

Original Article

MOLECULAR CHARACTERISATION OF BLOOD MEAL SOURCES IN *ANOPHELES MACULATUS* FROM A *PLASMODIUM KNOWLESI* TRANSMISSION AREA IN SEREMBAN, NEGERI SEMBILAN, MALAYSIA

Abdul Jabir **Jaafar**¹, Mohamad Nadzmi **Md Nadzri**², Ainul Huda **Khairul Azman**¹, Nurul Asmaa **Abdul Rahman**¹, Rezki Sabrina **Masse**^{1*}

¹ Medical Entomology Unit, Infectious Disease Research Centre, Institute for Medical Research (IMR), National Institutes of Health (NIH), Persiaran Setia Murni, Setia Alam, 40170 Shah Alam, Selangor, Malaysia

² Biomedical Epidemiology Unit, Special Resource Centre, Institute for Medical Research (IMR), National Institutes of Health (NIH), Persiaran Setia Murni, Setia Alam, 40170 Shah Alam, Selangor, Malaysia

Submission Date:

10 May 2026

Acceptance Date:

6 July 2026

Publication Date:

31 August 2026

***Corresponding author:**

Rezki Sabrina Masse,
rezki.sabrina@moh.gov.my
[Y](#)

DOI:

[https://doi.org/10.63719/i_mrj.2026.01\(SE\).06](https://doi.org/10.63719/i_mrj.2026.01(SE).06)

IMRJ © 2026. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction, provided the original author and source are credited.

ABSTRACT

Zoonotic malaria, particularly caused by *Plasmodium knowlesi*, has emerged as a major public health concern in Southeast Asia. Although Malaysia successfully eliminated indigenous human malaria transmission in 2018, the continued rise in zoonotic malaria cases remains a significant challenge to achieving the WHO malaria elimination certification. *Anopheles maculatus* has been experimentally shown to support the complete sporogonic development of *P. knowlesi* under laboratory conditions. As the primary vector of human malaria in Peninsular Malaysia, *An. maculatus* is also considered a potential vector of *P. knowlesi* in natural settings, given its high abundance in areas where human cases of knowlesi malaria have been reported. Therefore, this study aimed to determine the blood meal sources of *An. maculatus* collected using the human landing catch (HLC) method between March and June 2024. A total of 150 visibly non-engorged mosquitoes were subjected to nested PCR targeting vertebrate DNA, followed by species-specific PCR assays for the detection of human and macaque DNA. Of the 150 samples analysed, 70 (46.7%) were positive for vertebrate DNA. All 70 positive samples contained human DNA, while no macaque DNA was detected. These findings indicate a strong association with human blood meals among *An. maculatus* collected in the study area and provide insights into the host-feeding behaviour of this species in a zoonotic malaria-endemic setting. The findings contribute to a better understanding of local malaria transmission ecology and may support future vector surveillance and control strategies. Further studies across broader geographical and ecological settings are recommended to better characterise the blood-feeding sources of *An. maculatus* and their epidemiological significance in zoonotic malaria transmission.

KEYWORDS: Zoonotic malaria, *Anopheles maculatus*, *Plasmodium knowlesi*, host preference, COI gene

INTRODUCTION

Zoonotic malaria caused by *Plasmodium knowlesi* has become an important public health concern in Southeast Asia, particularly in Malaysia (1,2). Although Malaysia successfully eliminated indigenous human malaria transmission in 2018, zoonotic malaria cases continue to be reported annually and now constitute the majority of malaria notifications in the country (2,3). The complex transmission cycle involving macaque reservoir hosts, *Anopheles* mosquito vectors, and humans presents major challenges to malaria elimination efforts and vector control strategies (4,5).

Several *Anopheles* species, primarily belonging to the Leucosphyrus Group, have been incriminated as vectors of *P. knowlesi* in Malaysia, including *An. latens*, *An. cracens*, and *An. balabacensis* (6–8). However, multiple other *Anopheles* species coexist within zoonotic malaria-endemic settings, and are frequently encountered during entomological surveillance activities. Despite their abundance, many of these species remain poorly investigated for their potential epidemiological relevance in zoonotic malaria transmission (9).

Anopheles maculatus, the principal vector of human malaria in Peninsular Malaysia, is frequently collected in areas where human *P. knowlesi* infections are reported (10,11). Experimental studies have demonstrated that *An. maculatus* can support the complete sporogonic development of *P. knowlesi* under laboratory conditions, suggesting biological susceptibility to the parasite (12). Recently, Sebayang reported the detection of *P. knowlesi* DNA in wild-caught *An. maculatus* from North Sumatra, Indonesia, suggesting the potential epidemiological relevance of this species in zoonotic malaria transmission settings (13). In addition, its opportunistic host-seeking behaviour, combined with its ability to bite humans frequently when opportunities arise, may increase its epidemiological relevance in zoonotic malaria-endemic settings. (11,14). However, the ecological role of *An. maculatus* in areas with ongoing *P. knowlesi* transmission remains poorly understood.

The analysis of mosquito blood meal sources provides important insights into host-feeding behaviour and vector-host interactions that influence malaria transmission dynamics (15). In zoonotic malaria systems, identifying the extent of mosquito feeding on humans and macaques is particularly important for understanding potential bridge transmission between reservoir hosts and humans (16). Molecular detection of vertebrate blood meals has therefore become a valuable approach for investigating the ecological behaviour of mosquito species in endemic settings (17,18).

Despite the frequent collection of *An. maculatus* in *knowlesi* malaria-endemic areas, information on its blood-feeding behaviour in such settings remains limited. Therefore, this study aimed to determine the blood meal sources of *An. maculatus* collected from a locality with reported human cases of *knowlesi* malaria in Negeri Sembilan, Malaysia, using molecular detection methods targeting vertebrate, human, and macaque DNA.

MATERIALS AND METHODS

a) Study site and study duration

A cross-sectional study was conducted in Mukim Pantai, Seremban, Negeri Sembilan, Malaysia, from March to June 2024. The study area is characterised by hilly terrain, with secondary forest, plantations, ecotourism chalets, and village settlements. Residential areas comprised a mixture of permanent brick houses and traditional wooden houses, reflecting semi-rural mixed development. Domestic and stray dogs were commonly observed within the study area. Troops of long-tailed macaques (*Macaca fascicularis*) were also frequently observed during field sampling activities, indicating proximity between human settlements and potential zoonotic reservoir hosts. In the year preceding the study, three human cases of knowlesi malaria were reported from this locality, supporting its relevance as a zoonotic malaria-endemic setting (19).

b) Mosquito sampling technique

Anopheles mosquitoes were collected using the Human Landing Catch (HLC) method for three consecutive nights each month from March to June 2024. Collections were conducted between 18:00 and 00:00 hours at selected sampling sites within the study area. Although resting collections are conventionally preferred for blood meal analysis, recent studies have demonstrated that vertebrate host DNA may remain detectable in mosquitoes for up to 72 hours post-feeding. Therefore, molecular detection of residual vertebrate DNA from HLC-collected mosquitoes was used in this study as a proxy for recent host-feeding activity. To minimise potential bias associated with freshly blood-fed mosquitoes, only visibly non-engorged individuals were selected for molecular analysis as indicators of recent host-feeding behaviour. Mosquitoes exhibiting visible fresh blood meals were excluded to reduce the likelihood of detecting blood acquired during the sampling process.

c) Sample identification and processes

All collected *Anopheles* mosquitoes were morphologically identified using Reid's taxonomic keys under a stereomicroscope (20). Only female *An. maculatus* mosquitoes were included for subsequent analysis. A visual examination of abdominal status was performed to exclude visibly engorged individuals from the study. The abdomens of female *An. maculatus* mosquitoes were separated from the heads and thoraces under a dissecting microscope, and preserved in 95% ethanol. The dissected abdomens were transferred into sterile microcentrifuge tubes, transported to the Medical Entomology Unit, Institute for Medical Research (IMR), and stored at -20°C before genomic DNA extraction. A total of 150 female *An. maculatus* mosquitoes were selected for this study.

d) Host blood determination

Genomic DNA was extracted from the 150 homogenised mosquito abdomen samples using the DNeasy Blood and Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions. Extracted DNA was subjected to nested PCR targeting the vertebrate mitochondrial Cytochrome c Oxidase Subunit I (COI) gene to detect the presence of vertebrate host DNA. The primers (Table 1) and amplification protocol used for vertebrate DNA detection were adapted from Alcaide (21).

The first-round PCR amplification was performed in a final reaction volume of 25 μ L containing 5 μ L of DNA template, 1 $\times$ Green GoTaq reaction buffer (Promega), 3.6 mM MgCl₂ (Promega), 0.75 mM dNTP mixture (Promega), 0.5 μ M each of forward (M13BC-FW) and reverse (BCV-RV1) primers, and 0.25 U GoTaq DNA polymerase (Promega). Cycling conditions consisted an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 40 s, annealing at 45 °C for 1 min, and extension at 72°C for 1 min, with a final extension at 72 °C for 7 min. Extracted DNA from a human blood-fed laboratory strain of the *An. maculatus* mosquito was used as the positive control, while molecular-grade water served as the negative control. The positive and negative controls were included in all PCR assays.

For the second-round PCR, 3 μ L of first-round PCR product was used as template in a final reaction volume of 27 μ L. Reaction mixtures were prepared with identical reagent concentrations to those of the first-round PCR, except that 0.2 U of GoTaq DNA polymerase (Promega) was used with the primers M13-FW and BCV-RV2. Cycling conditions consisted of an initial denaturation at 94°C for 3 min, followed by a touchdown PCR protocol in which the annealing temperature decreased from 60°C to 45°C over 15 cycles (–1°C per cycle), with denaturation at 94°C for 40 s and extension at 72°C for 1 min. This process was followed by 24 additional cycles of 94°C for 40 s, 45°C for 40 s, and 72°C for 1 min, with a final extension at 72°C for 7 min. Positive and negative controls were included in all PCR assays. Extracted DNA from a human blood-fed laboratory strain of the *An. maculatus* mosquito was used as the positive control for humans, and extracted DNA from archived *Macaca fascicularis* blood was used as positive control for macaques, while molecular-grade water served as the negative control. PCR products were visualised on 1.5% agarose gel stained with RedSafe™ (iNtRON Biotechnology, South Korea) under UV illumination (UVP ChemStudio).

Samples positive for vertebrate DNA were subsequently subjected to species-specific PCR assays targeting human and macaque DNA using primers described by Gunathilaka (22). PCR amplifications for human and macaque DNA detections were performed separately in individual reaction tubes. Each reaction was carried out in a final volume of 27 μ L containing 3 μ L of DNA template, 1 $\times$ Green GoTaq reaction buffer (Promega), 3.6 mM MgCl₂ (Promega), 0.6 mM dNTP mixture (Promega), 0.75 μ M each of forward and reverse primers, and 0.25 U GoTaq DNA polymerase (Promega). Cycling conditions consisted of an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 1 min, and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were visualised on 1.5% agarose gel stained with RedSafe™ (iNtRON Biotechnology, South Korea) under UV illumination (UVP ChemStudio). PCR preparation, amplification, and post-PCR analyses were conducted in separate designated areas to minimise contamination.

Table 1. List of primers for PCR

PCR Assay	Primer Name	Sequence (5'–3')	Amplicon Size (bp)	Reference
Primary PCR ^a	M13BC-FW	5'-TGT AAA ACG ACG GCC AGT HAA YCA YAA RGA YAT YGG-3'		(21)
	BCV-RV1	5'-GCY CAN ACY ATN CCY ATR TA-3		
Secondary PCR ^a	M13-FW	5'-GTA AAA CGA CGG CCA GTG -3'	758	

	BCV-RV2	5'-ACY ATN CCY ATR TAN CCR AAN GG-3'		
Human DNA ^b	Human-F	5'-TTC GGC GCA TGA GCT GGA GTC C-3'	228	(22)
	Human-R	5'-TAT GCG GGG AAA CGC CAT ATC G-3'		
Macaque DNA ^b	Monkey-F	5'-CCT CTT TCC TGC TGC TAA TG-3'	222	(22)
	Monkey-R	5'-TTT GAT ACT GGG ATA TGG CG-3'		

^aVertebrate DNA detection via nested PCR

^bSpecies-specific DNA detection

e) Data analysis

Data were cross-checked, verified, and compiled into a single dataset using Microsoft Excel (Microsoft Corporation, USA). Descriptive statistical analyses were performed to determine the frequency and proportion of vertebrate DNA-positive samples and species-specific PCR detections. Detection rates for human and macaque DNA were calculated and expressed as percentages of the total vertebrate DNA-positive samples to describe the host-feeding sources of *An. maculatus* collected in the study area.

RESULTS

A total of 150 female *Anopheles maculatus* mosquitoes collected using the HLC method were included in this study. All selected mosquitoes were visibly non-engorged before molecular analysis. Nested PCR targeting vertebrate mitochondrial *COI* DNA detected vertebrate host DNA in 70 of 150 (46.7%) *An. maculatus* abdomen samples analysed. Representative agarose gel electrophoresis results confirming successful vertebrate PCR amplification are shown in Figure 1.

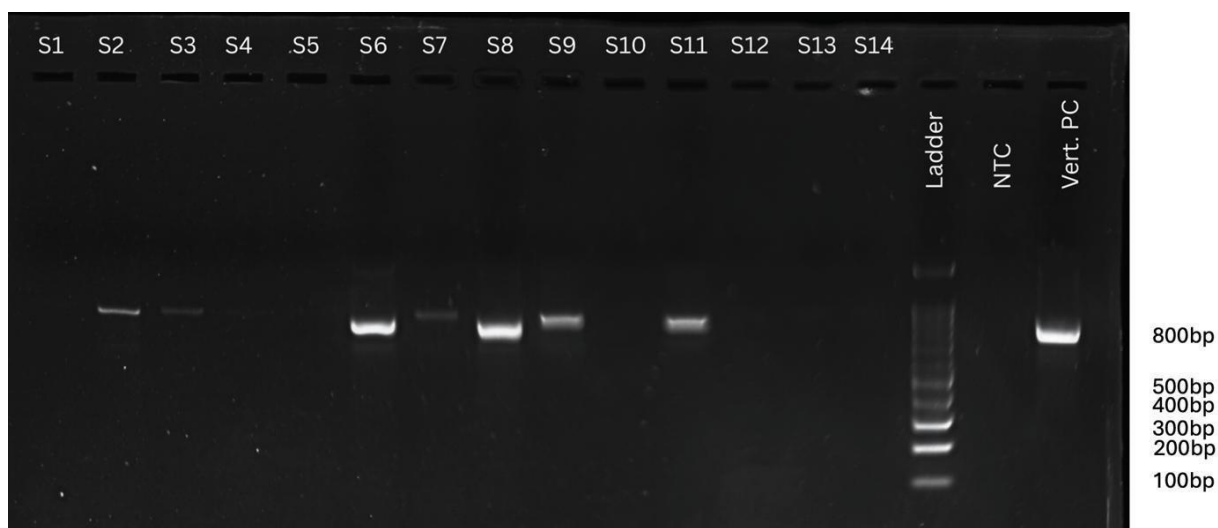


Figure 1. Representative agarose gel electrophoresis image of nested PCR amplification targeting vertebrate *COI* DNA from *An. maculatus*. Lanes S1–S14, representative mosquito samples, with samples S2, S3, S6, S7, S8, S9,

and S11 showing positive amplification; lane ladder, 100bp DNA ladder; lane NTC, no-template negative control; lane Vert. PC, vertebrate positive control. The expected amplicon size was 758 bp.

Species-specific PCR analysis subsequently detected human DNA in all vertebrate DNA-positive samples, whereas macaque DNA was not detected in any sample analysed. Representative agarose gel electrophoresis images of the species-specific PCR assays are presented in Figure 2.

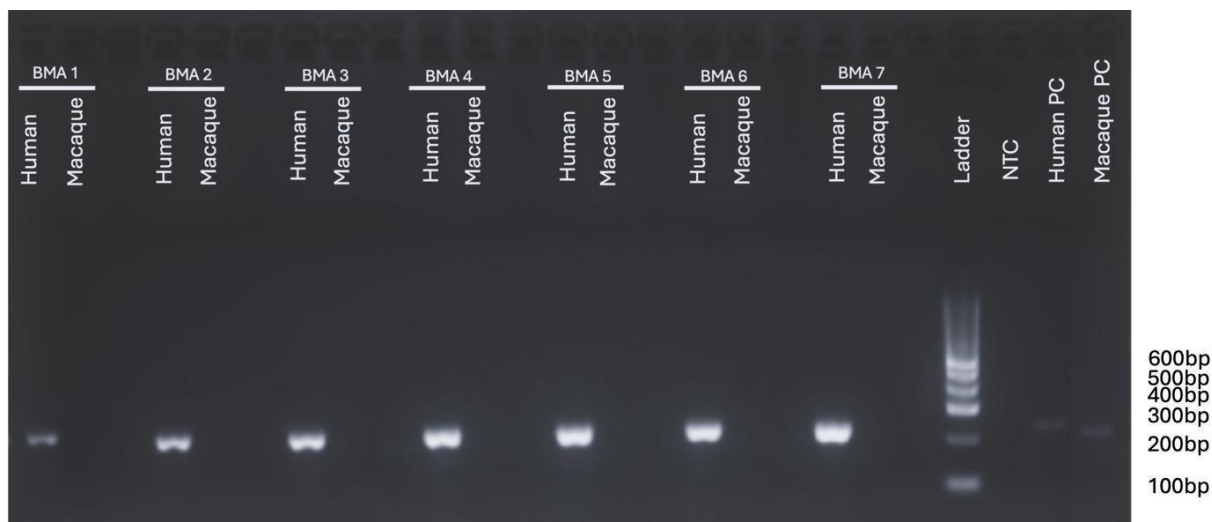


Figure 2. Representative agarose gel electrophoresis of species-specific PCR assays for human and macaque DNA detection in vertebrate DNA-positive *An. maculatus* samples. Lane BMA1 – BMA7, representative of vertebrate DNA-positive; lane ladder, 100-bp DNA ladder; lane NTC, no-template negative control; lanes Human PC, human positive control; lane Macaque PC, macaque positive control, representative samples. The expected amplicon sizes were around 228 bp for human DNA and 222 bp for macaque DNA.

The distribution of vertebrate and species-specific PCR results is summarised in Table 2.

Table 2. Detection of vertebrate, human, and macaque DNA in *Anopheles maculatus* collected between March and June 2024

Sampling Month	Total Samples Tested (n)	Vertebrate DNA Positive, n (%)	Human DNA Positive ^a , n (%)	Macaque DNA Positive ^a , n (%)
March 2024	50	24 (48.0)	24 (100.0)	0 (0.0)
April 2024	50	27 (54.0)	27 (100.0)	0 (0.0)
May 2024	30	6 (20.0)	6 (100.0)	0 (0.0)
June 2024	20	13 (65.0)	13 (100.0)	0 (0.0)
Total	150	70 (46.7)	70 (100.0)	0 (0.0)

^a Percentage for human and macaque DNA detection was calculated based on vertebrate DNA-positive samples only

DISCUSSION

The present study demonstrated the detection of vertebrate host DNA in visibly non-engorged *Anopheles maculatus* collected using the Human Landing Catch (HLC) method from a knowlesi malaria-endemic locality in Negeri Sembilan, Malaysia. Species-specific PCR analysis identified human DNA in all vertebrate DNA-positive samples, while macaque DNA was not detected in any sample analysed. These findings suggest human-associated feeding activity among *An. maculatus* within the study area and are consistent with previous reports describing this species as an anthropophilic mosquito with strong human-biting tendencies in Peninsular Malaysia and other Southeast Asian settings (11,23). As the principal vector of human malaria in Peninsular Malaysia, *An. maculatus* is commonly associated with peri-domestic and semi-rural environments where close human-vector contact may facilitate exposure to both human and zoonotic malaria transmission systems (10, 24). The predominance of human DNA observed in the present study is also comparable to findings reported by Jeyaprakasam (25), who identified human blood as the dominant vertebrate blood source among field-collected *Anopheles* mosquitoes in zoonotic malaria-endemic settings in Malaysia.

The absence of macaque DNA in all analysed samples should be interpreted cautiously and does not necessarily exclude the possibility of macaque feeding by *An. maculatus* under natural conditions. Although *An. maculatus* is generally regarded as an anthropophilic mosquito, previous studies have reported occasional collections of this species in macaque-baited traps, suggesting possible opportunistic attraction to macaque hosts under natural conditions (7). Similarly, studies from Thailand have reported that *An. maculatus* exhibits mixed anthropophilic and zoophilic feeding behaviour, with mosquitoes feeding on both humans and animal hosts depending on local ecological conditions (23). Several factors may have contributed to the study's findings, including biases in the HLC method towards collecting human-seeking mosquitoes, limited vertebrate host screening, temporal variation in host-feeding behaviour, and possible degradation of residual vertebrate DNA in non-engorged mosquitoes.

The exclusive detection of human DNA in this study should be interpreted with caution, as mosquitoes were collected using the human landing catch (HLC) method, which is inherently biased toward anthropophilic, host-seeking females. Consequently, HLC preferentially samples mosquitoes actively attracted to humans and may not fully represent the natural host-feeding behaviour of *An. maculatus*. To minimise the influence of blood meal acquired immediately during the capture, only visibly non-engorged mosquitoes were included in the molecular analysis. This approach was intended to decrease the likelihood of detecting DNA from a fresh blood bolus while allowing the detection of residual vertebrate host DNA from previous blood-feeding events. Previous studies have demonstrated that vertebrate host DNA can remain detectable in mosquitoes for up to 72 h after blood feeding, supporting the use of non-engorged mosquitoes for retrospective blood meal analysis (25). Nevertheless, because HLC selectively captures mosquitoes seeking human hosts, the predominance of human DNA observed in this study should not be interpreted as definitive evidence of an inherent preference for human hosts. Rather, the findings indicate that *An. maculatus* had previously fed on humans before being recaptured while host-seeking. Future investigations incorporating complementary collection methods, such as resting collections, together with a broader range of vertebrate host-specific assays, would provide a more comprehensive assessment of the natural blood-feeding behaviour of *An. maculatus*.

In addition, vertebrate DNA-positive samples that were negative for macaque DNA may potentially represent blood remnants from other vertebrate hosts that were not included in the species-specific screening assays as prioritisation was necessary and only human and macaque hosts were selected based on their relevance to the zoonotic transmission of *Plasmodium knowlesi*. The absence of macaque DNA detection may also reflect low-frequency macaque feeding events that were not captured within the present sample size. In zoonotic malaria ecosystems, mosquito host selection may vary according to ecological conditions, host availability, habitat disturbance, and the degree of human encroachment into forested environments (5,9). Therefore, the present findings should be interpreted as evidence of recent human-associated feeding activity rather than definitive host preference.

The frequent detection of human-associated vertebrate DNA in *An. maculatus* collected from a zoonotic malaria-endemic locality highlights the importance of mosquito-human interactions within the broader context of *P. knowlesi* transmission ecology. The semi-rural characteristics of the study area, including the proximity of human settlements to forested habitats and macaque populations, may favour peri-domestic mosquito-host interactions. Previous studies have suggested that zoonotic malaria transmission may increasingly occur outside deep forest environments, particularly in areas undergoing environmental modification and human encroachment (5,9). Mosquito species that frequently feed on humans within zoonotic malaria-endemic environments may increase the opportunities for parasite spillover between wildlife reservoirs and human populations. Although *An. maculatus* has not been incriminated as a natural vector of *P. knowlesi*, its widespread distribution, anthropophilic behaviour, and demonstrated susceptibility to *P. knowlesi* under experimental conditions warrant continued ecological surveillance in zoonotic malaria-endemic settings (7).

Blood meal analysis of mosquitoes is important for understanding host-feeding behaviour and assessing disease transmission risk. However, conventional resting-collection methods used for blood meal analysis are often operationally challenging in forested zoonotic malaria-endemic environments, where mosquito resting sites may be difficult to locate and access. Consequently, alternative molecular approaches using HLC-collected mosquitoes may provide useful supplementary ecological information regarding recent host-associated feeding activity. The present study demonstrated that vertebrate host DNA could still be detected in visibly non-engorged HLC-collected mosquitoes, supporting previous reports that residual blood meal DNA may remain detectable in mosquitoes for prolonged periods following feeding (21). This observation suggests that some mosquitoes may have been in the later stages of blood digestion, during which visible blood remnants were no longer apparent upon morphological examination, despite the persistence of detectable host DNA. In addition, detection of vertebrate DNA in visibly non-engorged mosquitoes may also reflect residual blood remnants from interrupted or partial feeding events. Although vertebrate DNA detection is likely to decrease progressively as blood digestion advances, molecular detection of residual vertebrate DNA from HLC-collected mosquitoes may still provide valuable insights into vector-host interactions in field settings. Such approaches may be particularly useful in zoonotic malaria-endemic areas where understanding mosquito-human contact is important for assessing local transmission ecology and guiding vector surveillance activities.

Several limitations should be acknowledged in the present study. First, the use of HLC collections may have preferentially selected mosquitoes actively seeking human hosts, potentially increasing the likelihood of detecting human-associated blood remnants. Second, only human and macaque DNA were targeted in the species-specific PCR assays, whereas *An. maculatus* may also feed on other vertebrate hosts present within the study area, including domestic animals and wildlife. In addition, the study was conducted within a single locality over a relatively short sampling period, which may not fully represent temporal or geographical variation in host-feeding behaviour. Future studies incorporating larger sample sizes, alternative mosquito collection methods such as resting catches and CDC light traps, broader vertebrate host screening, and simultaneous parasite detection assays would provide a more comprehensive understanding of the ecological and epidemiological relevance of *An. maculatus* in zoonotic malaria transmission settings.

CONCLUSION

In conclusion, the present study detected human-associated vertebrate DNA in non-engorged *Anopheles maculatus* collected using the HLC method in a *Plasmodium knowlesi*-endemic locality in Negeri Sembilan, Malaysia. These findings demonstrate that residual human-associated vertebrate DNA can be successfully detected in non-engorged *An. maculatus*, providing evidence of recent mosquito-host interactions, and supporting the utility of molecular blood meal analysis in non-engorged mosquitoes. However, because HLC preferentially samples anthropophilic, host-seeking mosquitoes, the predominance of human DNA should not be interpreted as definitive evidence of the natural host preference *An. maculatus*. Likewise, the absence of detectable macaque DNA does not necessarily indicate the absence of feeding interactions between *An. maculatus* and macaque hosts, as it may reflect methodological limitations, including sampling bias and the restricted range of vertebrate hosts included in the species-specific assays. Further studies incorporating complementary mosquito collection methods, broader vertebrate host screening, larger sample sizes, and more diverse ecological settings are warranted to better characterise the host-feeding ecology of *An. maculatus* and its potential role in the transmission dynamics of zoonotic malaria.

Supplementary Materials: Not applicable.

Data Availability: The datasets generated and/or analysed during the current study are available from the corresponding/first author upon reasonable request.

Author Contributions: A.J.J. (Sample collection; Molecular assays and Writing the manuscript), M.N.M.N., A.H.K.A. and N.A.A.R. (Sorting of samples, Assisting molecular assays, Tabulating data), R.S.M. (Identification of field samples; Data analysis, Reviewing manuscript).

Funding: This study is supported by the SEAMEO TROPMED Malaysia and the National Institutes of Health (NIH) Malaysia. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Institutional Review Board Statement: NMRR ID-26-00837-WME. Not applicable for Medical Research & Ethics Committee (MREC).

Informed Consent Statement: Human landing catch (HLC) collectors provided written informed consent prior to participation. Appropriate personal protective measures and malaria prophylaxis were provided throughout the sampling period.

Acknowledgements: We would like to thank the Director General of Health Malaysia and the Director, Institute for Medical Research (IMR) for their permission to publish this article. This study was supported by a grant from SEAMEO TROPMED Malaysia and NIH, Ministry of Health Malaysia.

Conflicts of Interest: Authors have no conflict of interest to declare.

CITATION

Jaafar, A. J., Md Nadzri, M. N., Khairul Azman, A. H., Abdul Rahman, N. A., & Masse, R. S. (2026). Molecular characterisation of blood meal sources in *Anopheles maculatus* from a *Plasmodium knowlesi* transmission area in Seremban, Negeri Sembilan, Malaysia. *International Medical Research Journal*, (Special Issue), 88–98. [https://doi.org/10.63719/imrj.2026.01\(SE\).06](https://doi.org/10.63719/imrj.2026.01(SE).06)

REFERENCES

1. Singh B, Kim Sung L, Matusop A, Radhakrishnan A, Shamsul SS, Cox-Singh J, et al. A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. *Lancet*. 2004;363(9414):1017–24. doi:10.1016/S0140-6736(04)15836-4.
2. World Health Organization. World malaria report 2024. Geneva: World Health Organization; 2024.
3. Ministry of Health Malaysia. Malaysia malaria surveillance report 2024. Putrajaya: Ministry of Health Malaysia; 2024.
4. Vythilingam I, NoorAzian YM, Huat TC, Jiram AI, Yusri YM, Azahari AH, et al. *Plasmodium knowlesi* in humans, macaques and mosquitoes in Peninsular Malaysia. *Parasit Vectors*. 2008;1:26. doi:10.1186/1756-3305-1-26
5. Fornace KM, Brock PM, Abidin TR, Grignard L, Herman LS, Chua TH, et al. Environmental risk factors and exposure to the zoonotic malaria parasite *Plasmodium knowlesi* across northern Sabah, Malaysia: a population-based cross-sectional survey. *Lancet Planet Health*. 2016;1:e179–86. doi:10.1016/s2542-5196(19)30045-2
6. Tan CH, Vythilingam I, Matusop A, Chan ST, Singh B. Bionomics of *Anopheles latens* in Kapit, Sarawak, Malaysian Borneo in relation to the transmission of zoonotic simian malaria parasite *Plasmodium knowlesi*. *Malar J*. 2008;7:52. doi:10.1186/1475-2875-7-52
7. Jiram AI, Vythilingam I, NoorAzian YM, Yusof YM, Azahari AH, Fong MY. Entomologic investigation of *Plasmodium knowlesi* vectors in Kuala Lipis, Pahang, Malaysia. *Malar J*. 2012 Jun 22;11:213. doi:10.1186/1475-2875-11-213
8. Wong ML, Chua TH, Leong CS, Khaw LT, Fornace K, Wan-Sulaiman WY, et al. Seasonal and spatial dynamics of the primary vector of *Plasmodium knowlesi* within a major transmission focus in Sabah, Malaysia. *PLoS Negl Trop Dis*. 2015;9(10):e0004135. doi:10.1371/journal.pntd.0004135
9. Manin BO, Ferguson HM, Vythilingam I, Fornace K, Drakeley CJ, Chua TH, et al. Investigating the contribution of peri-domestic transmission to risk of zoonotic malaria infection in humans. *PLoS Negl Trop Dis*. 2016;10(10):e0005064. doi:10.1371/journal.pntd.0005064
10. Wharton RH. The biology of *Anopheles hackeri* and *Anopheles maculatus* in relation to malaria transmission in Malaya. Kuala Lumpur: Institute for Medical Research; 1962.
11. Rahman WA, Adanan CR. Malaria and *Anopheles* mosquitoes in Malaysia. *Southeast Asian J Trop Med Public Health*. 1997;28(3):599–605. doi:10.1016/j.actatropica.2005.06.022
12. Collins WE, Warren M, Skinner JC, Richardson BB, Kears TS. Infection of *Anopheles maculatus* with *Plasmodium knowlesi*. *J Parasitol*. 1967;53(4):778–82.

13. Sebayang BF, van de Straat B, Kurniawan A, et al. Evidence incriminating *Anopheles maculatus* as a potential vector of *Plasmodium knowlesi* and *Plasmodium inui*. Sci Rep. 2026;16:6515. doi:10.1038/s41598-026-35946-z
14. Sinka ME, Bangs MJ, Manguin S, Chareonviriyaphap T, Patil AP, Temperley WH, Gething PW, Elyazar IR, Kabaria CW, Harbach RE, Hay SI. The dominant *Anopheles* vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionomic précis. Parasit Vectors. 2011 May 25;4:89. doi:10.1186/1756-3305-4-89.
15. Kent RJ. Molecular methods for arthropod bloodmeal identification and applications to ecological and vector-borne disease studies. Mol Ecol Resour. 2009;9(1):4–18. doi:10.1111/j.1755-0998.2008.02469.x
16. Fornace KM, Abidin TR, Alexander N, Brock P, Grigg MJ, Murphy A, William T, Menon J, Drakeley CJ, Cox J. Association between Landscape Factors and Spatial Patterns of *Plasmodium knowlesi* Infections in Sabah, Malaysia. Emerg Infect Dis. 2016 Feb;22(2):201-8. doi:10.3201/eid2202.150656.
17. Kent RJ, Norris DE. Identification of mammalian blood meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome b. Am J Trop Med Hyg. 2005;73(2):336–42.
18. Logue K, Keven JB, Cannon MV, Reimer L, Siba P, Walker ED, et al. Unbiased characterization of *Anopheles* mosquito blood meals by targeted high-throughput sequencing. PLoS Negl Trop Dis. 2016;10(3):e0004512. doi:10.1371/journal.pntd.0004512
19. Technical Meeting for the Malaria Elimination Strategic Plan, Vector-Borne Disease Control Sector, Disease Control Division, Ministry of Health Malaysia
20. Reid JA. 1968. *Anopheline Mosquitoes of Malaya and Borneo*. Studies from the Institute for Medical Research Malaysia No. 31.
21. Alcaide M, Rico C, Ruiz S, Soriguer R, Muñoz J, Figuerola J. Disentangling vector-borne transmission networks: a universal DNA barcoding method to identify vertebrate hosts from arthropod bloodmeals. PLoS One. 2009;4(9):e7092. doi:10.1371/journal.pone.0007092.
22. Gunathilaka N, Denipitiya T, Hapugoda M, Abeyewickreme W, Wickremasinghe R. Determination of the foraging behaviour and blood meal source of malaria vector mosquitoes in Trincomalee District of Sri Lanka using a multiplex real time polymerase chain reaction assay. Malar J. 2016;15:242. doi:10.1186/s12936-016-1279-5.
23. Muenworn V, Sungvornyothin S, Kongmee M, Polsomboon S, Bangs MJ, Akrathanakul P, et al. Biting activity and host preference of the malaria vectors *Anopheles maculatus* and *Anopheles sawadwongporni* (Diptera: Culicidae) in Thailand. J Vector Ecol. 2009;34(1):62-9. doi:10.1111/j.1948-7134.2009.00008.x.
24. Rahman WA, Adanan CR, Abu Hassan A. Species composition of adult *Anopheles* populations and their breeding habitats in Hulu Perak district, Peninsular Malaysia. Southeast Asian J Trop Med Public Health. 2002;33(3):547-50.
25. Jeyaprakasam NK, Low VL, Liew JWK, Pramasivan S, Wan-Sulaiman WY, Saeung A, et al. Blood meal analysis of *Anopheles* vectors of simian malaria based on laboratory and field studies. Sci Rep. 2022;12(1):354. doi:10.1038/s41598-021-04106-w.