

Round #1

Decision

by Alison Duncan, 2018-04-17 10:53

Manuscript: <https://doi.org/10.1101/207183>

Decision for preprint Vantaux et al

Changes made in response to the editor are highlighted in yellow in the revised version of the ms.

Vantaux et al investigate, whether Anopheles mosquitoes harbouring sporozoites, the stage of Plasmodium falciparum transmitted to the vertebrate host, are more attracted to, and likely to have fed on, human hosts. The study checked for the presence of Plasmodium in mosquitoes from 1) field, host-choice experiments for human or calf odours and 2) collections from occupied or unoccupied houses, or animal sheds. They show that both infected and uninfected mosquitoes are equally attracted to humans, but that mosquitoes harbouring sporozoites are more likely to have fed on humans. The experiment is coupled with an epidemiological model predicting that this preference could increase the number of infectious mosquito bites people receive by 250% (14 infectious bites compared to 4 if mosquitoes showed no host preference).

I enjoyed reading the manuscript and thought the experiments and epidemiological model provide a neat, novel body of work. Both Reviewers liked the manuscript, but request a number of revisions. I agree with both the Reviewers that the Methods would benefit from being fleshed out. More information is required about the model parameterisation.

Comment # 1: The methods would also be easier to follow if more information were provided in the last paragraph of the Introduction setting out the aims of the manuscript, and if the ‘Mosquito host preference’, ‘Mosquito blood-feeding pattern’ and ‘Mathematical model’ were more clearly highlighted as the major axes of the paper.

We agree and these aims have now been highlighted in the introduction (lines 70-76).

Comment # 2: The ‘Mosquito blood-feeding pattern’ section also needs a few sentences at the beginning describing the aims. Please state somewhere whether a live calf was placed in the tent for calf odours, and whether different volunteers and calves were used each night.

This has now been added (lines 107-110 and 119-126).

Comment # 3 =: Please be reminded that the following information must be included in final version of the preprint.

-Data must be available to readers after recommendation, in the preprint or through deposition in an open data repository, such as Zenodo, Dryad or institutional repositories, for example.

-Details of the quantitative analyses (e.g. data treatment and statistical scripts in R, bioinformatic pipeline scripts, etc.) in the recommended preprints must be available to readers in the text or as appendices or supplementary materials, for example.

We have now uploaded our data on zenodo and the analyses are available as supplementary material. See the data availability section lines 455-457.

Some very minor comments of my own:

Comment # 4: Lines 71 – 75: State more clearly that these are separate experiments.

We have now reformulated this paragraph to state more clearly the two different experiments (lines 70-76, line 90, 119, 140- 141, 265, 281).

Comment # 5: Lines 140 – 142: I would put this information at the beginning of this section.

This has now been moved to the beginning of the section (lines 140-145).

Comment # 6: Lines 229 – 231: Please state in the text or Figure legends how HBI is impacted by shelter type and village.

These have now been added to the text (lines 306-319).

Comment # 7: Line 293: Doesn't this also allow you to distinguish from an intrinsic parasite characteristic?

This allow to distinguish from an intrinsic mosquito characteristic (lines 383-384): mosquito host selection being different in the sporozoite-infected compared to the oocyst-infected individuals means that it is a parasite effect and not due to a mosquito higher intrinsic preference for human, otherwise both oocyst and sporozoite-infected mosquitoes would have been more likely to feed on humans compared to uninfected mosquitoes.

Comment # 8: There are some references missing from the discussion for example, lines 297, 312, 315, 333, 345 – 346,

These lines are very specifics and refer to the discussion of the results obtained as part of the present study. There is to our knowledge no previous studies exploring these questions and hence either supporting or questioning our results.

Comment # 9: Lines 323 – 328: Is anything known about mosquito phototactic behaviour?

Phototactic behavior in mosquitoes is, to our knowledge, poorly documented and we preferred to delete this hypothesis.

Reviews

Changes made in response to the first reviewer are highlighted in green in the revised version of the ms.

Reviewed by anonymous reviewer, 2018-03-02 15:06

The manuscript by Vantaux et al. provides evidence for mosquitoes, infected with *Plasmodium falciparum*, preferring to feed on humans. Interestingly, this preference is specific for mosquitoes infected with sporozoites, the mosquito-to-human transmissible stage. This is a well-written manuscript, using a combination of field work, behavioural experiments and theoretical modeling.

The authors measure mosquito preference for humans versus domestic animals, using three different assays: odour-baited entry traps; odour-baited double net traps and collection of indoor resting mosquitoes. In the first two assays, the mosquitoes are attracted to either calf or human odours. In the latter assay, indoor resting mosquitoes were collected in the morning from human dwellings, unoccupied houses and animal sheds. Mosquitoes for these assays were field mosquitoes from three different locations. All mosquitoes were dissected and ELISA or PCR were used to determine their infection status and the host on which the mosquito fed.

My main criticisms are the following:

- The main result of the paper is that in the collected indoor resting mosquitoes there is an increased preference for humans in the mosquito population that is infected with sporozoites. However, it is unclear to me whether this result is or not fully dependent on the mosquitoes collected in the human dwellings. This is because the sample size varies a lot between the different shelter types. From fig. S4: 1731 mosquitoes from human dwellings; 198 from unoccupied houses; 399 from animal sheds. Thus the sample size in unoccupied houses and animal sheds is 11 and 23% of what the authors have for human dwellings. As the authors state in the main text that there is no interaction between shelter type and infection status, the effects of each variable can be tested separately. Thus, it is key to know whether the human blood index (HBI) is significantly affected by infection status within each shelter type.

This is a really good point and all numbers mentioned by Referee 1 are correct. The sporozoite-infected individuals were more anthropophilic across all shelter type (see figure below). Because of low sample sizes in both animal sheds and unoccupied houses, the observed increased anthropophagy in sporozoite-infected individuals (figure below) was not statistically significant when analyzed separately. However, when pooling samples from unoccupied houses and animal sheds together (i.e. total of 198 + 399 samples), the difference became statistically significant ($X^2 = 7.457$, $df = 2$, $p\text{-value} = 0.02403$). Therefore, the overall pattern was not driven only by human dwellings but by all shelter types.

In addition, because sample size in the uninfected group ($n=2001$) was much higher than in both sporozoite-infected ($n = 209$) and oocyst-infected individuals ($n=118$) we ran a second set

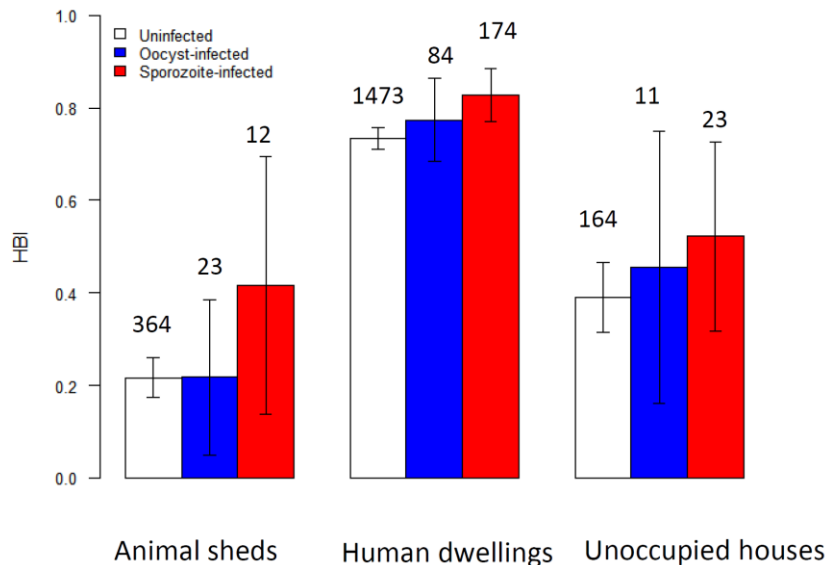
of analyses using a subset of 150 random uninfected individuals. This approach normalizes statistical power to test for statistically significant differences in HBI across heterogeneous sample sets (using the function `n_sample` of the `dplyr` package in R). With this approach we confirmed a significant increase in anthropophagy in sporozoite-infected individuals compared to both oocyst-infected individuals and uninfected individuals (LRT $X^2= 11.6$, $P= 0.003$).

All pairwise comparisons using the `glht` function of the `multcomp` package in R:

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
spz - oocyst == 0	-0.65392	0.25226	-2.592	0.02570 *
uninfected - oocyst == 0	0.06674	0.25473	0.262	0.96283
uninfected - spz == 0	0.72066	0.23525	3.063	0.00614 **

These results have been added lines 289-297.



- The authors model how the entomological inoculation rate depends on the HBI. However, the model appears to use fixed values of HBI whereas considerable variation is observed in the results from the field experiments. As the authors state that the ODE equation system is simulated throughout the season, would it be possible to allow HBI to vary (around some mean value) or at least discuss the implications of adding such variation

We thank the reviewer for this comment. We agree that there is significant heterogeneity in HBI. We therefore ran additional simulations accounting for HBI variation by considering minimum and maximum values of the HBI confidence interval of oocyst-infected mosquitoes instead of its mean. The new figure (see below) has been updated in the main text (figure 4, line 368-370).

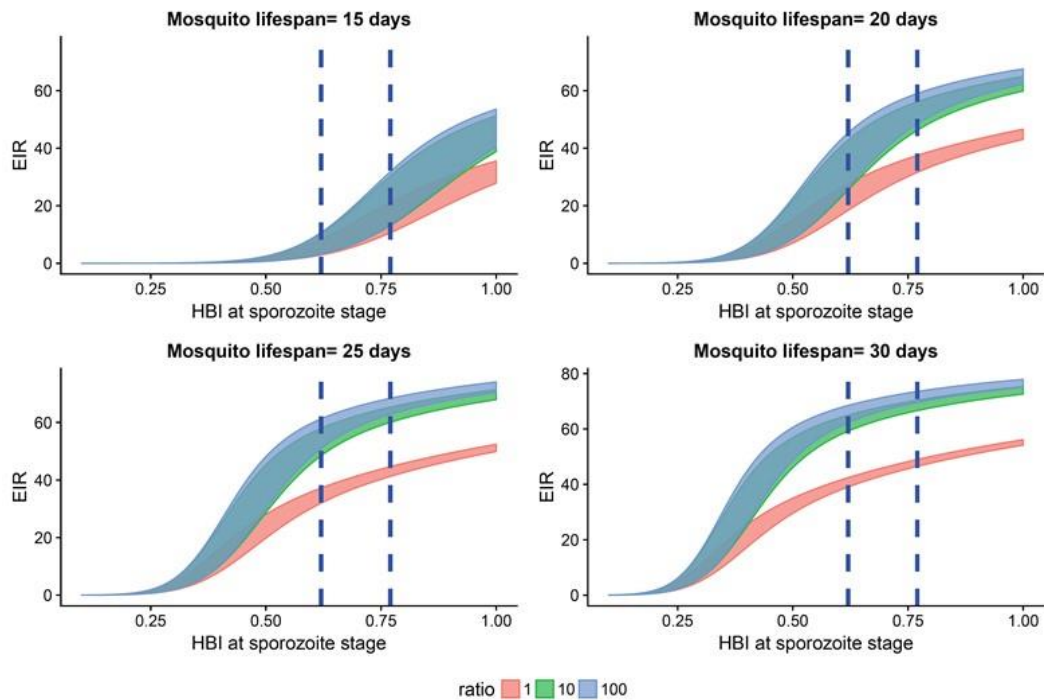


Figure 4. Expected epidemiological consequences of HBI variation for different values of mosquito lifespan and mosquito/human ratio. X axis represents the range of values considered for the HBI of infectious (sporozoite-infected) mosquitoes and the Y axis is the Entomological Inoculation Rate (EIR, number of infectious bites received by a person over one year) when the HBI of susceptible (uninfected and oocyst-infected) is 0.62. The ribbons represent the possible EIR values for different HBI of sporozoite-infected mosquitoes according to the confidence interval of HBI in oocyst-infected mosquitoes ($63.6\% \pm 5.7\%$) and for different values of mosquito-to-human ratio. The dashed lines represent the value considered for susceptible mosquitoes (0.62) and the value measure for sporozoite-infected mosquitoes (0.77). Ratio=adult mosquito/human densities.

- The methods would benefit from more detail. Particularly in the section “laboratory processing of samples” where the genes/proteins used in PCR and ELISA assays should be given. Also, to help replication, product codes/brands should be added

The methods have now been presented in more details, including genes, proteins and brands used.

- In the discussion the authors should be more clear in their discussion of the difference between the assays of the odour-baited traps and the indoor resting mosquitoes. This seems to be done from line 309 onwards, but the authors should make the comparison more explicit

We have reorganized the discussion section to make the distinction between these two experiments more clear (see lines 376-383, see also response to editor’s comment #4).

Minor points:

- methods model section: not all parameters are described, please add them all. line 183: I think there must be a typo, it should be “Ih”?

This has been fixed (lines 243-249).

- results: when including means +/- error measure, please state what is the error measure

This has now been added lines 228-229.

- Figures: could the authors please add the lower half of the error bars

The lower half of the error bars have been added in all the figures:

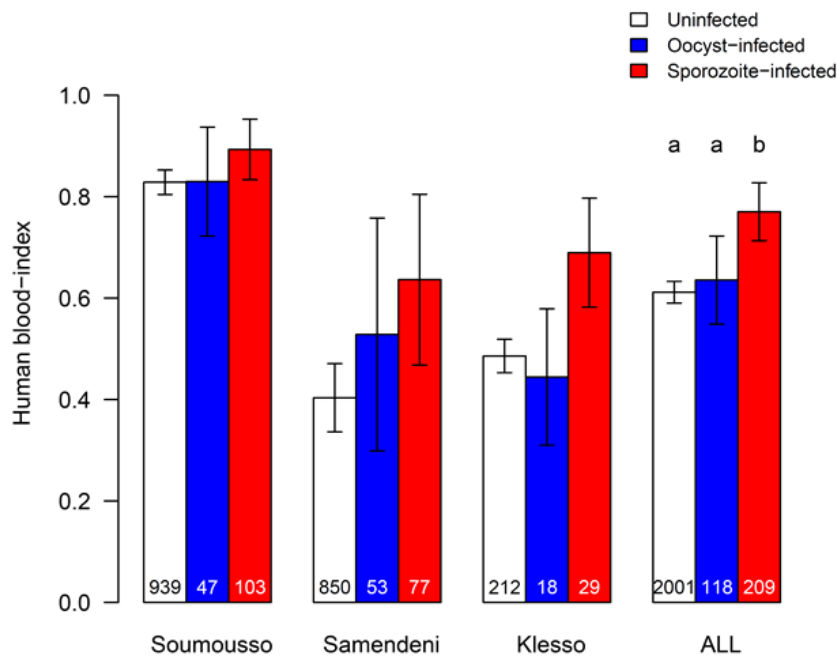


Figure 2. *Effect of infection status on the human-blood index of Anopheles gambiae s. l. females expressed as the number of females fed on human out of the total number of blood-fed females for the three sampled villages. Data show proportion ± 95% confidence interval. Numbers in bars indicate the total numbers of mosquitoes. Different letters indicate differences between infection status (Chi-square post-hoc tests: sporozoite-infected vs. oocyst-infected females $X^2_1=6.1$, $P=0.013$; sporozoite-infected vs. uninfected females $X^2_1=19.4$, $P<0.0001$; oocyst-infected vs. uninfected females $X^2_1=0.18$, $P= 0.67$).*

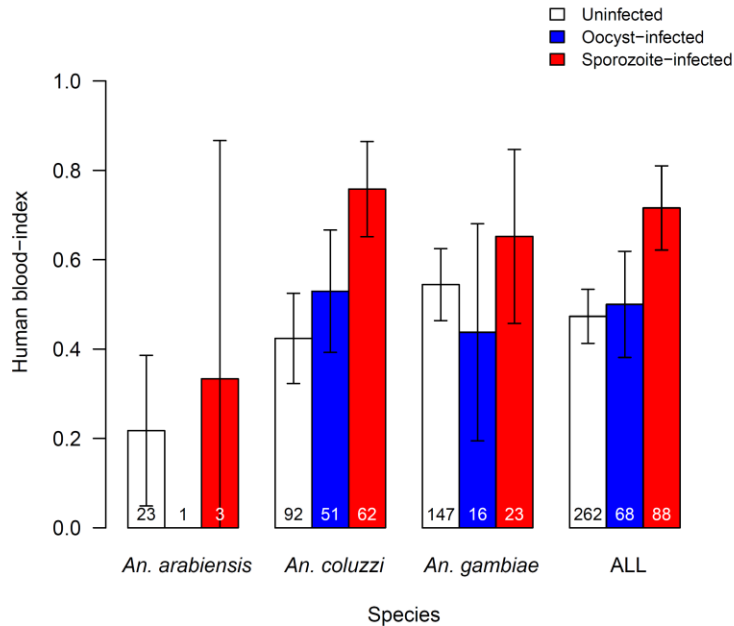


Figure 3. Effect of infection status and Anopheles species sensu stricto on the human-blood index expressed as the proportion of females fed on human or human and animal out of the total of blood-fed females. Data show proportion \pm 95% confidence interval. Numbers in bars indicate the total numbers of mosquitoes.

- line 209: “with a significant attraction toward human odours” - This does not appear to be the case for sporozoite-infected mosquitoes - given the stats in front

We have now reworded the sentence (lines 271-276).

line 225: I find the statement of a 24% increase a bit strange. Given that the HBI is itself a percentage, isn't the percent increase just $HBI(\text{sporozoite}) - HBI(\text{uninfected})$, rather than one over the other, as I assume the authors have done?

We calculated the percentage change with the following formula: $\frac{\text{Percentage sporozoite} - \text{Percentage (uninfected+oocyst-infected)}}{\text{Percentage (uninfected+oocyst-infected)}} * 100$ which gives us the percent increase. The formula $HBI(\text{sporozoite}) - HBI(\text{uninfected})$ only gives the net increase.

- line 281: "The plain lines show how EIR changes according to HBI..." instead of "The plain lines show the evolution of EIR according to HBI..."

We are now presenting the different value of EIR according to the confidence interval of HBI in oocyst-infected mosquitoes and have thus modified the sentence accordingly (line 369).

- line 324: if this was the case, one should expect a significant interaction between infection and shelter type?

A significant interaction between infection and shelter type would be expected if parasite would manipulate endophagic/philic behavior based on human emitted odors. However, “infectious mosquitoes may exhibit an enhanced tendency to enter (or a decrease tendency to exit) house interstices regardless of emitted odors” (lines 412-413).

Reviewed by Olivier Restif, 2018-03-02 15:10

Changes made in response to Olivier Restif are highlighted in blue in the revised version of the ms.

Review of "Field evidence for manipulation of mosquito host selection by the human malaria parasite, Plasmodium falciparum" by Vantaux et al for PCI Evol Biol.

Manuscript downloaded from BioRxiv

(<https://www.biorxiv.org/content/early/2018/02/26/207183>) on 6 March 2018.

This is an original, innovative and interesting study of the effect of Plasmodium infection on mosquito blood feeding behaviour in a natural setting. The hypothesis and methods were clearly presented, and the results presented support the authors' conclusions. The use of a mathematical model to predict the epidemiological consequence of the observed behavioural differences is a useful addition.

I have found no major issues, but I would like to see a better justification of the model's assumptions and parameter values. Here I list suggested corrections which would help improve the clarity of the manuscript. Line numbers refer to the pdf version on BioRxiv.

- 1.50: "reduced feeding attempts seem beneficial to the parasite": is that a theoretical prediction or an observation? and does it only apply to the oocyst stage?

This is a theoretical prediction explaining the observations from the previous sentences that oocyst-infected mosquitoes were less likely to attempt feeding. This reduced feeding attempts would limit transmission during the sporozoite stage. We have reformulated this sentence to make it clearer (lines 46-49).

- 1.95: If possible, include Figure S2 (a and b) in the main text, as it's very helpful for readers not familiar with the traps.

The figure has now been included to the main text (Figure 1).

- 1.131: how large and representative was the subset? Is that sufficient to rule out other Plasmodium species in all the oocyst-infected mosquitoes in the study?

The subset consisted of 20 midguts of a total of 118 oocyst-infected individuals (about 20% of the total sample size)(see lines174-176). In this endemic area, > 95% of infections in humans belong to P. falciparum. Other occurring Plasmodium species transmitted by An. gambiae sl. are P. malariae and P. ovale. Similar to P. falciparum, these two species are also highly human specific. Therefore, the same predictions as those made for P. falciparum can be made regarding the occurrence of specific manipulation in these two species. In other words, genotypes of P. malariae or P. ovale able to increase biting of their vectors on human are expected to be favoured by natural selection.

Other non-human Plasmodium species in the area (bird and lizard malaria species) are transmitted by Culex and/or Aedes mosquitoes, not An. gambiae sl.

- 1.133: are the ELISA and qPCR assays used specific to the sporozoite stage?

ELISA were specific to the sporozoite stage as they target a specific surface protein of sporozoite. Furthermore, for both ELISA and qPCR assays, only heads and thoraces were used to not screen midgut's oocyst which are in the mosquito abdomen and is the usual method implemented to screen for Plasmodium sp. sporozoite.

- 1.175: replace "a SIR model" with "a compartmental model for Plasmodium transmission between humans and mosquitoes".

The terms have now been replaced (line 232).

- 1.176: please provide a reference for the validation of this particular model. In particular, is it valid to ignore latent infection in humans (Eh), and ignore human births and deaths?

This is an interesting point. Actually, we are looking at the pathogen dynamics over one season. Therefore, at this time scale, we think that ignoring human demography and human latency period will definitely not change qualitatively our results.

- *In addition, I'm surprised to see two different frequency-dependent terms for human-to-mosquito transmission and mosquito-to-human transmission: the former has Nm as the denominator and the latter has N_h. Both should be fractions of the same total number of mosquito bites. Although this would not affect the results as long as the two population sizes are constant, it seems to me that the equations are inconsistent.*

This is also an interesting point. First, as the reviewer points out, it will not affect the results since the two population sizes are constant. Nevertheless, it depends on how we define transmission patterns. If we would have studied a directly-transmitted pathogen with frequency-dependent transmission, we would have used the two population sizes as

denominators. For vector-borne diseases, we generally used only one denominator, assuming that only vectors are doing the contact despite the fact that human behaviors could definitely affect contact probability. Therefore, we would prefer to keep the formulation as it is because we think it is more general, but we can definitely change it if the reviewer is more comfortable with classic formulation for vector-borne diseases.

- 1.185: Table S1 is very succinct. Please provide justifications (and references) for the chosen parameter values and ranges.

These are classical parameters and values of malaria transmission models. We have now added references where some of these parameters have been measured (line 247).

- 1.188: It's not clear from the formula how the 1-year duration was accounted for, as I_m represents the instantaneous number of infected mosquitoes. Did you allow the model to reach its steady state first?

We have chosen to use the term I_m after one year of simulation. We prefer to keep this in order to show an EIR that would represent the proportion of Infectious mosquito at the end of a given season. Studying number of infectious vectors at the equilibrium will impose to introduce a seasonality in mosquito population dynamics, as well as considering human demography and immunity buildup in humans.

- Figures 1 and 2: I'm not a big fan of bar plots (unless they show count data), and in this case they hide the bottom halves of the confidence intervals, which is not helpful.

We have now added the bottom halves of all the confidence intervals.

- 1.263: echoing my comment about Table S1, I'd like to see a justification for the choice of values for mosquito lifespans and densities.

As for previous comment, we based mosquito lifespans and densities values on previously observed data.

1.264: 0.62 is the mean of the HBI measured in three locations, which range from 0.4 to 0.8. It would be worth showing the effect of this variation too.

This comment was also raised by referee 1 (see response to referee 1's comment #2).

Figure 3: please add a legend for the red, grey and black lines.

The legend of the ribbons (which are replacing the lines) has now been added.