Analysis of *Anopheles arabiensis* Blood Feeding Behavior in Southern Zambia during the Two Years after Introduction of Insecticide-Treated Bed Nets

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Abstract. *Anopheles arabiensis* mosquitoes are the primary vector responsible for *Plasmodium falciparum* transmission in Macha, Zambia. Because insecticide-treated bed nets (ITNs) have the potential to alter host feeding behavior, the extent of the zoophilic and exophagic tendencies of the vector was evaluated during the two rainy seasons after ITN introduction. Centers for Disease Control light traps, paired indoor/outdoor human landing catches, and outdoor cattle-baited collections were used to assess potential changes in host preference. Results support the hypothesis that *An. arabiensis* mosquitoes in Macha remain highly anthropophilic despite high ITN use. *Anopheles arabiensis* mosquitoes in Macha appear to be relatively exophagic and have been caught biting outdoors immediately after sunset and before sunrise, potentially circumventing some of the protective effects of ITNs.

INTRODUCTION

Since 2003, the National Malaria Control Program in Zambia has redoubled its efforts to reduce the country's malaria burden. In 2003, the Ministry of Health introduced intermittent presumptive treatment of pregnant women and adopted artemisinin combination therapy as the standard treatment of uncomplicated malaria.¹ In 2004, the National Malaria Control Program added the implementation of large-scale integrated vector control efforts, including indoor residual house spraying, larviciding, environmental management, and the distribution of insecticide-treated bed nets (ITNs).² By 2007, more than 5.3 million ITNs were distributed throughout the country free of charge.² The Macha area of Southern Province received 4,800 long-lasting ITNs impregnated with deltamethrin (Thuma PE, unpublished data).

The major vector of *Plasmodium falciparum* parasites in the Macha area are *Anopheles arabiensis* Patton mosquitoes.³ In contrast to the other major malaria vectors in sub-Saharan Africa, *An. gambiae* s.s. Giles and *An. funestus* s.s. Giles, which are primarily anthropophilic, endophagic, and endophilic,^{4,5} *An. arabiensis* is more variable its foraging behavior. Although the vector can be found feeding and resting indoors in some localities,^{6–11} in other areas it is mainly exophagic^{12–15} and exophilic.^{12,16,17} Additionally, *An. arabiensis* has been reported to be highly anthropophilic in some regions,^{9,18–21} including southern Zambia,^{3,22} whereas elsewhere it predominantly displays zoophagic foraging tendencies.^{16,17,23,24} Furthermore, human blood indices (HBIs) for the vector have been shown to vary even between households in the same village based on cattle ownership.^{17,25}

The pyrethroid insecticides that are used to impregnate ITNs affect anopheline mosquitoes in a number of ways. Implementation of ITNs may reduce vector survival and suppress vector populations.^{26–28} These nets can also alter foraging behavior. It has been reported that ITNs can shift anopheline biting outdoors,^{26,29} earlier in the evening,^{26,29,30} or to alternate hosts.^{28,30,31} Additionally, ITNs may have contact irritancy and/

or non-contact excito-repellency effects, decreasing the numbers of *An. gambiae* s.l. that enter sleeping houses and causing those that do enter to exit more quickly.^{32,33} The goal of this study was to assess the foraging behavior of *An. arabiensis* in Macha during the two years after large-scale ITN introduction.

MATERIALS AND METHODS

Mosquito collecting and handling. *Anopheles arabiensis* were captured from December through May during the 2007–2008 and 2008–2009 rainy seasons after ITN introduction near the Johns Hopkins Malaria Research Institute field station. This field site is located at 16.39292°S, 26.79061°E in the Southern Province of Zambia. Collections were performed in two village-areas, Chidakwa and Namwalinda, situated 5 km and 10 km from the field station, respectively. After capture, all mosquitoes were killed by freezing, identified morphologically,³⁴ and packaged on silica gel desiccant (Fisher Scientific, Fair Lawn, NJ) and cotton in individual tubes for stable storage and transport.

The degree of exophagy of *An. arabiensis* and the activity level of the vector throughout the night was assessed by paired indoor/outdoor human landing catches (HLCs). Teams of two HLC collectors stationed inside and outside seven selected sleeping houses captured mosquitoes by manual aspiration, keeping hourly collections in separately labeled paper cups. Collections were performed from 8:00 PM to 6:00 AM during the 2007–2008 season and were extended to 7:00 PM to 7:00 AM during the 2008–2009 season. Collections were performed for 246 trap nights. Collectors were rotated between collection locations each night.

Anthropophily of *An. arabiensis* was examined by paired outdoor HLC and cattle-baited trap (CBT) collections performed simultaneously at three different households over 72 nights for 216 trap nights. Outdoor HLC and CBT collections were conducted by teams of two trained field personnel from dusk until dawn. The CBTs were conducted in Chidakwa and Namwalinda at seven households. Each CBT was a kraal, made of felled trees and branches, just large enough to hold one calf. After being led and tied into the kraal, the calf was covered with a double bed-sized untreated bed net. The net was hung above the kraal so that it lay on the outside of the pen, resting approximately 30 cm off the ground. The upper portion of the net was fashioned into a funnel that collected

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most of the resting mosquitoes and was cinched closed in the morning. The calf was then released and the remaining mosquitoes were collected by aspirator.

Additionally, anthropophily was assessed by determining the human blood indices of engorged *An. arabiensis* collected by Centers for Disease Control and Prevention (CDC) light traps during each season. The CDC traps were hung next to occupied beds protected by bed nets. If there was more than one bed in a trapping room, the other occupants were instructed to also use bed nets. Household members were shown how to turn on the trap before they went to sleep and how to cinch the bag closed and disconnect the battery in the morning. Trapping was performed in approximately 10 households in both village-areas multiple times per month.

Bed net use survey. At the peak of each rainy season, household censuses were performed in Chidakwa and Namwalinda. The total numbers of people who slept in each structure and the number of people who slept under an ITN the previous night were enumerated during the 2007–2008 and 2008–2009 rainy seasons. Additionally, during the second year, the ages and sexes of those not sleeping under a bed net were noted. Results were averaged across three months each year to account for movements between sleeping structures. An odds ratio was calculated to determine if there was a gender bias in bed net use.

DNA isolation and polymerase chain reaction. Heads/thoraces were separated from mosquito abdomens so that DNA could be extracted from each separately. Prior to homogenization, specimens were rehydrated at room temperature for 10 minutes in 20 μ L of double-distilled water. DNA was extracted by using a modified salt procedure³⁵ and resuspended in 50 μ L of double-distilled water. DNA from engorged abdomens was used as template to determine host source by polymerase chain reaction.^{22,35} DNA from head/thoraces was used to confirm species by using the diagnostic procedure of Scott et al.³⁶ and also to screen for *P. falciparum*. *Plasmodium* infection status was assessed by using a nested PCR.³⁷

Data analysis. Mosquito collections were modeled as overdispersed Poisson populations.^{38–40} Comparisons of CBT and outdoor HLC collections and comparisons of indoor and outdoor HLCs were performed using separate negative binomial regression analyses in STATA version 10 (Stata Corp., College Station, TX). Mosquito counts were assumed to follow a Poisson distribution with an overdispersion parameter to account for increased variance as compared with the mean. The log of the expected counts was modeled as the function $\log(E(Y_{ijk})) = \alpha + \beta X + \epsilon_{ijk}$, where Y_{ijk} is the monthly mosquito count for household i in month j and year k , and X are the covariates of sampling method, collection year, and collection month. This method enabled standard errors to be adjusted for correlated observations within collection households.

RESULTS

Across the three months during the 2007–2008 rainy season when household censuses were conducted, an average of 343 persons in 43 households were interviewed about their bed net use. During the 2008–2009 rainy season, an average of 305 persons in 39 households were surveyed. Every household owned at least one ITN. During the first year 82% of people self-reported sleeping under a bed net the previous night. Bed net coverage was 87% in Chidakwa ($n = 159$) and 78%

in Namwalinda ($n = 184$). Coverage was still high the following year, but the proportion of people self-reporting bed net use decreased to 75% overall, 85% in Chidakwa ($n = 149$) and 65% in Namwalinda ($n = 156$). More detailed surveys were conducted during the 2008–2009 season. The odds of a female reporting to have slept under a bed net were 1.87 (95% confidence interval [CI] = 1.11–3.16) times that of a male. The age group reporting the largest number of individuals not sleeping under a bed net was persons 5–14 years of age. Only one child less than 1 year of age was reported to not have slept under a net (Figure 1).

Prior to the distribution of ITNs, *An. arabiensis* in Macha was highly anthropophilic; published HBIs were 0.872 and 0.923 for indoor-resting mosquitoes.^{3,22} This study found that the vector remained highly anthropophilic in the Macha region during the two years after the introduction of ITNs. Outdoor HLC teams caught nearly double the numbers of *An. arabiensis* as paired CBTs, although CBTs caught more mosquitoes overall. In total, CBTs caught 10,002 mosquitoes, and outdoor HLCs caught 1,878. *Anopheles arabiensis* catch totals were 285 and 147 for outdoor HLCs and CBTs, respectively. A negative binomial regression analysis demonstrated that the average number of *An. arabiensis* caught by a HLC pair was approximately 2.2 (95% CI = 0.99–4.9) times the number captured by a CBT collection.

Additionally, high HBIs were calculated for blood fed mosquitoes captured by CDC light trap (Table 1). Although everyone who slept in a room where CDC light trapping occurred was instructed to use a bed net, a sizeable percentage of *An. arabiensis* in the traps were blood fed. During the 2007–2008 season, 28% of the mosquitoes captured were engorged. Likewise, during the 2008–2009 season, 25% were blood fed. Human blood indices of 0.94 and 0.96 were determined for the two consecutive years, with the remaining blood meals found to be from cattle, dogs, and goats (Table 1). Two *An. funestus* s.s., one unfed and one engorged on human blood, were also captured by CDC light trap during the 2008–2009 season. None of the *An. arabiensis* collected by HLC, CBT, or CDC light trap were positive for *P. falciparum* sporozoites.

Over the course of this study, *An. arabiensis* consistently displayed exophagic behavior (Figure 2). During the first year, outdoor HLC pairs captured 279 *An. arabiensis*, and indoor teams caught 130 specimens. The following year 96 *An. arabiensis* were caught outside, and 61 were captured inside. A negative binomial regression analysis showed that outdoor HLCs

Ages of Individuals Not Protected by a Bed Net

FIGURE 1. Individuals not protected by a bed net during the 2008–2009 season, partitioned by age class, Macha, Zambia.

TABLE 1

Abdominal status and host source of engorged *Anopheles arabiensis* mosquitoes collected by Centers for Disease Control light trap, Macha, Zambia

Season	Unfed	Gravid	Blood fed	Host source, no (%)			
				Human	Cow	Dog	Goat
2007–2008	588	8	235	204 (94)	14 (6)	0	0
2008–2009	681	38	233	204 (96)	4 (1.9)	3 (1.4)	2 (0.9)

caught an average of 1.7 (95% CI = 1.2–2.5) times as many *An. arabiensis* as indoor HLCs. Furthermore, throughout the night there was consistently more biting activity outdoors (Figure 3). *Anopheles arabiensis* began biting indoors and outdoors by 7:00 PM and continued to be active until 6:00 AM inside and 7:00 AM outside. Peak biting for the vector occurred between 10:00 PM and 2:00 AM, decreasing thereafter. A total of 14% of *An. arabiensis* biting occurred before 10:00 PM.

DISCUSSION

The foraging behavior of *An. arabiensis* in Macha, Zambia during the two years after introduction of ITNs was assessed in this study. Over the two-year period, the vector remained highly anthropophilic, as shown by high HBIs from CDC light trap collections and paired outdoor HLC and CBT comparisons. This study is the second report of a direct comparison between HLCs and CBTs for *An. arabiensis*. As underscored previously,¹² the relative efficiency of the two techniques cannot be known. It may be that more mosquitoes were attracted to the cattle bait but that the trap was less efficient at catching them than the human collectors. However, when taken in conjunction with the HBI data, there is strong support for continued anthropophily in *An. arabiensis* in the Macha region. There have been reports of shifts in foraging by anophelines to alternate hosts after introduction of ITNs in India,²⁸ Papua New Guinea³⁰ and Kenya.³¹ However, similar to the data from Macha, other investigations of *An. gambiae* complex mosquitoes in Africa have not seen this effect.^{26,29,41}

Other potential behavioral changes that have been observed in anophelines with the introduction of ITNs are shifts toward outdoor and/or earlier biting. Like other aspects of its behavior, the nightly biting activity of *An. arabiensis* varies dramatically across Africa. Peak biting after midnight has been

observed in Senegal,^{9,10} Chad,⁸ and Kenya.²⁴ However, in Mozambique¹³ and Tanzania,¹⁴ high activity levels were seen as early as 9:00 PM, and in Ethiopia peak biting was seen from 7:00 PM until 9:00 PM.⁴² In Macha, *An. arabiensis* biting occurs throughout the night, with peak activity starting before midnight at approximately 10:00 PM. Although most persons have gone to bed by this hour, it is important to note that 14% of *An. arabiensis* biting occurs prior to this time, when residents are finishing dinner and preparing for bed and are not protected by ITNs.

It is difficult to interpret whether ITNs are having an effect on *An. arabiensis* biting times in Macha because similar collections were not performed in the pre-intervention year. Likewise, it is unknown whether the high degree of exophagy observed in the population is a result of ITN introduction. *Anopheles arabiensis* in Macha might be inherently exophagic as it is elsewhere,^{12–15} or the vector might have adapted rapidly to feeding outdoors because indoor hosts are now mostly inaccessible. However, it is likely that the exophagy is not caused by a decrease in *An. arabiensis* entering behavior. Centers for Disease Control and Prevention light traps have been shown to catch similar numbers of mosquitoes when placed next to either an untreated bed net or ITN in Macha,⁴³ which suggests that nets treated with deltamethrin produce little non-contact repellency.

The numbers of *An. arabiensis* captured by CBTs and HLCs were significantly reduced during the second year of trapping. However, this was probably not caused by community-wide suppression of the vector as reported for *An. gambiae* s.l. populations in Kenya²⁷ and Tanzania²⁶ because the total numbers of mosquitoes captured, including zoophilic culicines collected by CBT, were also reduced. Instead, differences in mosquito densities are likely a result of interannual variation in rainfall because *An. arabiensis* numbers have been shown to fluctuate greatly with the amount and frequency of precipitation

FIGURE 2. Seasonality of *Anopheles arabiensis* mosquitoes caught by human landing catch pairs during the 2007–2008 and 2008–2009 rainy seasons, Macha, Zambia. Bars indicate monthly means plus one standard error.

FIGURE 3. Total *Anopheles arabiensis* mosquitoes caught each hour by human landing catch pairs December–April, Macha, Zambia.

received in the Macha area.³ Furthermore, it is evident that rainfall greatly influences *An. funestus* s.s. in Macha. Prior to an extended drought during the 2004–2005 rainy season, *An. funestus* s.s. was a recognized vector in the region. However, since that drought year it had not been observed until this report with the collection of two specimens by CDC light trapping during the 2008–2009 season.

Overall, CDC light traps in Macha captured a high percentage (26.5%) of blood fed *An. arabiensis*, with greater than 93% of the blood meals coming from human hosts, even though all occupants of trapping houses were instructed to use a bed net. Other studies have found CDC light traps to catch between 1.8% and 9% blood fed *An. gambiae* s.l. when used in conjunction with bed nets.^{44–47} Even when used without nets, the numbers of engorged *An. gambiae* s.l. captured by CDC trap have been lower than the proportions reported here.^{43,46} The high percentage of blood fed mosquitoes collected from CDC traps in Macha might be a consequence of capturing some fraction of exophagic but endophilic *An. arabiensis*. Additionally, it is possible that the host sources of the blood meals are occupants of the trapping rooms. Perhaps bed nets are being used improperly or the vector might be feeding through the bed net. Although none of the mosquitoes

collected for this study were sporozoite positive, during the two rainy seasons of trapping after introduction of ITNs, malaria cases continued to be seen at the Macha Mission Hospital. To further decrease malaria transmission, there needs to be a better understanding of who is being bitten, and therefore, who is at greatest risk for contracting malaria or being a gametocyte donor.

It is evident that regardless of whether shifts in feeding location or biting times by *An. arabiensis* in Macha have occurred, the vector remains anthropophilic. This preference, in combination with the exophagic behavior of the vector and potential for early evening biting, might abrogate ITN protection as intervention use continues. Therefore, the foraging behavior of *An. arabiensis* in the Macha region will need to be monitored during future rainy seasons.

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