

Anopheles donaldi (Barbirostris Group) As A Potential Vector For Plasmodium knowlesi In Sarawak, Malaysia

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INTRODUCTION

Plasmodium knowlesi is a significant cause of zoonotic (simian) malaria in Sarawak. As of 2018, only one study had been conducted in Sarawak to identify vectors of *P. knowlesi*, in which *Anopheles latens* was identified as the vector in Kapit, Sarawak. A comprehensive entomological investigation was subsequently carried out in Semena, Limbang, following the report of an imported case of malaria caused by the *P. falciparum* parasite in March 2018. This investigation suggests a new potential vector for *P. knowlesi* in Sarawak.

OBJECTIVE

To identify the vector species in Semena, Limbang and to determine the presence and species of malaria parasite in mosquitoes sampled.

MATERIALS AND METHODS

(i) Study Sites

The study was carried out in the Limbang District of northern Sarawak, Malaysian Borneo (Fig. 1).



Figure 1: Map of RH Semena, Limbang, Sarawak

(ii) Larva Survey

By using dipping and pipetting method, a number of dipping and total number of *Anopheles* larva collected were recorded.

(iii) Mosquito Sampling, Identification and Dissection

Mosquito collection was carried out by four collectors over a period of three days in April 2018. Mosquitoes were collected using both the human landing catch method and the resting catch method. The collection took place from 1800 to 2400 hours (Fig. 3).

All mosquitoes were identified morphologically via microscope. Anopheline mosquitoes were dissected to determine the presence of oocysts and sporozoites.

(iii) DNA Extraction and Detection of Plasmodium sp.

Oocyst-positive slides, along with undissected *Anopheles* mosquitoes were transported to Institute of Medical Research (IMR) for species confirmation and to detect the presence of malaria parasites using molecular techniques.



Figure 2a: An abandoned fish pond identified as a breeding site for *Anopheles donaldi* sp.

Figure 2b-f: Potential breeding sites for *Anopheles* mosquitoes.



Figure 3: Workflow for sampling adult mosquito

REFERENCES

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- Joshua X.D. Ang, Singh, B. et al. (2020). New vectors in northern Sarawak, Malaysian Borneo, for the zoonotic malaria parasite, *Plasmodium knowlesi*. *Parasites Vectors* 13, 472.
- Vythilingam I, Tan CH, Asmad M. et al. (2018). Natural transmission of *Plasmodium knowlesi* to humans by *Anopheles latens* in Sarawak, Malaysia.

RESULTS

A total of 117 female *Anopheles* were successfully examined. Of these, 29 were identified as *Anopheles donaldi* by morphology, while the reminder consisted of *An. leucosphyrus* spp; *An. barbirostris* spp and *An. kochi*.

Through dissection, one *An. donaldi* (from *An. barbirostris* group) was found to be oocyst-positive on midgut (I 322) and confirmed as *Plasmodium knowlesi* through molecular analysis.

All of the mosquitoes sampled were pooled into 36 tubes plus 1 additional tube from oocyst-positive slide.

Out of 37 tubes, 4 abdominal tubes and 2 thoracic tubes (including the slide) were positive for *P. knowlesi* and 6 abdominal tubes 1 thoracic tube were found positive for *P. vivax*.

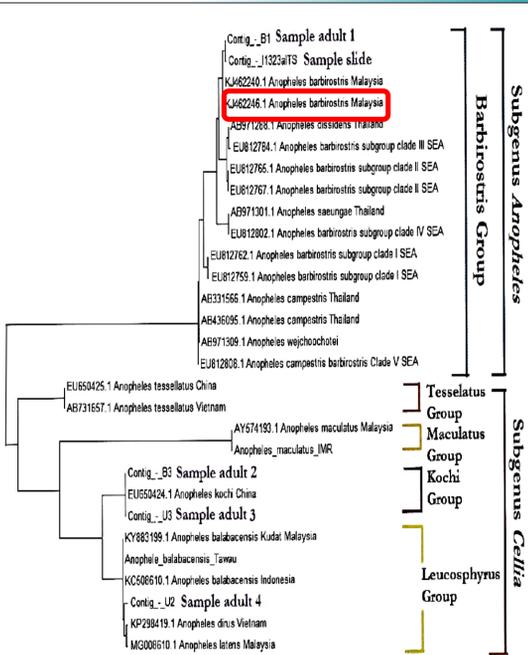


Figure 4: Phylogenetic tree for *Anopheles* spp.

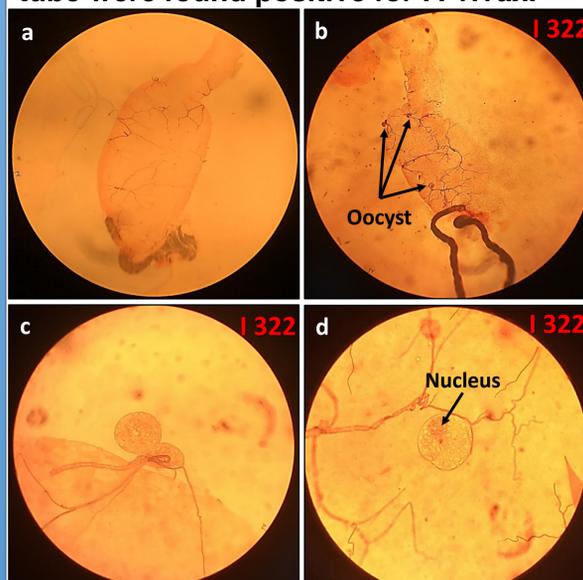


Fig. 5a,5b: Shows non-infected midgut vs infected midgut. Fig 5b,c,d: Shows oocyst on midgut under 40x lens in sample ID I 322.

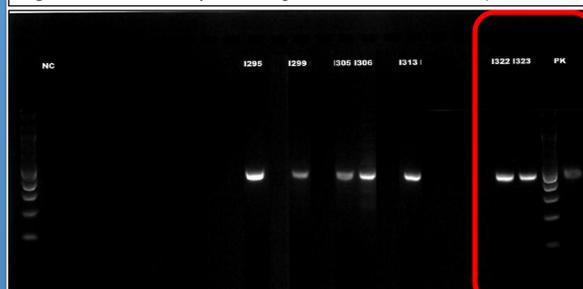


Fig. 6: Bands show Positive samples of *Plasmodium knowlesi*.

RECORD NUM	SAMPLES NUM. *5 mosquitoes/ 1 tube	POSITIVE / NEGATIVE Malaria Parasites	JKN ID MOSQUITOES SPP.	IMR ID MOSQUITOES SPP
I 286	SW G 001	Negative		
I 287	SW S 001	Negative		
I 288	SW G 002	Negative		
I 289	SW S 002	Negative		
I 290	SW S 003	Positive <i>P. vivax</i>		
I 291	SW S 003	Negative		
I 292	SW G 004	Negative		
I 293	SW S 004	Negative		
I 294	SW G 005	Positive <i>P. vivax</i>		
I 295	SW S 005	Positive <i>P. knowlesi</i>		
I 296	SW G 006	Negative		
I 297	SW S 006	Negative		
I 298	SW G 007	Negative		
I 299	SW S 007	Positive <i>P. knowlesi</i>		
I 300	SW G 008	Positive <i>P. vivax</i>		
I 301	SW S 008	Negative		
I 302	SW G 009	Positive <i>P. vivax</i>		
I 303	SW S 009	Negative		
I 304	SW G 010	Positive <i>P. vivax</i>		
I 305	SW S 010	Positive <i>P. knowlesi</i>		
I 306	SW G 011	Positive <i>P. knowlesi</i>		
I 307	SW S 011	Negative		
I 308	SW G 012	Negative		
I 309	SW S 012	Negative		
I 310	SW G 013	Negative		
I 311	SW S 013	Negative		
I 312	SW G 014	Negative		
I 313	SW S 014	Positive <i>P. knowlesi</i>		
I 314	SW G 015	Negative		
I 315	SW S 015	Negative		
I 316	SW G 016	Negative		
I 317	SW S 016	Positive <i>P. vivax</i>		
I 318	SW G 017	Negative		
I 319	SW S 017	Negative		
I 320	SW G 018	Negative		
I 321	SW S 018	Negative		
I 322	SW G 019 (slide)	Positive <i>P. knowlesi</i>	<i>An. donaldi</i> sp.	<i>An. barbirostris</i> group
I 323	SW S 019 (slide)	Positive <i>P. knowlesi</i>	<i>An. donaldi</i> sp.	<i>An. barbirostris</i> group

*SW: Sarawak ; G : Midgut ; S : Salivary glands

Table 1: Shows result of species identification by DNA sequencing and PCR methods.

DISCUSSION AND CONCLUSION

- Through dissection method, this studies has found infective *Anopheles donaldi* with well present oocyst in midgut. The Plasmodium species is confirmed as *Plasmodium knowlesi* through molecular techniques.
- P. knowlesi* was also detected in the salivary glands of the same sample (ID I 322) through molecular techniques (i.e PCR).
- Until 2018, the presence of *Plasmodium knowlesi* in *Anopheles donaldi* (Barbirostris group) had never been reported during previous entomological field studies in the state of Sarawak.
- However, a more recent study conducted in 2020 also discovered *P. knowlesi* in *Anopheles donaldi* in Lawas, Sarawak.
- As for the conclusion, in-depth studies need to be conducted across all districts in Sarawak to confirm whether *Anopheles* species other than *An. leucosphyrus* group have the potential to serve as vectors for *P. knowlesi*.
- In addition, the detection of both *P. vivax* and *P. knowlesi* in the abdomen and thoracic part of mosquitoes indicates that Semena is highly vulnerable to malaria transmission and need continuous control activities.

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