

General comments:

This is a really nice study that investigate the role of the soft tick, *Ornithodoros maritimus*, as a potential vector of *Babesia* sp. YLG. This study relies on tick sampling during 4 successive years on the islet Carteau which constitutes a simplified environment with only one vertebrate host and one tick species. Analyses are based on tick dissection to isolate different organs involved in pathogen transmission (salivary glands and ovaries) and test for the presence of Babesia DNA. The molecular tests also involved a control of potential contamination of tick organs by potentially infected host blood present in the caecum. This is a nice study, well written and thus easy to read. One of my major concern, which will be easily corrected, is the absence of any statistical test, despite mentions of differences in infection rates. Some clarifications are also needed throughout the manuscript.

Once these issues are addressed, I believe that this preprint can be recommended in PCI Infections.

Specific comments:

-L110: "*nymphs had undergone metamorphosis before they were analyzed* ». I believe that this means larvae having molted into nymphs. It sounds as if nymphs were able to undergo a molt without blood feeding. Please clarify.

-L121 : Figure1 : « *C : Caeca* ». I think that this is misleading to use C here to abbreviate Caecum since C is also the name of one picture with salivary glands.

-L 148-149 : Is it a nested PCR as for the amplification of 18S from YLG ?

-L180-180 : How were ticks chosen since you mention that they represent only a small fraction of the ticks that were present in sampled nests ? Randomly ? Preferentially females ?

L181 : How do you define active nests ?

L194 : « *The 18S rRNA gene of O. maritimus was detected in all DNA extracts.* » Which DNA extracts are they ? extract from all organs ? Caecum ?

L196 : «*26.1% in 2019 (6/23 tested ticks), 15.9% in 2020 (10/63 tested ticks), 39.5% in 2021 (15/38 197 tested ticks) and 45% in 2022 (9/20 tested ticks)* ». Is the infection rate significantly different between years ?

L 197 : « *All tick stages were found infected, females (31.8%, 198 34/107), males (26.7%, 4/15) and nymphs (9.1%, 2/22).* » Is it significantly different ?

L198-199 « *Positive ticks came from 18/38 of the identified 199 sampled nests (4/6 in 2019, 9/17 in 2020, 5/6 in 2021, unknown in 2022)* ». I don't understand how you can compute this ratio since the nest origin was not recorded. I see that you summed the number of positive nests in 2019, 2020 and 2021 over (I guess) a total number of nests sampled in 2019, 2020, 2021 and 2022. This does not seem the right way to do it. You could calculate this ratio over 2019, 2020 and 2021, excluding 2022.

L206-207 : « *Prevalence of positive salivary glands tended to vary among life stages: females (15/103, 14.6%), males 207 (1/12, 8.3%) and nymphs (1/22, 4.5%).* » Did you test the statistical differences between stages ? For example you could simply use **prop.test** in R

L 209-210 « *However, for three, Babesia sp. YLG DNA was not found in the gut content, suggesting that the salivary glands were truly infected and the positive result was not due to a contamination by*

*infected gut content.* » I don't understand the difference with the 17 salivary glands mentioned before (L204) where the parasite was detected without traces of gut contamination.

L 215 Figure 3 : « *F : newly moulted females* ». They are not mentioned in the material and methods. Is it DNA extraction from the whole tick ?

L233 : « *Cox1 gene was successfully amplified from 33 of the 60 organs in which the Babesia sp. YLG 18S rRNA gene was detected (17 salivary glands, 10 ovaries, 6 female endospermatophores, 1 male genitalia and 26 caeca).* » The number of positive caecum are not mentioned before

L238-239 : « *on 17 fully characterized sequences* » It is not clear why they are some fully characterized sequence, what it means. From Table 1, we can see that some SNP positions are not characterized. Is it due to sequencing issue. Why ?

L239-240 « *7 "a" variants, 5 "b" variants, 5 "c" variants, 3 "d" variants and 1 "e" variant were counted.* » This does not sum up to 17 sequences. From Table 1, I understand that for some ticks, they are 2 potential variants (that are read in the same sequence ?) . Therefore, you may state that you obtained 21 sequences. At least, there is a need for further explanations with those sequences and variants.

L 241-242 : « *but sometimes different combinations of haplotypes were detected within the same tick* ». This is already mentioned line 236-237

L 309 : « *A. vespertilionis* » You should also write the entire genus name.

L 313 : Malhobo et al 2021 : This reference is missing from the reference list.

L 314-315 : « *O. capensis* ». Write the genus name. Ornithodoros I guess (you have a Otobius just before so it may be confusing)

L 327-328 : « *to the oocyte maturation stage at the time of infection allowing or not parasite penetration into oocytes.* » Would you have any bibliographic reference to support this hypothesis ?

L 330-331 : « *YLG can be transmitted vertically in its soft tick vector and thus whether ticks can maintain local infection rates, at least from one year to the next.* » Is it really required that there is vertical transmission for ticks to maintain local infection from one year to the next ? I guess that the longevity of soft ticks may allow them to transmit pathogens from one host at year N-1 to another host at year N. I see that this idea is presented in your last paragraph. It may be useful to mention it here.

L 390 : « *Maxime Duhaydon* ». I guess this is Maxime Duhayon ?

L 566 : « **SM3.** *Number of each organ type of Ornithodoros maritimus collected after dissection and used to test for the presence of Babesia sp YLG (number of ticks in brackets).* » In Mat & Meth, you mentioned the dissection of caecum. Are they dissected in all tested ticks ? If so, could you state it clearly ?