Co-infections mask pathogen-specific associations with the gut microbiota in wild voles

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Abstract:

Research Highlight: Brila, I., Lavirinienko, A., Tukalenko, E., Kallio, E. R., Mappes, T. & Watts, P. C. (2022). Idiosyncratic effects of coinfection on the association between systemic pathogens and the gut microbiota of a wild rodent, the bank vole (*Myodes glareolus*). *Journal of Animal Ecology,* https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2656.13869. Interactions between pathogens and host-associated microbial communities can influence host fitness, disease progression and pathogen emergence. The vast majority of studies characterize interactions between single pathogens and bacterial commensals, yet co-infections with multiple pathogens are the norm in nature. In their paper on pathogen-microbiome interactions, Brila et al. (2022) examine how co-infections with four systemic pathogens associate with the gut microbiota in wild bank voles. Building on a series of tests, the authors show that excluding co-infection information from statistical models masks pathogen-specific patterns and confounds interpretations. This paper advances on previous studies by generating surveillance data on a phylogenetically diverse suite of vole pathogens to address the question as to whether pathogens exhibit unique or universal associations with gut commensals. They report that even bacterial pathogens with similar transmission ecology have divergent associations with gut microbes, and highlight that a mechanistic understanding of host-pathogen interactions is necessary for decoding the diverse consequences for gut microbial communities.

Main text:

Animals and plants harbour complex suites of microbes that span the parasitism-mutualism continuum. To date, microbiomes have largely been studied in the context of their interactions with host biology (e.g., host diet or genotype), yet interactions between microbes themselves have profound consequences for host fitness (Gould et al., 2018), disease progression (O'Keeffe et al., 2021), and pathogen evolution and emergence (Drew et al., 2021; Frederickson & Reese, 2021). Pathogenic microbes are particularly important for shaping interaction networks because they are often uniquely able to suppress competitors (Amaro & Martín-González, 2021) and can hijack the host immune system in a way that affects target and non-target microbes (Kamada et al., 2013). Among studies that characterize interactions between commensal and pathogenic microbes, most explore the effect of single pathogens. In nature, however, co-infections with multiple pathogens are the rule rather than the exception (Hoarau et al., 2020). Generalizing microbial interactions in the face of such complexity remains a major challenge, yet is essential to advance our understanding of how host-associated microbiomes shape host ecology and evolution.

In their recent publication in the *Journal of Animal Ecology*, Brila et al. (2022) investigate associations between co-infecting pathogens and commensal gut microbes in wild bank voles *Myodes glareolus*. The authors pair surveillance data on four systemic pathogenic microbes (two bacterial, one protozoan, and one viral) with faecal microbiota profiling (Figure 1A). Systemic pathogens that infect host blood and tissue present an intriguing case study because they cannot directly interact with commensals. In other words, systemic pathogens cannot outright compete with commensals for niches and nutrients. Any observed associations are therefore assumed to be mediated through pathogen effects on host immunity, although shared responses to other processes (e.g., depressed immunity following an environmental stressor) may provide an additional explanation. Their hypotheses were guided by a recently proposed framework that aims to classify types of interactions between co-infecting pathogens and gut microbiota (Schmid et

al., 2022). This framework states that any effects on the gut microbiota arising from co-infections can either be neutral, implying no interference with the effect of the co-infecting pathogen, antagonistic, whereby co-infecting pathogens have opposite effects on microbial phenotypes, or synergistic, which describe additive effects of multiple pathogens. Brila et al. hypothesized that co-infections would result in antagonistic associations, because interactions with host immunity are often pathogen-specific (Hawley & Altizer, 2011).

In their study, over half of the 192 voles sampled were infected with at least one systemic pathogen, and almost 20% were co-infected with various combinations of the four pathogens. Overall, they identified weak, antagonistic associations between different pathogens and gut microbial phenotypes. Crucially, accounting for co-infections revealed pathogen-specific associations that would otherwise have gone undetected. For example, only single infections with *Puumala orthohantavirus* were associated with higher α-diversity compared to uninfected individuals, whilst additional infections negated this effect (Figure 1B). Associations between the gut microbiota and one bacterial pathogen, *Anaplasma phagocytophilum,* tended to override any associations with other co-infecting pathogens, while co-infections involving the other three pathogens generated antagonistic associations that masked pathogen-specific associations with ß-diversity (Figure 1C).

Pathogen-specific associations with α- and ß-diversity were reflected by differential abundances of specific microbial taxa between infection categories. The study identified substantially more differentially abundant genera when considering co-infections, and patterns were largely pathogen- and infection-specific. For instance, a member of *Barnesiella*, a genus found frequently in gut microbial communities of healthy humans, was reduced in animals with single *Puumala orthohantavirus* infection, but not in co-infected individuals. Although a taxonomically diverse suite of microbes was differentially abundant between infection groups, pathogen-specific associations

were particularly common for members of the family *Lachnospiraceae* and *Ruminococcaceae* (Figure 1D). Nevertheless, some genera belonging to these families demonstrated both positive and negative associations with particular pathogens.

A noteworthy finding from this study is that the two tick-born bacterial pathogens, *A. phagocytophilum* and *Borrelia burgdorferi,* demonstrated different associations with the gut microbiota. This is potentially surprising because one might expect similar associations between pathogens belonging to the same kingdom and transmitted through the same vector. However, despite these ecological similarities, differential effects may be mediated through pathogen-specific interactions with host immunity. The intracellular bacterium *A. phagocytophilum* mainly interacts with agents of host innate immune functions, specifically neutrophils, whereas *Borrelia* pathogens modify the balance of antibodies IgM and IgG to subvert T-dependent B cell responses (Tracy & Baumgarth, 2017). These differences highlight that host immune mechanisms are likely more important than pathogen phylogeny or ecology for predicting pathogen interactions with commensal microbes.

A mechanistic understanding of these findings will require further studies that incorporate additional information, including infection intensity, the order of infections, and host immune parameters. Data on infection intensity may help distinguish between chronic and acute infections, which is relevant for shaping the type of immune response mounted by the host. Similarly, the order of infections may play a role in how sequentially co-infecting pathogens interact with each other, host immunity and microbiota (Halliday et al., 2017), and certain immune parameters are particularly relevant for shaping both the gut microbiota and pathogen dynamics (Schmid et al., 2023). Bank voles are an ideal model organism to study these processes experimentally and in the wild because they host a range of pathogens, which differ in the duration

and intensity of infection and their transmission ecology, including those that are likely co-transmitted or sequentially transmitted from the same vector (Moutailler et al., 2016).

Brila et al. provide support for pathogen-specific associations with the commensal microbiota. Disentangling causality and identifying generalizable associations between pathogenic microbes and parasites and host-associated microbiomes (e.g., categorizing pathogen-microbiota and microbiota-microbiota associations by their phylogenetic, functional and ecological properties) present future challenges for the field of microbial ecology (e.g., (Roche et al., 2022)). Given recent evidence that microbial interactions between commensals and pathogens are profoundly important for shaping both host fitness and pathogen evolution, studies such as this that integrate surveillance-style methods to pinpoint microbial interactions with a diverse suite of pathogens are sorely needed. Coupling surveillance results with carefully designed experiments will aid future research to further disentangle mechanistically how pathogenic and commensal microbes interact.

Author contributions

Both authors contributed equally to the manuscript.

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Conflict of interest

The authors have no conflict of interest to declare

Figure

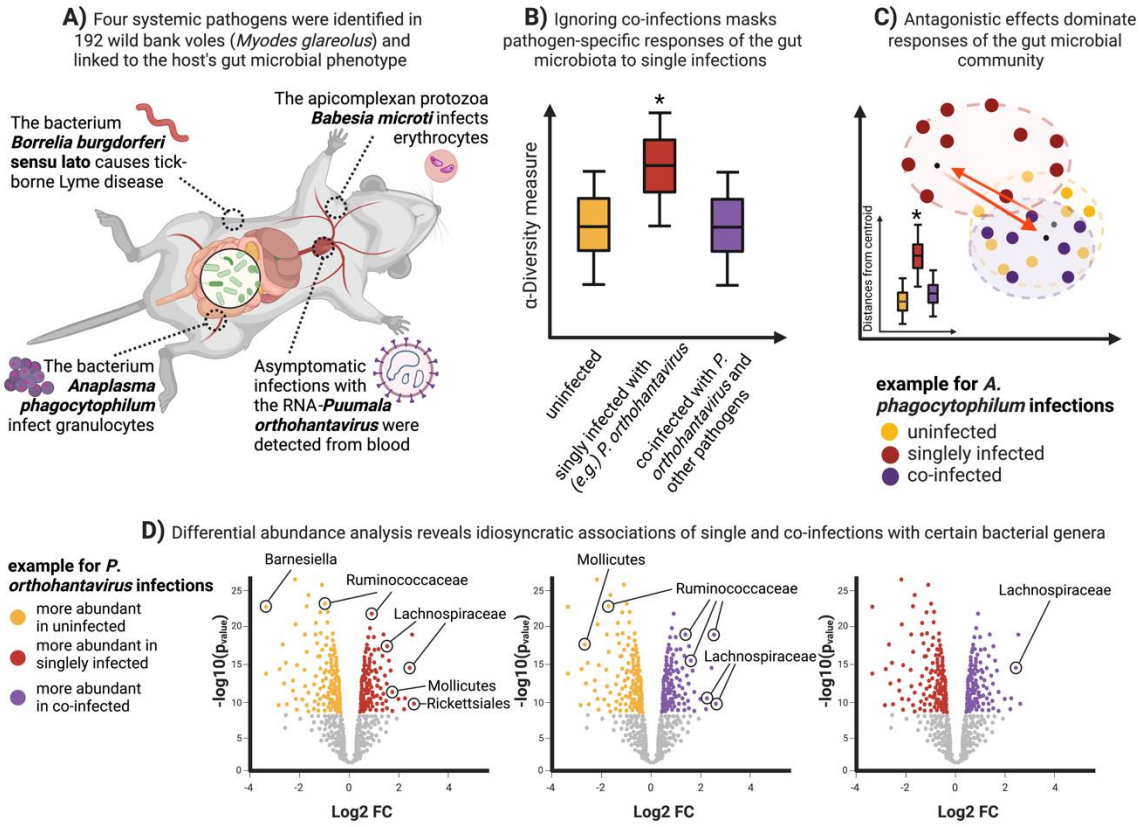

Figure 1. Graphical summary of methods and findings from Brila et al. 2022.

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