Succinctus

Characterising interactions between co-infecting parasites using age-intensity profiles

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A B S T R A C T

Interactions between co-infecting parasite species can impact transmission. Whether co-infection is beneficial or detrimental to a target parasite, and whether the mechanism involves changes in host susceptibility or parasite clearance, can be difficult to assess. We demonstrate the potential for host age-parasite intensity curves to allow assessment of these factors. A model is developed to generate predictions and test these predictions using helminth parasites of white-tailed deer (*Odocoileus virginianus*). We identify three beneficial interactions involving five helminth species, including susceptibility and clearance-based mechanisms. Our results suggest that analysis of age-intensity data represents a new tool for assessing the nature and strength of co-infecting parasite interactions.

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Interactions between parasite species occurring within individual hosts, although cryptic, are increasingly recognised as powerful forces shaping the fitness and transmissibility of parasite species (Ulrich and Schmid-Hempel, 2012; Griffiths et al., 2014; Ezenwa and Jolles, 2015; Budischak et al., 2018). Parasite interactions can be mediated by host resources or the host immune system (Pedersen and Fenton, 2007), but traits of the co-infecting parasites (e.g., resources used, site of infection, specific immune response triggered) determine how these resource- and immune-mediated interactions translate into fitness outcomes for the parasites. Recently, considerable progress has been made in terms of detecting whether interspecific interactions are beneficial or detrimental for a parasite species (Fenton et al., 2010; Telfer et al., 2010; Johnson and Buller, 2011; Lello et al., 2018). However, commonly used approaches for detecting interactions, such as testing for pairwise correlations between parasites in cross-sectional data, are often unreliable (Fenton et al., 2014), highlighting the need for new quantitative tools to detect parasite interactions.

Beyond determining the beneficial or detrimental nature of interactions between parasites, identifying the precise parameters affected by co-infection (e.g. host susceptibility, host infectiousness, parasite clearance rate, disease-induced mortality) has proven more elusive. This is especially true in non-experimental

contexts. For example, when co-infection with one parasite has a positive effect on another, even though the general mechanism might be known (e.g., immune-mediated facilitation), it can be difficult to determine whether the observed facilitation is due to an increase in host susceptibility to the target parasite or a decrease in the parasite clearance rate following infection (Lello et al., 2018). However, information on the precise parameters affected are often necessary to predict how co-infection with one parasite might affect the population dynamics of another, to interpret patterns of heterogeneity in infection and to assess potential control strategies. Here, we present a framework for assessing whether parasite interactions operate via changes in host susceptibility or clearance rate. Our approach can use either longitudinal or cross-sectional sampling of host individuals to infer whether a background (co-infecting) parasite increases or decreases the transmission potential of a focal parasite. We apply this approach to helminth communities of white-tailed deer (*Odocoileus virginianus*) as a test case.

First, our method makes use of age-stratified data on helminth intensity (number of parasites per host) to identify parasites showing a convex age-intensity profile, where intensity increases with host age in the early phase of host lifespan but decreases later in life. Convex age intensity profiles are well-described in host-helminth systems (Bundy et al., 1988; Cattadori et al., 2008; Lello et al., 2018), and there are several mechanisms that can generate this pattern including parasite-induced host mortality, acquired

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immunity, and age-related changes in parasite susceptibility or exposure (Woolhouse, 1998; Wilson et al., 2002). Note that some of these mechanisms could increase intensity with age (e.g., age-dependent exposure), provided the net effect is a reduced force of infection. The simplest model that generates convex age-intensity profiles for a single parasite species assumes that hosts experience a diminishing age-dependent force of infection and constant parasite clearance rate, so that the number of parasite individuals in a host (intensity, y) changes with host age (A) according to $\frac{dy}{dA} = \lambda e^{-\lambda A} - vy$, where λ is the maximal force of infection (experienced by uninfected newborn hosts) and v is the clearance rate. This results in an age-specific intensity of $y = \frac{\lambda}{\lambda - v}(e^{-\lambda A} - e^{-vA})$. Alternative models, in which acquired immunity affects clearance, generate broadly similar results (Supplementary File S1) compared with this more simple model.

Second, we assumed that the process generating the convex age-intensity profile is modified by a background parasite species, leading to a specific set of predictions about how age-intensity relationships should vary depending on the parameter driving the interaction between parasites. Hosts with the background parasite may have a higher or lower value of either λ or v. This reflects facilitative or antagonistic interactions between the parasite species that affect either susceptibility to (λ) or clearance of (v) the focal parasite. Since convex age-intensity patterns can be characterised as the peak intensity and peak host age at which that intensity occurs, and changes in these peaks ('peak shifts') have been used to infer differential transmission both within and among host populations (Anderson and May, 1985; Woolhouse, 1998; Cattadori et al., 2005), we used the direction of peak shifts in co-infected hosts to distinguish the type and strength of the interaction occurring between background and focal parasites (Fig. 1).

Finally, we applied our method to data on helminth infections of white-tailed deer in the United States, collected as part of the Herd Health survey (Yabsley et al., 2003). These data comprise adult helminth records (species and abundance) for known-aged animals sacrificed between 1973 and 2003. Eighteen helminth spe-

cies were recorded from over 2000 host individuals. Of these, two helminths, *Ostertagia dikmansi* and *Trichostrongylus axei*, exhibited convex age-intensity profiles (Fig. 2). Consequently, *O. dikmansi* and *T. axei* were treated as focal parasites in our study. All other parasite species served as 'background' parasites.

For each focal parasite, we explored potential interactions with 17 background parasite species. This was done by sub-setting the full data set to only hosts infected by the focal parasite, and then creating two groups within each host age class. One group had the highest levels of intensity of the background parasite, the other had the lowest levels of intensity. This allowed two age-intensity data sets to be created per focal-background parasite pair (i.e., with high and low intensity background infection), with equal numbers of hosts overall and within age classes. Balancing data across background infection levels and age classes was desirable from a curve-fitting perspective to determine peak intensity as a function of host age, which was achieved by fitting loess curves to $\log_{10}$(intensity) versus age data. Potentially interacting parasite pairs were excluded from further analysis if one or both of the age-intensity curves (at high and low intensity infection with the background parasite) was not convex, which prevents a peak from being identified. Further, parasite pairs were excluded if the background parasite had low prevalence (<25%) since this resulted in both the high and low background intensity groups being dominated by intensity values of zero, meaning the two groups lacked contrast. Twenty-three out of 34 parasite pairs were excluded based on the first criterion above, and eight of the remaining 23 pairs were excluded based on the second.

Three focal-background parasite pairs had good contrast between low and high background intensity levels and generated peak shifts, with peak intensity varying between the high versus low intensity levels. In all three cases, the background parasite species was beneficial to the focal parasite species as illustrated by the presence of all three points in the positive quadrants of relative peak age-intensity space (Fig. 3, cf. Fig. 1C). For focal parasite *O. dikmansi*, hosts that had relatively high intensity infections with
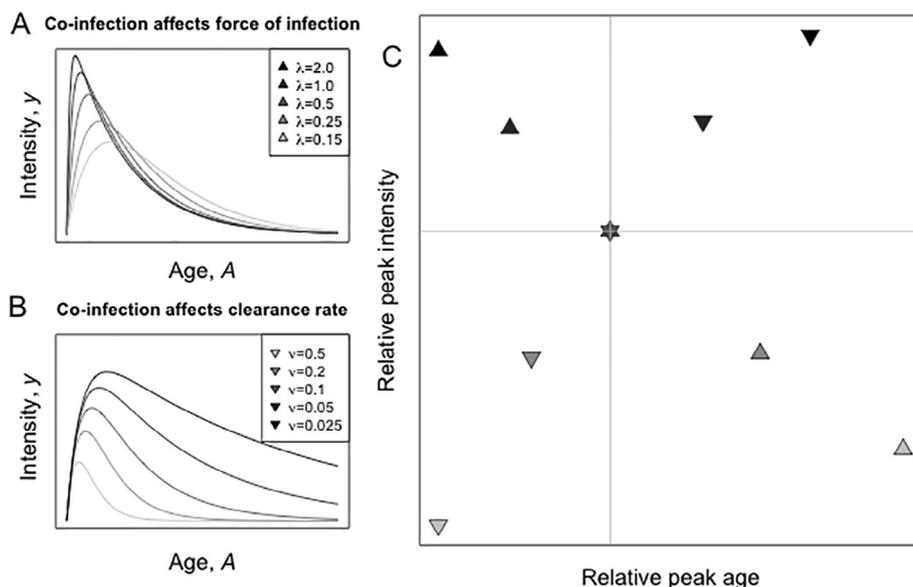
Fig. 1. Relationship between parasite intensity and host age for co-infected hosts experiencing different types of parasite interactions. (A and B) Predicted parasite intensity (y) as a function of host age (A) according to the model: $y = \frac{\lambda}{\lambda - v}(e^{-\lambda A} - e^{-vA})$, where λ is the maximal susceptibility and v is the clearance rate. Grey-scale lines assume that co-infection results in a different susceptibility (A) or parasite clearance rate (B) relative to a reference scenario (mid-grey, $\lambda = 0.5$, $v = 0.1$). (C) The corresponding characteristic shifts in age at peak intensity, and peak intensity according to whether susceptibility or clearance rates are increased or decreased relative to the reference scenario. Quadrants demarcate the effect of co-infection as: increased susceptibility (upper left), reduced clearance rate (upper right), reduced susceptibility (lower right), increased clearance rate (lower left). Grey scale and orientation of triangles correspond to the legends in A and B. For upward-pointing triangles, $v = 0.1$, and for downward-pointing triangles, $\lambda = 0.5$.
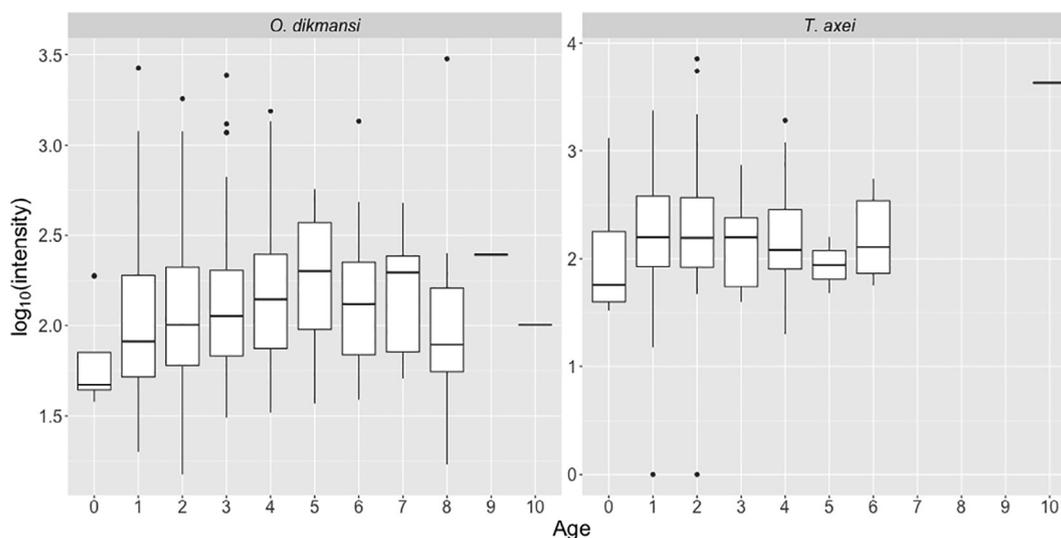
Fig. 2. Intensity data for adult *Ostertagia dikmansi* and *Trichostrongylus axei* parasites quantified in white-tailed deer of various ages.
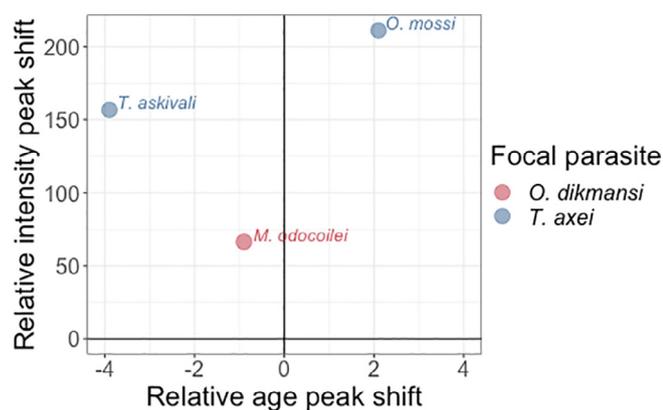

Fig. 3. Relative peak shifts in age (years) and intensity for two focal parasites, *Ostertagia dikmansi* (circle) and *Trichostrongylus axei* (triangle), depending on the level of infection of co-infecting parasites (zero/low intensity versus high intensity). For the zero/low intensity co-infection group, relative age at peak intensity and relative peak intensity are represented by the origin, whereas the high intensity co-infection groups are indicated by shapes. For *O. dikmansi* the average intensity (across all host ages) of *Mazamastrongylus odocoilei* was 240 in the low intensity group and 1575 in the high intensity group. For *T. axei* the average intensity of *Trichostrongylus askivali* in low and high intensity groups was 21 and 777, respectively, and for *T. axei* the average intensity of *Ostertagia mossi* in low and high intensity groups was 154 and 990, respectively.

Mazamastrongylus odocoilei reached peak intensity approximately 1 year earlier than hosts with relatively low intensity *M. odocoilei* infections, and the peak intensity was approximately 60 worms higher in the high background intensity group. Similarly, focal parasite *T. axei*, also benefited from background infection with either *Trichostrongylus askivali* or *Ostertagia mossi*. In the case of *T. askivali* co-infection, *T. axei* intensity peaked 4 years earlier with an intensity approximately 120 worms higher than the low background intensity group. In the case of *O. mossi*, *T. axei* intensity peaked 2 years later in the high background infection group with an intensity approximately 250 worms higher.

While all three parasite interactions benefited the focal parasite species, our method further reveals that two were associated with increased susceptibility (higher and earlier peak intensity) and one was associated with reduced clearance (higher and later peak intensity). This inference is based on our theoretical prediction showing that when parasite interactions are facilitative (i.e., peak

intensity is increased relative to a host population infected with only one parasite) the age of the peak reveals information about the mechanism: susceptibility-based facilitation exhibits an earlier peak and clearance-based facilitation exhibits a later peak (Fig. 1). The interactions were also of different magnitudes, with focal parasite *O. dikmansi* reaping the smallest benefit of co-infection and *O. mossi* conferring the strongest facilitative effect.

Our conclusion that during co-infection *O. dikamansi* and *T. axei* benefit from the presence of *M. odocoilei* and *T. askivali/O. mossi*, respectively, is supported by emerging evidence on the effects of economically important helminths on the immune system of ruminant hosts. For example, a well-studied relative of *O. mossi*, *Teladorsagia circumcincta*, has been shown to modulate the immune response of sheep hosts in a way that can facilitate parasite survival (McNeilly et al., 2013). Similarly, a congener of *T. askivali*, *Trichostrongylus colubriformis*, which infects various livestock species as well as white-tailed deer (Prestwood et al., 1976), produces a protein with high homology to known immunomodulatory peptides (Riffkin et al., 2000), which may enhance its survival within the host. Such immunomodulatory mechanisms should also benefit co-infecting parasites, helping to explain the patterns we observed for parasites of deer. While immunomodulatory effects may generally account for the beneficial effects of co-infection we observed, the current paucity of information on the specific effects of most ruminant helminths on the host immune response precludes any hypotheses about why some co-infection benefits accrued from increases in susceptibility of the host to infection, while others were due to reduced clearance of the parasite by the host. Applying our novel method to parasite systems with better-characterised effects on the host immune response (e.g., mouse helminths; Reynolds et al., 2012; Grencis et al., 2014) would help further validate our approach.

The approach presented here relies on reasonably high quality data on species-level parasite intensity as a function of host age. If assessing age is difficult for a particular host species, or if parasite species identity cannot easily be determined (e.g., if relying on faecal egg counts to estimate intensity) then this approach may not be suitable for inferring the nature and strength of interspecific parasite interactions. In the case of cross-sectional sampling of hosts, as we have used in the data component of this study, there may be other unmeasured covariates driving age-intensity patterns, which means that any conclusions should be taken as preliminary evidence of interactions, warranting more

extensive research. However, a conservative approach of retaining all potential hypotheses (clearance-only, susceptibility-only, or both) seems prudent. Furthermore, hosts are often infected with multiple background parasites, which can potentially mask the role of any particular interspecific interaction. However, it is unlikely that co-infection by multiple background parasite species also results in them sharing similar intensities across all host individuals. Additionally, co-infection may reduce host life span, especially for those individuals with high worm burdens, such that samples of relatively old hosts may be biassed towards low intensity values. This is likely not an issue in our data since co-infected groups had higher peak intensities, but is a potential limitation of the approach. In spite of the caveats associated with this idea, we contend that the ability to identify potential within-host parasite interaction mechanisms from age-intensity data is a promising addition to the suite of techniques aimed at understanding the nature and consequences of co-infection. Future work combining this peak shift-based approach with controlled experimental studies can provide a strong test of this new method.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2019.11.001.

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