Measuring the exposure of primate reservoir hosts to mosquito vectors in Malaysian Borneo

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Abstract

Several vector-borne pathogens of primates have potential for human spillover. An example is the simian malaria *Plasmodium knowlesi* which is now a major public health problem in Malaysia. Characterization of exposure to mosquito vectors is essential for assessment of the force of infection within wild primate populations, however few methods exist to do so. Here we demonstrate the use of thermal imaging and Mosquito Magnet Independence Traps (MMIT) to assess the abundance, diversity and infection rates in mosquitoes host seeking near long-tailed macaque (*Macaca fascicularis*) sleeping sites in the Lower Kinabatangan Wildlife Sanctuary, Malaysian Borneo. The primary *Plasmodium knowlesi* vector, *Anopheles balabacensis*, was trapped at higher abundance near sleeping sites than control trees. Although none of the *An. balabacensis* collected (n=15) were positive for *P. knowlesi*, two were infected with another primate malaria *Plasmodium inui*. Analysis of macaque stools from sleeping sites confirmed a high prevalence of *Plasmodium* infection, suspected to be *P. inui*. *Plasmodium inui* infections have not yet been reported in humans, but its presence in *An. balabacensis* here and previously in human-biting collections highlight its potential for spillover. We advocate the use of MMITs for non-invasive sampling of mosquito vectors that host seek on wild primate populations.

Introduction

Non-human primates (NHPs) are reservoirs of vector-borne pathogens that can infect humans. Some already pose significant public health problems, such as the monkey malaria parasites *Plasmodium simium* (1) and *P. brasilianum* (2) in South America. Several human vector borne diseases have sylvatic origins, including Yellow Fever, Zika and Dengue (3,4) alongside other lesser known viruses with potential to emerge (5). Understanding the force of infection in wild primate populations is crucial for assessment of potential for human spillovers, and the possibility of disrupting transmission in primate populations to mitigate against this. Such assessment requires estimating primate exposure to mosquito vectors, however there are few practical methods to do this. Current methods are invasive by relying on captive monkeys used as baits in traps, and may not reflect exposure in a natural population. There would be great value in finding a non-invasive and representative method for characterising NHP exposure to mosquito vectors.

One of the most notable primate Vector-borne diseases (VBD) of public health significance in SE Asia is *Plasmodium knowlesi*, a zoonotic malaria whose natural hosts are long-tailed and pig-tailed macaques and *Presbitis* leaf-monkeys, transmitted by Leucosphyrus group *Anopheles* (6–9). Since

first detection of human cases in 2004 (10), *P. knowlesi* has become the most common cause of malaria in people in Malaysian Borneo (11). In 2014, human cases of another macaque malaria, *P. cynomolgi*, were also reported in Malaysia (12,13). Other macaque malarias (*P. coatneyi*, *P. fieldi* and *P. inui* (14,15)) have been detected in mosquito vectors in Malaysian Borneo, but are not yet known to have infected humans. Additional VBDs circulate in NHPs in Malaysia that can infect people; e.g. sylvatic Dengue in macaques, leaf monkeys and orangutans (5,16,17), and the filarial worm *Brugia malayi* in leaf monkeys. (18,19). This wide range of potential VBDs necessitates surveillance of mosquitoes biting NHP to anticipate future spillover risk.

Characterization of VBD transmission in primates has been hindered by logistical and ethical constraints. To date there are a limited range of tools for studying NHP exposure to VBDs; most being invasive by requiring blood sampling (20–22). Alternative non-invasive methods for detecting malaria parasite DNA in faecal samples (23–29) are promising but are yet to be widely applied and optimized. Similar constraints apply to assessment of primate exposure to mosquito vectors. This has generally been conducted through “Baited Traps” in which monkeys are placed in cages inside a net with gaps to allow mosquitoes attracted to enter but not leave (7,30–32). Contemporary animal welfare regulations for working with captive primates often make such approaches unfeasible. Alternative less invasive approaches such as “e-nets” in which macaques are held in larger cages and have their odour collected and channelled to attract mosquitoes are logistically challenging and yield few vectors (33). Finally, all methods that require the use of a host ‘bait’ require capture of wild primates or handling of captive individuals; both of which are invasive and could cause distress. Identification of less invasive methods for sampling the vector population that host seeks on wild primate populations would be of great value.

So far most investigation of the mosquito vectors of macaque VBDs have been conducted in areas near human settlements (30–33) which may not be reflective of natural transmission cycles within primate populations in the absence of humans. Knowledge of the range of parasite and vector species responsible for monkey to monkey transmission in undisturbed settings is necessary to identify future spillover risks, and assess the feasibility of disrupting transmission in primate reservoir populations as a strategy to reduce this.

Here we evaluated the use of commercially available Mosquito Magnet Independence Traps (MMIT) to passively sample malaria vectors host seeking in the vicinity of long-tailed macaques within the Lower Kinabatangan Wildlife Sanctuary (LKWS) Sabah, Malaysia. Aims were to assess the performance of the MMIT in terms of the abundance and diversity of potential vector species captured near macaque roosts versus uninhabited trees, and whether infection rates in vectors caught near roost sites were reflective of infection prevalence in macaques as assessed from faecal samples. We also investigated relationships between malaria vector abundance in MMITs, macaque troop size as measured with thermal imagery, and environmental factors. Whilst Mosquito Magnet traps have been investigated for passive surveillance of human malaria vectors (34–40), to our knowledge this is the first time it has been evaluated in a wild monkey population.

Methods

Study site

This study was conducted at the Danau Girang Field Centre (DGFC), Lot 6 of the LKWS (5°24'49.93" N, 118°02'18.58" E) (Fig. 1). The LKWS is a protected secondary disturbed forest area (ranging from 10 - 60 years old) that contains primary to secondary lowland dipterocarp forest, mangrove and oil palm plantations (41,42). The sanctuary spans 27000ha (43), and hosts ten primate species including the two reservoir hosts of *P. knowlesi*; long-tailed macaques (*Macaca fascicularis*), and pig-tailed macaques (*Macaca nemestrina*). In 2002, population densities (per km²) were estimated as 16.82

for *M. fascicularis* and 3.30 for *M. nemestrina* (36). The nearest human settlement is at least 15 km downstream from DGFC.

[insert Figure 1 here]

HLC vs MMIT trap comparison

A pilot study was performed to confirm the MMIT (Mosquito Magnet, model: MM3200, supplier: Syarikat Thiam Siong Sdn Bhd, Sabah) was capable of sampling *Anopheles* in this environment (Fig. S1). The MMIT was compared with the standard Human Landing Catch (HLC) method; which is known to be efficient for sampling the *P. knowlesi* vector *An. balabacensis* (14,30,32). The MMIT lures mosquitoes using mammalian odour bait (CO₂ and octenol), heat and water vapour (36,39). The MMIT was modified to run off batteries (4 x 1.5V) and on locally available gas (30% propane: 70% butane).

Each night, one HLC and one MMIT site were selected; with stations ~100m apart on one of three walking trails (Fig. S2). The following night the HLC and MMIT switched sites in a cross over design. This was repeated for ten nights of collections. Hourly collections were conducted from 18:00 – 00:00 hrs to coincide with the peak biting time of *An. balabacensis* (18:00 - 20:00 hrs (14,44)). One person performed HLC accompanied by an assistant. Each hour comprised 45 minutes of trapping and 15 minutes break. The MMIT was switched off during the break and the collection net replaced.

Use of MMIT for sampling vectors near macaque sleeping sites

Mosquito sampling using MMIT was conducted along a 20km section of the Kinabatangan river (560km, Fig. 1). Trees (*Colona*, *Nauclea subdita*, *Pterospermum acerifolium*, *Kleinhovia hospita* and *Ficus*) of a minimum 20m depth lining the river bank (45) are used as sleeping sites for several primate species (46,47), including long-tailed macaques. The study site was divided into ten 2km transects (Fig. S3). The home range of long-tailed macaques in this reserve was estimated as 1.25km² in a previous survey (47). To avoid repeated sampling near the same macaque troop, sampling was conducted in different 2km transects each night. Mosquito sampling took place in each transect once every ten nights; with the transect selected randomly using the Random UX app. Sampling was conducted in blocks of five nights with a one night break; resulting in 38 sampling nights between September to November of 2017. Each block was sampled 3 - 4 times during this period, with traps placed on alternate sides of the river on each visit.

Upon arrival at the selected transect (17:30 hrs), the river banks were scanned with a thermal imaging camera to identify potential macaque troops by driving slowly up and down the river. When the camera indicated presence of a troop, binoculars were used to inspect trees for long-tailed macaques. Once the presence of roosting macaques was confirmed, a MMIT was placed near the bottom of their sleeping tree (conditional on bank being accessible, Fig. S4). Macaques generally moved from the selected tree to higher up in the canopy or deeper inside the forest as the boat approached, but would return after the trap was placed. A second MMIT was placed at least 100m away at a 'control' tree that was of similar structure and species, but uninhabited by macaques or other primates. This 'control' tree enabled differentiation of mosquitoes specifically host seeking in the vicinity of macaques.

Mosquitoes were collected at macaque sleeping and control sites each night from 18:00 – 06:00 hrs. Before sunrise and movement of macaques (approximately 05:30 hrs), the number of macaques sleeping in the tree where the MMIT was placed were counted from the boat using the thermal camera. Daily rainfall data (collected by rain gauge) was provided by DGFC.

Mosquito processing

Mosquitoes were stored at -20°C for approximately 12 hrs then identified to genera and species where possible (48–51). Leucosphyrus group *Anopheles* were identified using Sallum *et al* (2005) (52). All identified mosquitoes were stored in 95% ethanol. Molecular analysis was performed on Leucosphyrus group *Anopheles*, *An. barbirostris* gp. (*An. barbirostris* and *An. donaldi*), *An. epiroticus* and *An. tessellatus* (malaria vectors in Sabah or elsewhere in SE Asia, (44,50,53–56)) to screen for *Plasmodium* infections using the method described in (57).

Macaque Faecal collection

Each morning after emptying MMIT traps, the ground within a 20m radius of sleeping trees was inspected for the presence of fresh macaque stools (see Supplementary Methods S1). Stool samples were homogenized in RNAlater solution then stored at -20°C.

DNA was extracted from 200 µl of each stool solution using the QIAamp DNA Stool Mini Kit. DNA was eluted in 100 µl buffer AE and stored at -20 °C. Samples were screened by PCR for detection of DNA from the *Plasmodium* genus (see Supplementary Methods S2). *Plasmodium* positive samples were then screened to test for the specific presence of *P. knowlesi* following the method of Kawai *et al* (29).

Statistical analysis

Data were analysed using R statistical programming software (3.4.2) with packages lme4 and multcomp. Generalized Linear Mixed Models (GLMMs) were used to compare the abundance of mosquitoes in HLC and MMIT; with comparisons made for all mosquitoes and just *Anopheles*. Negative binomial GLMMs were used to account for overdispersion in mosquito count data (58). The response variable was the mean abundance of (i) all mosquitoes (ii) *Anopheles* per night. The main fixed effect was trap type with random effects fit for date and trail. A post hoc Tukeys' test was used to assess differences in mosquito abundances between traps. The vegan package was used to measure *Anopheles* diversity in HLC and MMIT catches. Four diversity indices were calculated: species richness, rarefied species richness, Simpson's index and the Shannon index (57).

Sampling of mosquitoes near trees where macaques were sleeping was conducted for 38 nights. On a few occasions, macaques or other primates were present at the control site in the mornings or the traps stopped working overnight due to failure of gas supply or batteries. Excluding these scenarios, data was available from 33 nights of sampling at control trees and 34 nights at trees with sleeping macaques. With this data, GLMMs were constructed to test for differences in the response variables of 1) *Anopheles* abundance 2) *An. balabacensis* abundance and 3) *An. donaldi* abundance between macaque sleeping sites and control trees. A negative binomial distribution was used with date and river transect set as random effects. Models tested for associations between mosquito abundance and macaque presence and abundance, and rainfall on the day of sampling. The significance of each variable was tested by backward elimination using likelihood ratio tests. Post-hoc Tukey's tests were performed to assess differences in mosquito abundance between sleeping sites and control collections.

On nine sampling nights, the datalogger failed to record the average nightly temperature. Thus a subset of data (28 nights at control trees and 29 nights at sleeping sites) for which mean nightly temperatures were available was used to test for associations between temperature and mosquito abundance. Here the impact of temperature on three mosquito subgroups were investigated as above.

Results

HLC vs MMIT trap comparison

Both HLC and MMITs collected mosquitoes belonging to the same eight genera (Table S1). Mosquitoes were identified to species level where possible, however due to time constraints, priority was given to *Anopheles*, *Culex* and *Mansonia*. *Aedes* and *Uranotaenia* mosquitoes were mostly identified to subgenus. In general, mosquitoes trapped by HLC were in better condition for morphological identification than those trapped in the MMIT because key characteristics necessary for species determination such as hairs and scales were better preserved.

Almost all *Anopheles* caught in the HLC could be speciated, except one individual that was missing features to distinguish between *An. barbirostris* or *An. donaldi*. Two *Anopheles* from MMIT collections (3.2 % of total) could not be placed to a subgenus. Five *Anopheles* species were collected by HLC compared to 8 species with MMIT (Table 1). *Anopheles* diversity was higher in MMIT than HLC collections (Table 2). Both methods trapped the *P. knowlesi* vectors *An. balabacensis* and *An. donaldi*; with a higher proportion of these being caught by HLC (80.5 %, $n = 29$) than MMIT (72.6 %, $n = 45$).

Although mosquito numbers tended higher in MMIT than HLC collections, the mean nightly abundance was not significantly different (Tukey's test: $P = 0.39$, Fig. 2A). Similarly, the mean nightly abundance of *Anopheles* did not vary between trapping methods (Tukey's test: $P = 0.210$, Fig. 2A). *Anopheles donaldi* was most abundant between 18:00 – 20:00 hrs (Fig. 2C), whereas *An. balabacensis* biting rates were relatively constant between 18:00 - 23:00 hrs with none collected between 23:00 -24:00 hrs (Fig. 2B).

[insert Figure 2 here]

MMIT to sample Anopheles host seeking near macaques

Overall, 11400 mosquitoes from eight genera were collected in MMITs placed near macaque sleeping sites and control trees (Table S2). Both malaria vector species, *An. balabacensis* and *An. donaldi* were trapped at sleeping sites and control trees.

Combining over all species in the genera, the mean nightly abundance of *Anopheles spp.* was not significantly associated with the presence ($X^2 = 0.45$, $df = 1$, $P = 0.50$) or number of macaques ($X^2 = 0.62$, $df = 1$, $P = 0.43$) at a tree, or with daily rainfall ($X^2 = 0.67$, $df = 1$, $P = 0.41$) (Fig. S5). Within the subset of data for which full temperature recordings were available, a strong positive association was detected between mean nightly *Anopheles* abundance and temperature ($X^2 = 6.46$, $df = 1$, $P = 0.01$, Fig. 3).

[insert Figure 3 here]

The primate malaria vector *An. balabacensis*, however, was significantly impacted by the presence of macaques at sampling sites. The mean abundance of *An. balabacensis* was significantly higher near macaque roost sites than at control trees ($X^2 = 8.25$, $df = 1$, $P < 0.01$, Fig. 4A), but was not related to number present ($X^2 = 1.28$, $df = 1$, $P = 0.26$) or daily rainfall ($X^2 = 0.42$, $df = 1$, $P = 0.52$, Fig. 4B and C). Sample sizes of *An. balabacensis* within the subset of data where temperature was available were not sufficient for robust analysis.

[insert Figure 4 here]

The total number of *An. donaldi*, caught at macaque sleeping sites ($n = 106$) was lower than at control trees ($n = 211$, Table 3); however this difference was only marginally significant (Tukeys: $P = 0.052$). The abundance of *An. donaldi* was not dependent on the presence or absence of macaques ($\chi^2 = 0.92$, $df = 1$, $P = 0.34$), the number of macaques present ($\chi^2 = 0.13$, $df = 1$, $P = 0.72$) or the daily rainfall ($\chi^2 = 0.23$, $df = 1$, $P = 0.63$) (Figure S6). Within the subset of data for which full temperature recordings were available, *An. donaldi* abundance was positively associated with mean daily temperature ($\chi^2 = 5.86$, $df = 1$, $P = 0.02$, Figure S7).

Plasmodium infections in mosquitoes and macaque stools

Eighty-one *Anopheles* collected in the initial HLC versus MMIT trap comparison were tested for malaria (*An. donaldi* = 61, *An. balabacensis* = 7, *An. barbirostris/donaldi* = 5, *An. tessellatus* = 5, *An. celia* group = 1, *An. unknown* = 2). Of these, one tested positive for *Plasmodium* infection ($n = 1/81$). In the larger study using MMITs at macaque sleeping sites and control trees, 398 *Anopheles* were tested for malaria (Barbirostris group = 373 (including *An. barbirostris* (2) and *An. donaldi* (317)), *An. balabacensis* = 15, *An. epiroticus* = 1, *An. tessellatus* = 2 and unidentified *Anopheles* species = 7, Table 3). Of these, one tested positive for *Plasmodium* ($n = 1/398$). Both infections were confirmed to be *P. inui*. Both infections were detected in *An. balabacensis*, representing an overall infection rate of 9% ($n = 2/22$) in this vector species.

Of the 46 long-tailed macaque faecal samples collected, 17 (37%) tested positive for *Plasmodium*. However in the subsequent round of PCR analysis to test for *P. knowlesi*, none were positive. Samples were not screened for other malaria species, thus the identity of *Plasmodium* infections remains unknown.

Discussion and Conclusions

This study to our knowledge provides the first evaluation of the use of MMIT for sampling the mosquitoes that host seek on wild primates. We show that the abundance of all mosquitoes (pooled across genera), and *Anopheles* in particular was similar in collections made by MMITs and the HLC gold standard. Our results confirm that the MMIT can be used as an indirect exposure-free alternative to the HLC. MMIT placed below macaque roosting trees collected several known vectors of zoonotic and human malaria (*An. balabacensis*, *An. donaldi* and *An. barbirostris*). While *Anopheles* density was not higher overall at trees with than without macaques; the abundance of the confirmed primate vector *An. balabacensis* was significantly higher near macaque sleeping sites. This implies *An. balabacensis* were actively host seeking on macaques. Macaque density was very high in the study area, with analysis of their faecal samples indicating a high prevalence of *Plasmodium* infection (37%). However, the zoonotic malaria *P. knowlesi* was not detected in either vector or macaque samples here; suggesting transmission was primarily of other simian parasite species. Mosquito infections were confirmed to be *P. inui*; a primate malaria parasite that has not been documented in humans. Despite the absence of *P. knowlesi*, the ability of MMITs to detect known vector species feeding at macaque sleeping sites highlights its value for non-invasive monitoring of primate exposure to mosquito vectors. This tool could thus provide opportunity to study the transmission of *P. knowlesi* as well as other primate VBDs in wild primate populations.

The abundance of *Anopheles* spp. was similar in MMIT and HLC collections, although the MMIT caught more species (8 vs 5 in the HLC). The greater diversity of Anophelines in the MMIT compared to HLCs, also seen in a Venezuelan setting (59) may be due to the use of a general R-octenol bait that attracts both anthropophilic and zoophilic mosquitoes (60), and/or that it releases a higher concentration of host cues than a single human collector. Here only one individual performed HLC, but it is well known that volatile emissions vary between people (61). Further investigation using multiple participants in HLC is required for more robust evaluation of the relative performance of

MMIT and HLC methods. However, given the MMIT has an advantage of enabling passive sampling and collected just as many *Anopheles* as HLC, it may be a more practical approach for sampling of malaria vectors host seeking on wild primates.

The primary *P. knowlesi* vector in Sabah, *An. balabacensis*, was detected at significantly higher numbers near trees with than without macaques; indicating this species has a propensity for this host type. This finding is in line with a host choice study involving baited electrocuting nets where *An. balabacensis* fed on both monkey and human hosts (33). However, there was no significant relationship between *An. balabacensis* abundance and the number of macaques at the sleeping site. Macaque troop size varied across sampling nights from 2 – 47 individuals (average ~14), thus incorporating substantial variability for detecting an association with mosquito density. Studies examining the influence of host density on malaria vector density have found correlation (positive and negative depending on vector species) between adult Anopheline density and the density of humans (62,63). The lack of association here, however may be the result of the odour plume of even one macaque being sufficient to lure *An. balabacensis*. Alternatively, mosquitoes could be attracted to the trees themselves. Macaques are known to revisit sleeping trees (64) thus macaque odour cues could build up around a site, signalling a reliable bloodmeal source for vectors. Additionally, there could be environmental characteristics not measured here that contributed to higher *An. balabacensis* abundances at macaque sleeping sites.

Associations between *Anopheles* abundance in MMIT collections and rainfall and temperature were not detected. Higher temperature and rainfall has been demonstrated to increase mosquito abundances in MMITS used in the Brazilian rainforest (38). No association was detected between temperature or rainfall and *An. balabacensis*; but ability to test for this was limited by small sample sizes. To investigate seasonal fluctuations in vector abundance with rainfall and temperature, we recommend more intensive longitudinal sampling across a full year to increase sample sizes and capture the extremes of environmental variation.

The primary focus of this study was investigation of *P. knowlesi* transmission within its wildlife reservoir in the absence of humans. However, malaria infections were detected in only two *An. balabacensis* and in both cases it was *P. inui*. *Plasmodium inui* is commonly found in wild macaques (65) and can infect humans under experimental conditions (via blood transfusion or infected mosquito bites in the lab (66). However natural human infections of *P. inui* have not been documented. Relatively high rates of *P. inui* and other primate malarias (*P. cynomolgi*, *P. fieldi* and *P. coatneyi*) have been described in *An. balabacensis* in village settings in Sabah (15). Thus, people are regularly exposed to these parasites in peri-domestic as well as forest settings; raising the possibility that *P. inui* could pose a significant risk for zoonotic spillover in the future.

The absence of *P. knowlesi* infection in mosquito vectors was matched with its absence in the macaque population. Although *Plasmodium* DNA was detected in more than a third of macaque stool samples, but none of these samples were identified as *P. knowlesi*. We hypothesise these infections were most likely *P. inui* based on its confirmation in the local *An. balabacensis* population. *Plasmodium knowlesi* prevalence in macaques has been reported at 6.9 % and 30 % in Peninsular Malaysia (31,67), and 20 % and 86.6 % in Sarawak (68,69). However these estimates were derived from analysis of macaque blood samples which have greater sensitivity to detect low density infections than the faecal screening method used here (70). However, another study also based on analysis of macaque blood samples reported a much lower prevalence of *P. knowlesi* (0.4 % (71)); indicating macaque infection rates are naturally heterogeneous. Researchers often assume that the force of *P. knowlesi* infection coming from macaques to humans is high throughout Sabah; given one study found *P. knowlesi* infection in 20% of the faeces collected from wild long-tailed macaques within the Kudat District, the hotspot of human infection in 2013 - 2014 (Salgado-Lynn, unpublished

data). In the same study, 80% of the blood samples of macaques from a different district in Sabah were positive for *Plasmodium*, 66% of which were positive for *P. knowlesi*. However the apparent absence of *P. knowlesi* infection here indicates the force of infection may vary considerably between wild reservoir populations. Therefore, recent efforts to generate *P. knowlesi* risk maps based on macaque distribution (79–81) may be limited by failure to incorporate underlying variation in infection prevalence within macaque populations. Furthermore, blanket control policies based on macaque culling may be both ethically questionable and have limited impact.

Here we demonstrate the suitability of MMIT for sampling mosquitoes host seeking in the vicinity of macaques and advocate its use as a tool for monitoring vector borne infections circulating in wild primate populations. In addition to its use for investigation into vector ecology associated with macaque to macaque pathogen transmission, it is a reliable alternative to performing HLC to study vectors feeding on people and removes the need to expose volunteers to potentially infectious mosquito bites. As yet there are no records of natural *P. inui* infections in people in Malaysia, however with the detection of the parasite in *An. balabacensis* here and in this vector species from collections nearby homes (15,72,73) people are likely frequently being exposed to this parasite and therefore warrants close surveillance of its potential for spillover.

List of abbreviations

HLC – Human Landing Catch

MMIT- Mosquito Magnet Independence Trap

LKWS- Lower Kinabatangan Wildlife Sanctuary

GLMM- Generalised Linear Mixed Models

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Tables

Table 1. *Anopheles* mosquitoes caught by Mosquito Magnet Independence Trap (MMIT) and human-landing catch (HLC) over ten nights of trap comparison study in Lower Kinabatangan Wildlife Sanctuary, Sabah.

	Human-landing catch (HLC)	Mosquito Magnet Independence Trap (MMIT)
<i>Anopheles</i>	36	62
<i>An. balabacensis</i>	2	5
<i>An. barbirostris</i>	0	2
<i>An. barbirostris/donaldi</i>	1	5
<i>An. barumbrosus</i>	0	2
<i>An. cellia</i> subgenus	0	1
<i>An. donaldi</i>	27	40
<i>An. kochi</i>	0	1
<i>An. montanus</i>	2	1
<i>An. roperi</i>	1	1
<i>An. tessellatus</i>	3	2
Unknown <i>Anopheles</i> spp.	0	2

Table 2. Measures of diversity in *Anopheles* species from Mosquito Magnet Independence Trap (MMIT) and human-landing catch (HLC) collections from a ten-night trap comparison study in Lower Kinabatangan Wildlife Sanctuary, Sabah.

Trap Type	Abundance	Species richness	Rarefied species richness	Shannon Index	Simpson's Index
HLC	35	5	2.93	0.84	0.39
MMIT	54	8	3.23	1.03	0.44

Table 3. *Anopheles* mosquitoes caught with Mosquito Magnet Independence Trap (MMIT) at trees with and without sleeping macaques (control trees) within the Lower Kinabatangan Wildlife Sanctuary, Sabah.

	Macaque sleeping sites (34 nights)	Control trees (33 nights)
<i>Anopheles</i>	250	476
<i>An. balabacensis</i>	13	2
<i>Barbirostris</i> gp	122	251
<i>An. barbirostris</i>	0	2
<i>An. donaldi</i>	106	211
<i>An. epiroticus</i>	1	0
<i>An. gigas</i>	1	0
<i>An. montanus</i>	2	2
<i>An. roperi</i>	0	1
<i>An. tessellatus</i>	2	0
<i>An. umbrosus</i> gp	1	2
Unknown <i>Anopheles</i> spp.	2	5

List of Figures

Figure 1. Map of Sabah indicating the location of the Danau Girang Field Centre (red) along the Kinabatangan river (blue). Green areas indicate boundaries of the Lower Kinabatangan Wildlife Sanctuary (Lots 1 - 10) and black lines show administrative districts.

Figure 2.A) Mean abundance of mosquitoes caught per night by Human landing catch (HLC) and Mosquito Magnet Independence Trap (MMIT) as predicted by negative binomial generalised linear mixed models (GLMM). Error bars represent 95% confidence intervals B) *An. balabacensis* and C) *An. donaldi* trapped per hour by human-landing catch (HLC) and Mosquito Magnet Independence Trap (MMIT).

Figure 3. Predicted relationship between mean *Anopheles* abundance collected by Mosquito Magnet Independence Traps (MMIT) and average nightly temperature (from the subset of 28 sampling nights at control trees and 29 sampling nights at macaque sleeping sites for which environmental data were available). Points indicate observed data, with the line indicating the predicted association. Dashed lines represent upper and lower 95% confidence intervals.

Figure 4. Influence of A) macaque presence/absence, B) number of macaques present and C) daily rainfall on the mean nightly *An. balabacensis* abundance collected by Mosquito Magnet Independence Traps (MMIT). Points indicate observed data in B and C, with the line indicating the predicted association. Error bars and dashed lines are 95% confidence intervals and * represents $P < 0.05$.

List of Supplementary Files

Figure S1. A Mosquito Magnet Independence Trap (MMIT, left) and a view of the mosquito collection net within the MMIT (right).

Figure S2. Map of forest trails surrounding the main building of Danau Girang Field Centre in the Lower Kinabatangan Wildlife Sanctuary. Boxes indicate the sites used on *Ficus* (wet lowland forest), Kingfisher (wet lowland forest) and Kayu Malam (dry lowland forest) walking trails for human-landing catch (HLC) and Mosquito Magnet Independence Trap (MMIT) evaluation of collecting *Anopheles*. Blue lines depict bodies of water.

Figure S3. The 20km stretch of the Kinabatangan River surrounding Danau Girang Field Centre where macaque roosting sites and control trees were selected for mosquito collection. Purple dots indicate the boundary of each 2km transect that could be randomly selected for mosquito sampling on each night.

Figure S4. The Mosquito Magnet Independence Trap (MMIT) in position on the riverbank at the base of a *Ficus* (fig) tree to be used by a long-tailed macaque troop as their overnight resting place.

Figure S5. Influence of A) macaque presence/absence, B) number of macaques present and C) daily rainfall on the mean nightly *Anopheles* abundance collected by Mosquito Magnet Independence Traps (MMIT). Points indicate observed data in B and C, with the line indicating the predicted association. Error bars and dashed lines are 95% confidence intervals and * represents $P < 0.05$.

Figure S6. Influence of A) macaque presence/absence, B) number of macaques present and C) daily rainfall on the mean nightly *An. donaldi* abundance collected by Mosquito Magnet Independence Traps (MMIT). Points indicate observed data in B and C, with the line indicating the predicted association. Error bars and dashed lines are 95% confidence intervals and * represents $P < 0.05$.

Figure S7. Predicted relationship between mean *An. donaldi* abundance collected by Mosquito Magnet Independence Traps (MMIT) and average nightly temperature (from the subset of 28 sampling nights at control trees and 29 sampling nights at macaque sleeping sites for which environmental data were available). Points indicate observed data, with the line indicating the predicted association. Dashed lines represent upper and lower 95% confidence intervals.

Table S1. Mosquitoes caught by Mosquito Magnet Independence Trap (MMIT) and human-landing catch (HLC) over ten nights of trap comparison study in Lower Kinabatangan Wildlife Sanctuary, Sabah.

Table S2. Mosquitoes caught with Mosquito Magnet Independence Trap (MMIT) at trees with and without sleeping macaques (control trees) within the Lower Kinabatangan Wildlife Sanctuary, Sabah.

A Trap Evaluation **B** *An. balabacensis* hourly biting activity **C** *An. donaldi* hourly biting activity

A *An. balabacensis*

B *An. balabacensis*

C *An. balabacensis*