction, the model framework used here is extremely flexible. In addition to pathogen interactions the model may simulate many systems where interaction is indirect. For example, the model could also represent apparent competition in certain predator-prey systems with specialist and generalist predators.

RESULTS

Does seasonality alter host immune-mediated interactions between pathogens?

Using our model (see Methods) we examined whether seasonality *per se* could alter host immune mediated interaction between pathogens. A simple two pathogen simulation was undertaken where pathogen 1 (P_1) was allowed to have either a positive or negative effect upon pathogen 2 (P_2), by varying the strength of the interaction, (γ_{12}) between -2 and 2, while pathogen 2 had no effect on pathogen 1 ($\gamma_{21}=0$). The immune decay rate (δ) was set to 4.6, which reduced the value of the immune parameter at time step t to 1% by time step t+1 (1 month later), assuming that no further increase in immunity had occurred. The simulations were conducted both with and without seasonality and the mean pathogen values of P_2 after the initial transient period were compared.

The addition of seasonality changed the mean pathogen abundances, even in the absence of interspecific pathogen interactions ($\gamma_{12}=0$). However, the percentage change in the average P_2 numbers at different levels of γ_{12}, compared to the average at $\gamma_{12}=0$ for the same model, were similar for the seasonal and non-seasonal models (the largest difference in the percentage change in P_2 between the two models was 2.0 %). Therefore, assuming that the sine function is a reasonable representation of natural seasonality we may conclude that seasonality *per se* has a negligible effect upon the impact of interspecific pathogen interactions when the seasonal abundance changes are synchronous.

How does temporal asynchrony between pathogens affect the impact of their interaction?

While seasonality *per se* may be of little consequence to interspecific pathogen interactions, differences in the timing of seasonal abundance between pathogen species may play a greater role. For instance, when the two pathogens are completely out of phase with one another we might expect there to be a much reduced interaction between them. We therefore assessed the influence of such temporal difference in seasonal abundance upon the interaction, by varying the timing of the peak in uptake of P_2 (g_2 in equation 3) from 0 (complete synchrony in uptake between the parasites) up to an 11 month lag between the species' peaks.

As might be expected, when the immune response was very short-lived (e.g. δ=4.6, such that immunity decays by 99% in one time step, the least effect of P_2 on P_1 occurred at approximately the point when the two pathogens were most out of phase (around 5.5 months; fig 1a) and the strongest interaction (the greatest suppression of P_2) occurred when the two species were perfectly in phase (g_2 = 0; fig 1a). Under these conditions the immune-mediated impact of P_1 on P_2 is instantaneous and transient, so the strength of the interaction at any point in time is completely determined by current parasite levels.

How does immune memory alter the interaction between species?

Although for very short lived immune responses (i.e. at high immune decay rates) the relationship between the two interacting pathogens appears simple (i.e. seasonal asynchrony reduces the effect of the interaction), in reality immune responses are typically

much longer lived due to the creation of immune memory. Reducing the immune decay rate (i.e. δ=0.76, 0.18 and 0.07, reducing immunity by 99% after 6, 24 and 60 months respectively) substantially increased the overall effect of P_1 on P_2 (note the different y-axes scales on figs. 1 a-d), because lower immune decay rates result in immune response levels at any point in time being made up of current immunity plus the integral of prior immunity over time. However, an additional and unexpected consequence of this reduction in immune decay was that the point of least effect for P_1 on P_2, (i.e. the lag at which the peak of the mean P_2 value occurs) was not at 5.5 months (as for δ=4.6) when the two pathogens were most out of phase (fig. 1 b-d). When immunity was long lived both the shape of the effect curve and the points of least and greatest interaction between the two pathogens changed. The least effect of P_1 on P_2 occurred at approximately 6 months for δ=0.76, 4.75 months for δ=0.18 and 4 months for δ=0.07, with the point of greatest interaction occurring 6 months later (fig. 1 b-d). Therefore, when immunity is long lived, pathogens may actually interact most strongly when they are seasonally out of phase with each other.

In order to understand this apparently counterintuitive relationship (i.e. that P_1 can have more effect on P_2 when the two parasites are out of phase than when they are in phase) we must examine the relationship between the effector species (P_1) and its immunity (I_1). At high immune decay rates (δ=4.6) P_1 cycles almost synchronously with I_1 (fig 2a). However, when immune decay rates are low (δ=0.07), there is a phase shift and I_1 cycles asynchronously with P_1 (fig 2b). At low immune decay rates there is always a relatively high value for I_1, and this keeps both P_1 and P_2 at low values. Since the 'growth rate' of I_1 is

dependent upon the value of P_1, low P_1 values result in slow I_1 growth rate, thereby pushing the immunity out of phase with the pathogen.

Can immune-mediated interactions alter the cyclic dynamics of a pathogen?

The model reveals that the interplay between seasonality and immune memory can alter the timing of the peak effect of an immune-mediated interspecific pathogen interaction. Further, slow immune decay rates have the effect of shifting the immune response such that it is out of phase with the pathogen against which it is produced. Therefore, another question arises as to whether these effects can change the seasonal dynamics of the affected species. To test this we again varied the seasonal lag in uptake between the two pathogen species but this time we allowed the seasonal dynamics of the effector species P_1 to vary (i.e. by changing g_1, while keeping g_2 fixed) and observed the impact on the temporal dynamics of P_2. Both the timing of the seasonal peak of P_2 and the shape of the seasonal abundance curve can be markedly altered by changing the seasonal peak of P_1 (fig. 3). Therefore, peak shifts resulting from pathogen interactions could potentially force pathogens, which would normally cycle in phase, to be pushed apart, effectively producing completely different seasonal patterns for each pathogen.

Can changes in seasonal abundance due to interspecific interaction be observed in a natural pathogen system?

The model clearly predicts that a sufficiently strong immune-mediated interspecific pathogen interaction should be detectable as a shift in the seasonal abundance pattern of an affected species. In earlier work we presented evidence of interaction between the gut

helminths of the adult wild rabbit (*Orcytolagus cuniculus*) [4]. In particular, we demonstrated that *Graphidum strigosum* showed a substantial reduction in intensity (-29%) when *Trichostrongylus retortaeformis* was present. Further, we hypothesised that the relationship between *T. retortaeformis* and *G. strigosum* must be indirect, as the latter is upstream (in the gut) from the former and thus direct interaction is not feasible. The most likely mechanism for this interaction was therefore mediation through host immunity. These two species also show clear seasonal abundance patterns, which may be approximated by a fitted sine wave function. In order to assess whether we could detect the interaction between these two species as a shift in the seasonal abundance of *G strigosum,* we divided the adult rabbit data (myxomatosis negative only) into *T. retortaeformis* infected (n=1423) and uninfected (n=313) rabbits. Using the non-linear least squares regression in the SPLUS statistical package we fitted a sine wave to the *G. strigosum* abundance by month for both data sets. The sine wave had the form:

$$y = c + a * \text{Sin}((x + g)/h) \qquad (1)$$

where y is the raw data for *G. strigosum* (with or without *T. retortaeformis*) in each month; x is month (where Jan = 0 and December = 11) and h= 11/(2*π), which constrains the sine wave to a complete single cycle of exactly 12 months in length. The parameter c is the constant, or centre point, of the sine wave and parameter a determines the amplitude of the wave. Parameter g determines the wave's position along the x axis and, as such, it determines the timing of the seasonal peak of *G. strigosum*. The fitted sine waves (fig. 4) reveal that the seasonal peak for *G. strigosum* in the presence of *T. retortaeformis* occurs 2

weeks later than that for *G. strigosum* alone. To determine the statistical significance of this shift we created a bootstrapping procedure in the Mathematica computing package which generated 1000 values of g at random from normal distributions based on the estimates of g obtained from the SPLUS analyses for *G. strigosum* with and without *T. retortaeformis* co-infection. For each of these bootstrapped values of g the timing of the peak in abundance was calculated by differentiating equation 1 with respect to x (month), setting the equation equal to zero and solving for x. An ANOVA then compared the 1000 peak time values for each data subset, revealing a highly significant difference between the peaks (F ratio 2819.11, df 1,1000, $p<0.0001$) of the two data subsets, such that the presence of *T. retortaeformis* induces a significant delay in the peak timing of *G. strigosum* abundance. This analysis demonstrates that a shift in pathogen seasonal abundance induced by an interspecific pathogen interaction is detectable in natural data, as the model predicts.

DISCUSSION

Our work suggests that the interplay between seasonality and immune function can have substantial effects upon both the strength and timing of interaction between species and potentially on the seasonal abundance pattern of the affected species. In practical terms this suggests that when there is immune memory, pathogens may interact with one another even if they have entirely different seasonal abundance patterns. Further, when immune memory is long lived, pathogens may interact more strongly when they display different seasonal abundance patterns than when they cycle synchronously. Finally, pathogens interacting via host immunity may shift one another's seasonal abundance patterns. This model prediction is upheld by examination of the interaction between *T. retortaeformis* and

G. strigosum in a natural wild rabbit data set. By examining these data we found a significant shift in the seasonal abundance of *G. strigosum* in the presence of *T. retortaeformis*. This not only confirms the relevance of the model predictions but also provides another method for seeking evidence for interactions in real data. Evidence for the presence and / or effects of interspecific pathogen interactions, in natural systems, is very rare. The provision of any novel methodology for identifying interaction from wild host data is therefore a very useful step forward.

The simplicity of the chosen model format should make it applicable to a wide range of pathogen systems as few assumptions regarding the type of system are made. The sine function chosen to model seasonality is also applicable to other simple forms of temporal cycle. Additionally, it is the immune decay rate which causes the temporal shifts and this is merely made visible by the cyclic function, therefore any function which extrinsically forces cycles, as does the sine wave, should allow the phase shifts to occur. The immunity in our model acts in a similar manner to a predator and immune decay rate is therefore analogous to a predator death rate. As stated earlier, the model could therefore be viewed as a two prey, two predator system, with one generalist and one specialist predator (i.e. I_1 and I_2 respectively). In this case the model indicates that apparent competition between two prey species, mediated via a generalist predator, can have effects over and above a simple reduction in prey numbers. Relatively long lived predators could act similarly to the long lived immunity and thus two prey species might be pushed out of temporal synchrony via predation. Alternatively, two prey species may have evolved to cycle out of phase in order to avoid competition for a particular resource. If predation were to push such species into

phase, then the apparent competition due to the predator could potentially be exacerbated by increasing direct competition for a particular resource.

The concept of shifting temporal behaviour, in either a pathogen or prey population, caused through apparent competition with a second species could profoundly affect the way interactions between species are examined. We conclude that pathogen interactions will not normally be prevented by cyclic pathogen dynamics. Indeed, it is clear that cyclic dynamics may themselves be altered by interaction and that host immunity and immune memory will play a complex and dynamic role in this process.

METHODS

Basic Model Structure

The basic model is an adaptation of that published in Lello *et al.* (2004) [4] with the exception that, for simplicity, the pathogen uptake term is altered to be a constant uptake rate (equation 1):

$$dP_i/dt = \Lambda_i e^{c_i I_i} - d_i P_i \qquad (2)$$

where P_i is density of pathogen species *i*, t is time, Λ_i is the constant uptake rate of pathogen species *i*, c_i is a constant which moderates the immune response against the pathogen, I_i is the immune response created against pathogen *i*, and d_i is the death rate of pathogen *i*. We assume seasonal variation in pathogen abundance is driven by seasonal variations in parasite transmission between hosts (which may incorporate seasonality in, for instance, contact rates between hosts, or mortality rates of free-living infective stages on pasture etc). We therefore modified the parasite uptake term by a sine function (equation 2)

$$dP_i/dt = \Lambda_i (1+\lambda_i \sin(2\pi(t+g_i)/\tau)) e^{c_i I_i} - d_i P_i \qquad (3)$$

where λ_i determines the amplitude of the seasonal wave and lies between 0 and 1, t is time, g_i is a phase shift and determines when the seasonal abundance peak for species *i* occurs and τ is the period.

An immune response is created against each pathogen and may be moderated (increased or decreased) by a second species (equation 3).

$$dI_i/dt = \alpha_i P_i - \gamma_{ji} P_j - \delta_i I_i \qquad (4)$$

where I_i is the immunity produced against pathogen *i*, α_i is the rate of production of immunity stimulated by pathogen *i*, γ_{ji} is the rate of production of immunity by pathogen *j* against pathogen *i*, P_j is the abundance of pathogen species *j* and δ_i is the rate of immunity decay. Interactions between pathogens are therefore incorporated by increasing or decreasing the production of immunity against one species due to the presence of a second species (i.e. varying the γ_{ji} values). Unless otherwise stated in the text, $\Lambda_i = 1000$, $c_i = -0.05$, $\lambda_i = 1$, $d_i = 1.5$, $g_i = 0$, $\tau = 12$, $\alpha_i = 1$, $\gamma_{12} = -0.5$ and $\delta_i = 4.6$, for all model runs. In all subsequent sections results are reported from the models after they have settled to stable dynamics.

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FIGURE LEGENDS

Figure 1. Effect of changing the rate of immune decay (δ) and the time lag between pathogen seasonal peaks upon the interaction between P_1 and P_2. As immune longevity increases a) δ=4.6, b) δ=0.76 c) δ=0.18 and d) δ=0.07, the point of least interaction, (i.e. when P_2 is at its highest average value) shifts away from the mid-point of the time lags. Additionally the shape of the curve also alters.

Figure 2. Abundance, after initial transient period, of pathogen species 1 (solid line) and level of the immunity produced against it (dashed line) through time with relatively rapid a) δ=4.6 and slow b) δ=0.07 immune decay rates.

Figure 3. Changes in pathogen 2 abundance dynamics obtained when the seasonality parameter (g_1) of pathogen 1 is altered. All values of $g_1 > 0$ result in a shift in the seasonal pattern of abundance for P_2 with the greatest effect being a 2 month shift in the peak abundance of P_2. This indicates that pathogen interactions could act to alter not only the numbers but also the seasonal patterns of an interacting species.

Figure 4. a) Fitted sine waves for the monthly abundance of the nematode Graphidium strigosum calculated from hosts without (solid line) and coinfected with (dashed line) the nematode Trichostrongylus retortaeformis, revealing the 2 week seasonal shift between the two sub-groups. Plots b and c show the same fitted lines for rabbits uninfected with T. retortaformis and coinfected rabbits, respectively, along with the raw G. strigosum abundance data for each group. For all graphs month 0 is January and 11 is December.