Peer Community In Infections

The cost of endosymbionts on the reproductive fitness of the soft tick *Ornithodoros moubata*

Angélique Gobet based on peer reviews by Tuomas Aivelo ? and **Luciana Raggi Hoyos**

Taraveau Florian, Pollet Thomas, Duhayon Maxime, Gardès Laëtitia, Jourdan-Pineau Hélène (2023) Influence of endosymbionts on the reproductive fitness of the tick *Ornithodoros moubata*. bioRxiv, ver. 3, peer-reviewed and recommended by Peer Community in Infections. https://doi.org/10.1101/2023.05.09.539061

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Ticks are amongst the most important pathogen vectors in medical and veterinary clinical settings worldwide (Dantas-Torres et al., 2012). Like other holobionts, ticks live in association with a diverse microbiota. It includes tick-borne pathogens (TBP) and other microorganisms that have a beneficial or detrimental effect on the physiology of the host and can also affect the transmission of TBP to animals or humans. In this microbiota, primary endosymbionts, which are obligatory and inheritable, play a role in tick reproduction, the host defense and adaptation to varying environmental conditions (Duron et al., 2018). However, the effect of the microbiota structure and of the endosymbionts on tick fitness and reproduction is not well known. The soft tick *Ornithodoros moubata*, a parasite known to transmit African swine fever virus (Vial, 2009), is known to host Francisella-like and Rickettsia endosymbionts (Duron et al., 2018). These endosymbionts carry genes involved in B vitamin synthesis which may be supplemented to the host (Bonnet & Pollet, 2021).

Here, the authors investigated the role of endosymbionts on the reproductive fitness of *Ornithodoros moubata* by conducting two experiments (Taraveau et al., 2023). First, they tested the effect of antibiotic treatment of 366 first-stage nymphs on the main endosymbionts Francisella-like and Rickettsia, and measured the endosymbionts presence overtime by qPCR. Second, they surveyed the effect of antibiotic treatment with or without the addition of B vitamins on the survival and reproductive fitness of 132 females over 50 days. This second experiment intended to identify whether the endosymbionts have an effect on the host reproduction or on its nutrition. The supplementation of B vitamin did not have a drastic effect on tick fitness or reproductive traits. However, antibiotic treatments reduced the presence of endosymbionts while increasing tick survival, suggesting a potential cost of hosting endosymbionts on the tick fitness.

The authors did a lot of work to thoroughly follow the propositions from Dr Raggi, Dr Aivelo and myself to reconstruct and to revise the manuscript. I believe that the manuscript now reads very well and the answers to the reviews also add some value to the manuscript. As Dr Aivelo pointed out, "this study follows the traditional path of so-called population perturbation studies, where ecologists have administered antibiotics or antihelminths to different animals and seen how the community changes and what effects this has on the host fitness and survival". As both reviewers stated, results from this study are valuable and provide important basic knowledge that will likely help conduct future experiments on tick microbiota. This recommendation is the result of the thorough reviewing work of Dr Aivelo and Dr Raggi which I warmly thank. **References**

Bonnet, S. I., & Pollet, T. (2021). Update on the intricate tango between tick microbiomes and tick-borne pathogens. Parasite Immunology, 43(5), e12813. https://doi.org/10.1111/pim.12813

Dantas-Torres, F., Chomel, B. B., & Otranto, D. (2012). Ticks and tick-borne diseases: A One Health perspective. Trends in Parasitology, 28(10), 437–446. https://doi.org/10.1016/j.pt.2012.07.003

Duron, O., Morel, O., Noël, V., Buysse, M., Binetruy, F., Lancelot, R., Loire, E., Ménard, C., Bouchez, O., Vavre, F., & Vial, L. (2018). Tick-Bacteria Mutualism Depends on B Vitamin Synthesis Pathways. Current Biology, 28(12), 1896-1902.e5. https://doi.org/10.1016/j.cub.2018.04.038

Taraveau, F., Pollet, T., Duhayon, M., Gardès, L., & Jourdan-Pineau, H. (2023). Influence of endosymbionts on the reproductive fitness of the tick Ornithodoros moubata. bioRxiv, ver.3, peer-reviewed and recommended by Peer Community in Infections. https://doi.org/10.1101/2023.05.09.539061

Vial, L. (2009). Biological and ecological characteristics of soft ticks (Ixodida: Argasidae) and their impact for predicting tick and associated disease distribution. Parasite, 16(3), 191–202. https://doi.org/10.1051/parasite/2009163191

Reviews

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2023.05.09.539061
Version of the preprint: 2

Authors' reply, 06 September 2023

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Decision by Angélique Gobet, posted 26 July 2023, validated 26 July 2023

Thank you for submitting your manuscript for a recommendation from PCI. I would like to apologize for the time taken to hand in the reviews and my recommendation. The two reviewers and I made some comments that I feel should be addressed to improve the manuscript. I hope the comments and suggestions will be of interest to the authors and help improve the manuscript. I look forward to receiving a revised version of the manuscript. General comments

Overall, the manuscript is well written but some parts may need some rearrangements to help the reading of the manuscript.

A general comment that has also been noted by Dr Raggi is that, when the authors used the term "microbiota", it is not clear whether they refer only to the targeted endosymbionts or to the whole microbial community associated with the ticks. In the text, it becomes clear only in the discussion L345-347, so the authors may be more specific earlier in the text.

The experimental set-up is very thorough but as suggested by Dr Aivelo, some rearrangements of the text may help the reading. For instance, in figure 1, the authors may assign a number to the different steps of the experiment and then refer to each step in the text.
As Dr Aivelo suggested, some parts of the discussion may be trimmed and rearranged in other parts of the manuscript to make the reading easier. Specific comments
Please find below some specific comments complementary to those from the 2 reviewers:
L19-21: This sentence may be rephrased. With the later sentence introducing the prevalence of the endosymbionts, it is not clear whether the authors followed the whole microbiota or only the two endosymbionts.
L87: Are the "main bacterial species" Francisella-like and Rickettsia endosymbionts? Please specify.
L92: Materials and methods should be written in the past tense.
L102: I was wondering if it would be of interest to specify if these stages were only female or of both sexes.
L103-104: Can the process of blood feeding be a source of potential DNA or microbial contamination that would impact the study? Were there adequate precautions taken?
L110-112: Are the antibiotics known to have an effect on the 2 targeted endosymbionts? Please specify somewhere in the text.
L113, L147: Was the Milli-Q water UV treated or treated in order to avoid DNA contamination? Would not molecular grade water be more adequate to further use a DNA-based molecular approach?
L156: It would be more informative to give a range of DNA concentration than the volume.
L358: There is a typing mistake: "target*ed".
L358-361: This information should be put earlier in the text to understand the antibiotic choice.
Figures 2, 3, 4: This is a minor comment but instead of writing "boxplot of", "histogram of", the authors may directly write the title, for instance: "Ratios of DNA concentration…".

Reviewed by Tuomas Aivelo, 27 June 2023

Taraveau et al are presenting a preprint on the study that they did on a soft tick species. The authors fed both adult and nymphs with a treatment of two different antibiotics and supplemental vitamin B and measured both the effects on the survival and the fitness of the adult ticks, but also the dynamics of two endosymbiont species within nymphs. They found that antibiotic treatments reduced the endosymbionts but also increased tick survival, whereas vitamin B supplements did not have a drastic effect.

The general problem with the tick microbiome studies is that the effect of the microbiome structure on the tick fitness is difficult to assess and would require laboratory studies such as this one to really tease apart the effects of different community members of tick microbiota. This study follows the traditional path of so-called population perturbation studies, where ecologist have administered antibiotics or antihelminths to different animals and seen how the community changes and what effects this has on the host fitness and survival. Thus, I see this study as valuable in providing important basic knowledge about specific members of the tick microbiota.

I found the study setting and analysis methods sound and reliable – and especially they are very thorough in covering different important aspects, such as the actual changes in endosymbiont abundance. I had a look at the raw data and the analysis scripts and they seemed ok. The one major concern that I have, and which maybe is not even major, but rather medium-sized, is about the structure of the discussion.

Currently, the reading of discussion is a bit arduous task. The discussion has a long passages (rows 338-372, 386-387) that are better suited to other parts, such as introduction or methods. It does not go to the heart of the studied issue, but rather meanders its way. The authors describe it as "interesting result" that endosymbionts increased in concentration after tick blood meal, though intuitively it seems like a sensible thing for endosymbiont to do. Similarly, the authors say that the antibiotic that the *Rickettsia* endosymbiont is resistant to did not affect the bacteria, whereas the antibiotic that the both endosymbionts are susceptible to did decrease endosymbiont DNA concentration. Again, hardly interesting in the grand scheme of things. The

truly interesting stuff starts at row 419, where the authors go to the discussion about the things related to the title of the manuscript. I agree with the authors discussion on their results here and there are some worthy observations. Indeed, the ticks have long lifecycles and they can also be influenced by the egg composition, for example. I would appreciate if the authors also outline here how they think that a better study which would take into account the whole lifecycle of a tick could be carried out.

Thus, my main feedback would be restructuring the discussion to highlight the core of the manuscript. The main findings can also be brought more to the forefront. There are also worthy technical and methodological comments, but they are now rather scattered. It would make sense to collect these together under a heading such as ”Implications to future studies” or so after the main discussion.

Minor issues:

- The title: I found the title a bit too long. Obviously scientific articles are always aiming for new insights, so it is a bit redundant. Maybe just ”Influence of endosymbionts on the reproductive fitness of the tick Ornithodoros moubata” (I am not a native speaker of English so maybe run that also through somebody who has a certain grasp of articles.)
- There is first a division of bacteria to environmental, primary endosymbionts and secondary endosymbionts, but other bacteria than primary endosymbionts are not much described. I think that both for the symmetry of the description and as a justficiation of your approach, you should also outline what environmental bacteria and what secondary endosymbionts are and especially why you are choosing primary endosymbionts as your target.
- Materials and methods: You have a different order of adults and nymphs here than in results. To make the article more readable, consider the order and consistency carefully. Similarly, in results, the end result (i.e., females in the end of reproduction) are shown first and then the reproduction metrics. I understand this order from the point of the clarity, as the endosymbiont DNA concentrations lay ground to your discussion later but that would need a restructuring of the methods.
- Rows 101-: I found the description of your protocol a little bit difficult to follow, whereas the figure 1 was clear. I suggest referring to Figure 1 earlier. Similarly, it is not clear how nymphs are selected: it seems that those are from the same adults as previously treated, but how do you make sure that the previous treatment does not affect these results?
- Row 181: I would expect that qPCR results would be quite comparable across persons doing the analysis. Was the effect size of this effect a substantial?
- Row 184: A standard formulation would be 1.602 x 10-5 and so on.
- Rows 184, 212, 213, 227, 228, Tables etc.: Consistent and sensible rounding of decimals is needed.
- Rows 208-216: I find it unnecessary to present here unstandardized results at all.
- Tables and figures: The use of statistical difference codes in tables is unnecessary as readers probably understand the numbers faster than figuring out what these asterisks mean. Thus, I would think that the gray background is enough to indicate a single level of significance. Then again, in figures, I would appreciate indications of statistical signicifance.
- In results: I would consider the method of presenting means of different measurements. They are now already in figures as graphically represented and you have also tables on the statistical analysis. For example, the first 50 days, when there is only a one significant variable, you could survive with a lot less of body text.
- Row 436: I am not fan of describing non-significant results as almost significant. If you decide on an analysis which introduces a clear cut-off, then you have to live with clear cut-off. So I would not ”consider carefully” those, but just not consider at all.
- Table S1 would make sense to be included in the main manuscript. It could also be incorporate to Figure 1.

Reviewed by **Luciana Raggi Hoyos**, 10 July 2023

A series of experiments over the endosymbiont community are performed, trying to evaluate endosymbiont absence after antibiotic treatments and the addition of B vitamins. I would not call ”microbiota” these two types of endosymbionts.

The main problem of the study is that they could not get rid of the total endosymbionts and that the experiment was in a short time (50 days). However, observations might be useful for future experiments.

Perhaps information goes better for a short communication.

Punctual suggestions:

27-28 - for ticks –> during the tick reproduction cycle

55 - Alimentation

62 - (Guizzo et al. 2017; Li, Zhang, et Zhu 2018; Zhang et al. 2017;

Zhong, Jasinskas, et Barbour 2007; Kurlovs et al. 2014) –> Maybe to cite reviews?, list of references is a bit long.

65 - Repeated line - “poorly known” as in line 45 “microbiota species remains unclear”

66 - short blood feedings ?? Is it correct ¨short¨?? *

67 - 69 - re-write sentence

69-71 - make one sentence from those 2

69-73 - these could be the first lines of the introduction

81-83 - creo queue están de más

89 - such a disruption –> such disruption

89 - vitamin B

90 - to identify whether part of the...or it t was a consequence of their nutritional role

98 - since –> until

102 - How are ticks engorged?

107 - How many treatments did you have?

108 - following a modified protocol (Duron et al. 2018) where the dilutions...

115 group –> groups

120-123 - These lines are maybe results

159 - B –> ß

197 - B vitamin –> vitamin B or B vitamins

354-357 - if it was ruled out I think more information is not needed –> Although, broadly used before,...

358 - target –> targeted

366 - for ticks or for this species?

368-372 - It is repeated in the introduction, take it off here or there, but as there were not really changes with vitamin I do not see the point to put it in here.

374 - I think you should re-state the results.

381 - those –> these

392-299 - these are results, not discussion

403 - symbiont –> symbionts

406 - to eliminate completely the microbiota –> Are you talking about general microbiota that you did not measure? or about these endosymbiotic community that you are analyzing?

412-418 - It is not clear, re-write

423 - I see in Fig 3 symbionts were indeed eliminated with

432-433 - bacterias –> bacteria

434 - Rickettsia is abundant but after Perez-Sardiña et al (2023) FLE do not seem to be very abundant

438-440 - needs reference

473 - I think this line should be in the introduction and in methods to clarify this since the beginning.

Other observations:

1. I would shorten the introduction. Some information could be more useful in discussion as the 57-58 lines.

There is more information than needed.

2. I think you should point out somewhere in introduction that Francisella synthesize B vitamins that are deficient in the blood meal of ticks and that it is maternally transmitted to all maturing tick oocytes.

3. You state that this work is focused on the microbiota –> you are not really studying microbiota, but endosymbiont community. There is a paper on O. moubata microbiota (Perez-Sardiña et al 2023, doi: 10.3389/fmicb.2023.1173609), it would be interesting to analyze that data.

4. Experimental setup is not clear enough, I would state clearly which is Experiment 1 and 2 and treatments in each experiment.

5. Cites have a wrong (e.g. Duron et Gottlieb 2020) format with "et" word –> &

6. It is necessary to review English grammar.

7. One interesting conclusion is that endosymbionts are not easily lost, and the it is important to analyze de whole microbiota.

8. Were statistically significant differences determined for each treatment by post hoc tests (Kruskal-Wallis and Mann-Witney-Wilcoxon)? As graphs are not labeled I suspect there are no differences between treatments anywhere.

Fig 3: it is not clear at which point of the experiment these data were taken

Fig 6: Is this graph including nymphs and adults or is only adults? what happens if you separate? do you see the same pattern in adults separated from nymphs?