Journal Name:	International Journal of TROPICAL DISEASE & Health
Manuscript Number:	**Ms_IJTDH_38190**
Title of the Manuscript:	**Vector competence of laboratory-reared Anopheles stephensi for Plasmodium vivax-infected blood from Pakistani patients**
Type of the Article	**Original Research Article**

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SDI Review Form 1.6

PART 1: Review Comments

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Compulsory REVISION comments	It is a good study but the manuscript could use some improvement. Besides, some clarifications and explanations are needed about the blood feeding otherwise it greatly diminishes the interest of the paper and the part about mosquito survival. **While I understand this work has not been done in Pakistan for the first time, the authors should add references of similar work. Besides, many of the references could be more recent:** -"Malar J. 2017; 16: 284. Published online 2017 Jul 11. doi: 10.1186/s12936-017-1931-8 PMCID: PMC5504555 Dynamics of *Plasmodium vivax* sporogony in wild *Anopheles stephensi* in a malaria-endemic region of Western India Praveen Balabaskaran Nina,#1 Ajeet Kumar Mohanty,#2 Shuvankar Ballav,2 Smita Vernekar,2 Sushma Bhinge,2 Maria D'souza,2 Jayashree Walke,1,3 Suresh Kumar Manoharan,1,3 Anjali Mascarenhas,1,3 Edwin Gomes,3 Laura Chery,1 Neena Valecha,4 Ashwani Kumar,2 and Pradipsinh K. Rathod1" -"Exp Parasitol. 2013 May;134(1):48-51. doi: 10.1016/j.exppara.2013.01.016. Epub 2013 Feb 4. Genotyping Plasmodium vivax isolates infecting Anopheles stephensi, an Asian main malaria vector. Gholizadeh S1, Zakeri S, Djadid ND." **Material and methods** 2.2 The authors write *"The patients having blood group O+, irrespective of the age and gender, were selected and 2-3 mL of their blood was taken in vacutainers (BD, Belgium) and stored at ambient temperature."* Was the potential effect of the age and gender factored in the analysis? 2.4 At what age was the blood feeding done and was there only one blood meal? Were they given the opportunity to lay eggs? Why don't add a group with just sugar solution? Were the blood quality and nutrients of both infected and uninfected blood measured? -> It could totally modify the results if it is more a donor effect for example. That is really lacking here and diminishes the significance of these results about mosquito survival. **In the results,** A mean should never been given without the standard error or standard deviation. Especially given the large variation between experiments. A table to complement the oocysts data would be welcome. Do you have any explanation for these large variations in sporozoites rates? You could add it in the discussion, as your goal here is to show competence. Table 2 is not really informative. I would just keep the total results after 10 days and add the proportions. Why did you stop at 10 days, as the cost of infection may only appear later on? Did you check these mosquitoes for infection? How similar was the infected and uninfected blood in terms of nutrients and so on? Please specify the statistics used for the survival analysis. More details about the results in general and the software and versions of the software used are necessary.	

On figure 4 replace “not feed” by “not fed”

In the discussion, there is a need to modify several parts:
_This is for an introduction and not the discussion:
“Malaria is one of the leading causes of death in the world. Transmission pattern of malaria parasite through Anopheles stephensi in Pakistan is still unknown. Sporozoite rate and corresponding vector competence of Anopheles stephensi mosquitoes remained unaddressed. The current study investigated vector competence and progression of P. vivax in laboratory reared Anopheles stephensi vector, as it constitutes around 70% of malaria cases in Pakistan.”

_I would start the discussion with this sentence:
“The data suggests that laboratory reared Anopheles stephensi is a suitable vector for P. vivax because oocysts and sporozoites develop only when both the parasite and vector are compatible with each other (20).”

_Some sentences don’t seem to be linked directly to the results and should be kept out the discussion or added in the introduction:
“A parasite specific protein (with chitinase activity) helps the gametocytes to localize into mid gut wall which is necessary for its progression (15).”
“Female mosquito feeds on blood of rodents or primates for its development which provides protein and iron. Hemoglobin and transferrin are the major sources of iron for female mosquito development (22).”

The third paragraph needs some work and you could add some sentences of the fifth paragraph. Otherwise the fifth paragraph is redundant and not useful. I would write a bit more clearly about the role of immunity here with recent references.
I would keep the first sentence, and then add, *“sporozoite rate reported here is in line with previously published data in India (10). Sporozoite rate of Anopheles darlingi and Anopheles culicifacies for P. falciparum and P. vivax were reported to be 41% and 56%, respectively (10, 19), which corroborate our findings.”*.
Then *“Around half of the mosquito population was negative for sporozoite in its salivary gland. It indicates that half of the mosquitoes who fed on infected blood might not receive gametocytes in their meal.”*
“Another explanation is that the mosquitoes, which did not show any progression of malaria parasite, might have retained their anti-parasite immunity even after infected blood meal. Despite that “circumsporozoite- and TRAP-related protein (CTRP) enhances the growth of parasite by manipulating the immune system of mosquito in its favour (17). Mosquito specific nitrous oxide (NO) shows anti-parasitic activity and blocks the parasite development (18).”

In addition, as the oocysts rate was superior to the sporozoites rate I would say that the parasite’s ability to develop in the parasite and the mosquito immune system are more reasonable explanations than “It indicates that half of the mosquitoes who fed on infected blood might not receive gametocytes in their meal.”. Besides, this could have been tested by dissecting mosquitoes after a blood meal to find out how many have ingested gametocytes or by using molecular analysis.

In the last paragraph, I would like to see more reflexion about the potential cost of infection. The results could have been clearer if the survival had been done until all mosquitoes died. I would suggest doing it next time.

Minor REVISION comments	Work a bit on the manuscript to correct some English mistakes and typos. The singular is used instead of the plural several times for example. The formatting of the references could be improved in the text too. Like in material and methods 2.4. **Abstract,** In the abstract it is written *"Plasmodium vivax is supposed to contribute around 70% of total malaria cases in Pakistan"* Whereas in the introduction it is written *"Pakistan is among three countries where Plasmodium vivax (P. vivax) infection cases exceed 80% of all malaria cases."*. It would be better to decide on one of these figures. Study model instead of *"as a model of study."* **In the key words:** The authors use the word vectorial capacity instead of vector competence. However, vectorial capacity is defined in the 2015 NAL Glossary (2014) by United States Department of Agriculture, National Agricultural Library; "The capability for disease transmission by a vector to a host, as influenced by behavioral, ecological and environmental factors, such as population density, host preference, feeding habits or frequency, duration of latent period, or longevity." Besides, vectorial capacity can also be defined by equations. That is why it is better to use vector competence here instead. **In the introduction** I would speak more about the vector instead of putting so much about it in the discussion. Indeed, you want to explain malaria cases linked to *Plasmodium vivax* so detailing malaria epidemiology in Pakistan is of interest but the vector too. **In the conclusion,** *"Blood-fed mosquitoes showed considerable competence for malaria parasite which is dependent on sporozoite rate rather than oocyst rate."* This sentence is not really clear, as it is obvious that both are important and that of course without sporozoites there can be no transmission. I would remove the following sentence as it is not connected to the present study: *"Effect of various ecological factors like deforestation, urbanization, industrial development, floods and earthquakes can also be investigated on malaria vectorial capacity of female Anopheles stephensi in Pakistan."*	
Optional/General comments		

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