Peer Community In Infections

Unveiling the complex interactions between members of gut microbiomes: a significant advance provided by an exhaustive study of wild bank voles

Thomas Pollet *based on peer reviews by* ***Jason Anders*** *and 1 anonymous reviewer*

Marie Bouilloud, Maxime Galan, Adelaide Dubois, Christophe Diagne, Philippe Marianneau, Benjamin Roche, Nathalie Charbonnel (2023) Three-way relationships between gut microbiota, helminth assemblages and bacterial infections in wild rodent populations. biorxiv, ver. 2, peer-reviewed and recommended by Peer Community in Infections.
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The gut of vertebrates is a host for hundreds or thousands of different species of microorganisms named the gut microbiome. This latter may differ greatly in natural environments between individuals, populations and species (1). The vertebrate gut microbiome plays key roles in host fitness through functions including nutrient acquisition, immunity and defense against infectious agents. While bank voles are small mammals potentially reservoirs of a large number of infectious agents, questions about the links between their gut microbiome and the presence of pathogens are scarcely addressed.

In this study, Bouilloud et al. (2) used complementary analyses of community and microbial ecology to (i) assess the variability of gut bacteriome diversity and composition in wild populations of the bank vole *Myodes glareolus* collected in four different sites in Eastern France and (ii) evaluate the three-way interactions between the gut bacteriota, the gastro-intestinal helminths and pathogenic bacteria detected in the spleen. Authors identified important variations of the gut bacteriota composition and diversity among bank voles mainly explained by sampling localities. They found positive correlations between the specific richness of both the gut bacteria and the helminth community, as well as between the composition of these two communities, even when accounting for the influence of geographical distance. The helminths *Aonchotheca murissylvatici,*

Heligmosomum mixtum and the bacteria *Bartonella* sp were the main taxa associated with the whole gut bacteria composition. Besides, changes in relative abundance of particular gut bacterial taxa were specifically associated with other helminths (*Mastophorus muris*, *Catenotaenia henttoneni*, *Paranoplocephala omphalodes* and *Trichuris arvicolae*) or pathogenic bacteria. Infections with *Neoehrlichia mikurensis, Orientia* sp, *Rickettsia* sp and *P. omphalodes* were especially associated with lower relative abundance of members of the family Erysipelotrichaceae (Firmicutes), while coinfections with higher number of bacterial infections were associated with lower relative abundance of members of the Bacteroidales family (Bacteroidetes).

As pointed out by both reviewers, this study represents a significant advance in the field. I would like to commend the authors for this enormous work. The amount of data, analyses and results is considerable which has sometimes complicated the understanding of the story at the beginning of the evaluation process. Thanks to constructive scientific interactions with both reviewers through the two rounds of evaluation, the authors have efficiently addressed the reviewer's concerns and improved the manuscript, making this great story easier to read. The innovative results of this study emphasize the complex interlinkages between gut bacteriome and infections in wild animal populations and I strongly recommend this article for publication In Peer Community Infections.

References:

(1) Vujkovic-Cvijin I, Sklar J, Jiang L, Natarajan L, Knight R, Belkaid Y (2020) Host variables confound gut microbiota studies of human disease. Nature, 587, 448–454. https://doi.org/10.1038/s41586-020-2881-9

(2) Bouilloud M, Galan M, Dubois A, Diagne C, Marianneau P, Roche B, Charbonnel N (2023) Three-way relationships between gut microbiota, helminth assemblages and bacterial infections in wild rodent populations. biorxiv, 2022.05.23.493084, ver. 2 peer-reviewed and recommended by Peer Community in Infections. https://doi.org/10.1101/2022.05.23.493084

Reviews

Evaluation round #2

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Version of the preprint: 1

Authors' reply, 27 January 2023

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Decision by Thomas Pollet, posted 16 January 2023, validated 17 January 2023

2nd round of revisions

Dear authors,

Thank you for submitting the first round of revisions. Both reviewers found you answered to most of their initial concerns. However, they noticed some issues remained that they would like to be addressed before the manuscript being accepted for publication.

Please address all these points and submit both a revised version of your manuscript and response letter.

I look forward to receiving your revised manuscript.

Yours sincerely
Thomas POLLET

Reviewed by Jason Anders, 13 January 2023

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Reviewed by anonymous reviewer 1, 04 January 2023

I found that the authors have answered my initial concerns mostly sufficiently. There are two larger issues and some minor issues that I would like to be still addressed.

First, there is still a small lack of clarity with interactions. I note that the authors are careful of not mixing their observed associations as interactions, which is commendable, but this might be worth stating explicitly. For example, in paragraph from rows 127 to 148 as it will be one of the main motives in discussion where this is raised multiple times.

Second, the lack of broader theoretical framework causes some problems in relation to the Introduction and Discussion. There are a number of lenses to look at the assembly process for three different communities (e.g., stochastic/adaptive , extrinsic/intrinsic, environmental/?, transmission/interactions, colonization/maintenance, systemic/direct, local/global in introduction only!). I would go once through these passages and consider whether there is still room to simplify the conceptual jungle.

Minor issues:

- Row 84: Is not the ”variation in microbial fitness” rather a consequence of the variation of microbes differential reproductive success rather than a driver of said variation? If you want to contrast for “stochastic processes”, then I would think something along the lines of “adaptive differences in microbes”.
- Row 85: Should be “host”.
- Row 84-90: I find everytime these classifications of different factors difficult. I would not consider parasite infection as “environmental factor”. In previous sentence you have said that it is an extrinsic feature, which makes sense.
- Row 102: or rather “…bacteriota interactions may thus be positive or negative (Loke & Lim, 2015), with…”?
- Row 118-126: The references should be used a bit more accurate. I would like to see a reference to systemic impact and for the claim that studies have focused on viruses, not on bacteria.
- Row 139: This claim should have a reference, maybe to a classic study.
- Row 515: I think that it might overstretching your results to say that you found that _individuals_ were clustered within distinct enterotypes. The discussion that you are presenting considers this to be the case, but you had only one sample per individual, so technically, you rather have just a snap-shot in time. I think the first important step here would be to figure out whether distinct enterotypes are longitudinally constant or whether intraindividual variation trumps here interindividual variation.
- Row 537: It is strange to say that geographic location shapes the variation in gut bacteriota. I would expect that it is abiotic or biotic factors related to the location that does the shaping as you write.
- Row 576: I do not understand this sentence. Do you mean to say that you did not found it?
- Row 646: I do not follow the second idea fully. Not considering interactions, I would think that gut microbiota is more shaped by host immune function and/or host diet, whereas gut helminths are more limited by invasion success – I think this would be the thinking along the “everything is everywhere” paradigm of microbial ecology. Then again, this maybe goes beyond the authors’ focus as there is no general framework on how to combine transmission/external factors to within-host interactions in shaping the gut community.

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2022.05.23.493084

Authors' reply, 15 December 2022

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Decision by Thomas Pollet, posted 25 August 2022

Revision needed

Dear authors,

Thank you for submitting your article in PCI infections. Your manuscript has been evaluated by two expert reviewers. Apologies for the time it has taken to reach this point. As underlined by both reviewers, I would like to point out the relevance of the questions addressed in this study and commend the authors for the large amount of work and data. This study would unquestionably be an important contribution to the field. However, as rightly pointed out by both reviewers, the main focus/story of the study is lost in the large amount of results and the manuscript should be modified/shortened to improve the readability of the article. Furthermore, several major remarks and comments will have to be considered and addressed before a final decision can be made.

Please address all these points and submit both a revised version of your manuscript and response letter.

I look forward to receiving your revised manuscript.

Yours sincerely

Thomas POLLET

Reviewed by Jason Anders, 05 July 2022

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Reviewed by anonymous reviewer 1, 19 August 2022

The authors have studied bank voles (Myodes glareoulus) from four different sites in Eastern France and they surveyed colon bacteriome (based on V4 region of 16S), spleen bacteriome (as gut microbiome) and gastrointestinal helminths (based on GI extraction and morphological identification). Then they did a substantial number of different statistical analyses, including alpha diversity in each community (with two metrics), the effect of rodent characteristics on these alpha diversities and the effect of alpha diversities on alpha diversities; beta diversity with a single metric, the effect of geographical distance on beta diversities and factors shaping beta diversities; changes in bacterial taxa explaining gut bacteriome dissimilarities; interactions between different communities including correlations of dissimilarity matrices, the effects of infectious statues on alpha diversity indices and changes in bacterial taxa explaining significant variables.

I find the study as an important characterization of gut bacteriomes, spleen pathobacteriomes and GI helminths and it is definitely a more and more trendy approach to combine different within-host communities and explore their associations. I find the methods convincing and well-executed and the deposited raw data and scripts seem to be usable. That being said, I feel that while I feel this manuscript has a substantial promise on this account, it is not very clear, what the actual aim is.

My major issue with the paper is its immense and sheer volume. It takes ages to read and it just has so much information on it that I get lost in the manuscript – even though I actually have to read it closely and to deliver this critique. The main issue is two-fold: there is a huge amount of data and specifically statistical tests without any clear headline results or larger narrative standing out from the results. This would make a difficult

writing in any context. Furthermore, it is not very clear to me what is the actual aim or focus here, or whether it is just a fishing expedition in the sea of potential statistical methods. As there is no actual theory-driven hypothesis tested here, the reader has to wonder what is the take-away message.

For example, the discussion outlines in the beginning (line 598-618) three main findings: bank vole gut bacteriome has same taxa as previously found, bank voles have abundant helminth infections and bank voles have zoonotic bacteria. I feel like the listing of trivialities sells short your great data set.

I am not sure what is the best way in improving the readability. Maybe move some of the statistics to supplement to focus the discussion. Not every piece of information needs to be discussed or presented here. At least the structure needs to be strengthened and focused.

Then there other more tangible major issues:

- I am not sure what is the ecological scale here. The title of the manuscript is a bit misleading. When I think about "small-scale geography", it brings to my mind kilometers, not a hundred kilometer. Why is this the scale? The authors refer to the geographical scale few times, but it is not clear to me what are the implications of this scale.
- The authors say in the abstract that they apply the concepts of community and microbial ecology. I am not sure that I agree that they apply the concepts. The introduction mostly deals about tangible physical interactions between host and their symbionts, but I do not really see any, e.g., community ecology framework. The introduction, for example, does not really make clear what counts as an interaction and what is an association and how to tell these apart. These two seem to be used sometimes interchangeably, even though they are not.
- While the interaction of GI helminths and gut bacterial microbiota is quite understandable, as they are physically in same place and can physically interact, it is not clarified how spleen pathobacteriome relates to this. It is in different place, so there should not be direct interactions? How does one expect the ecological associations to work on this occasion?
- The results are now discussing more statistics than biology. I would rather write out sentences in underlining what is the biological meaning (behind the statistical tests). For example, do not start sentence with a name of the statistical test, but rather the main result.
- There are so many results on different levels (gut bacteriome, pathogenome, helminths) and different tests, that for example outlining the positive and negative results in a single table would make this very much clearer. Now tracking any significant variables is very difficult and time-consuming.

Minor issues:

- Table 1: I would use "Full data" as a name for the first set of data. "Total" is now confusing. Also, the combination of absolute numbers and percentages is confusing.
- Line 218: "OTUs number of reads" should be something else?
- The in-text references are sometimes misformatted, when they are not in regular type, e.g, line 226, 252, 260,...
- Line 310: Should be "absence"?
- Line 354: "Quality control" rather than "filtration"?
- Line 592: What is the intricate thing here?
- Line 598: What is the new light here? You frame your study as mainly corroborating previous studies.