Heterogeneity and Changes in Inequality of Malaria Risk after Introduction of Insecticide-Treated Bed Nets in Macha, Zambia

Laura C. Norris* and Douglas E. Norris

W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins Malaria Research Institute,
Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland

Abstract. In 2007, the first free mass distribution of insecticide-treated bed nets (ITNs) occurred in southern Zambia. To determine the effect of ITNs on heterogeneity in biting rates, human DNA from *Anopheles arabiensis* blood meals was genotyped to determine the number of hosts that had contributed to the blood meals. The multiple feeding rate decreased from 18.9% pre-ITN to 9.1% post-ITN, suggesting that mosquito biting had focused onto a smaller fraction of the population. Pre-ITN, 20% of persons in a household provided 40% of blood meals, which increased to 59% post-ITN. To measure heterogeneity over a larger scale, mosquitoes were collected in 90 households in two village areas. Of these households, 25% contributed 78.1% of *An. arabiensis*, and households with high frequencies of *An. arabiensis* were significantly spatially clustered. The results indicate that substantial heterogeneity in malaria risk exists at local and household levels, and household-level heterogeneity may be influenced by interventions, such as ITNs.

INTRODUCTION

The Southern Province of Zambia has historically had hyperendemic transmission of *Plasmodium falciparum*,¹ although there has been a significant decrease in malaria cases since 2003 (Thuma P, unpublished data). The primary malaria vector in the Macha region of southern Zambia is *Anopheles arabiensis*, a member of the *An. gambiae* sensu lato species complex.² A previous study showed that 18.9% of *An. arabiensis* mosquitoes collected in Macha had taken blood meals from more than one human host during a single gonotrophic cycle,³ which is much higher than previously reported multiple feeding rates for *An. gambiae* sensu stricto.^{4,5} That study took place during the 2005–2006 and 2006–2007 rainy seasons, when there were no vector control measures in place.

In 2007, the Zambian government began free mass distribution of insecticide-treated nets (ITNs) for malaria control. As part of this distribution, the Macha area received 4,800 long-lasting insecticide-treated nets (LLINs). At the time of this study, approximately 75% of persons in the immediate Macha area report sleeping under an ITN.⁶ It was hypothesized that implementation of this physical and chemical barrier for malaria control would have an effect on *An. arabiensis* host species choice, shifting feeding to a more readily accessible host (e.g., cattle). However, a study comparing human landing collections against cattle-baited traps⁶ showed that *An. arabiensis* remained anthropophilic despite high LLIN coverage. In addition, Centers for Disease Control (CDC) light trap collections in households with treated and untreated bed nets showed no difference in the house-entering behavior of *An. arabiensis*.⁷ One possible explanation for continued high anthropophily could be that *An. arabiensis* mosquitoes still enter houses in search of human hosts, but have focused biting on the fraction of persons who are not protected by ITNs. We hypothesized that with the introduction of ITNs there would be

fewer accessible human hosts, causing the multiple feeding rate, as measured by human microsatellite alleles, to appear to decrease. If *An. arabiensis* were to continue taking multiple blood meals, they would be more likely to take both meals from the same host. Therefore, a primary goal of this study was to compare the proportion of female *An. arabiensis* that took multiple human blood meals before and after the introduction of insecticide treated bed nets.

If the use of ITNs by most of the population focuses bites on those who are unprotected, the extent of heterogeneity in malaria risk may increase. The extent of heterogeneity, or inequality of risk, in a disease system can profoundly affect overall transmission. Ecological modeling has shown that when arthropod disease vectors bite some fraction of the population at a greater rate, it causes the basic reproductive number (R_0) of the pathogen to increase up to 2–4 times.^{8,9} More recent modeling has shown that when immunity and finite human population size are taken into account, heterogeneity can sometimes decrease R_0 , by focusing infective bites on humans who are already infected. In settings where the annual entomologic inoculation rate e (number of infective bites per year) is less than 100, heterogeneity will decrease R_0 , and in areas with higher entomologic inoculation rates, heterogeneity can increase R_0 .¹⁰ Sources of heterogeneity in vector-borne disease transmission can be caused by variation in inherent attractiveness to mosquitoes,¹¹ age and body size,¹² proximity to vector breeding sites,¹³ bed net use and quality,¹⁴ and myriad other factors. Heterogeneity in disease risk can be present at all scales, from focal disease transmission within an entire region to variability in biting rates among individuals in a household. An important implication of this idea is that if one can identify the part of the population most at risk, malaria control measures can be better targeted at those groups or persons, and more efficiently control disease transmission.

A malaria risk model for the Macha study area, approximately 30 × 40 km, has shown that there is a large degree of inequality of risk across the region, mainly correlating with the presence of third-order streams, in which anopheline breeding sites are plentiful.¹⁵ However, there has been no study of heterogeneity at a more local scale. Within a household, individual human hosts vary in their attractiveness to mosquitoes.¹¹ Our hypothesis, that the introduction of ITNs would

*Address correspondence to Laura C. Norris, W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins School of Public Health, 615 North Wolfe Street, Baltimore, MD 21205. E-mail: lcnorris@ucdavis.edu

focus biting onto a smaller fraction of hosts, would have implications for heterogeneity in host choice within a household. Therefore, another aim of this work was to determine the extent of the heterogeneity caused by host selection at the individual level before and after the introduction of ITNs.

Finally, it has been shown in other locations that malaria vector densities can vary from household to household.¹³ To bridge the gap between heterogeneity at the regional scale and at the individual scale, we aimed to determine the extent of heterogeneity in *An. arabiensis* abundance at the village area scale.

MATERIALS AND METHODS

Study area. These studies were carried out at the Johns Hopkins Malaria Research Institute (JHMRI) field station in Macha, Zambia. The field station is located in Southern Province, at 16.39292°S, 26.79061°E and an elevation of approximately 1,100 meters above sea level, in a Miombo woodland ecotype. The catchment area studied by JHMRI is 1,200 km², with approximately 8,750 households. Rainfall varies by year, but averages 600–1,000 mm in the Southern Province.¹⁶ There is a single rainy season each year (November–April), followed by a cool dry season (April–August) and a hot dry season (August–November). Mosquitoes were collected during the rainy season in the villages of Chidakwa, Lupata, and Namwalinda. Chidakwa and Lupata are 3 km from JHMRI, and Namwalinda is 10 km away. The population is Bantu Tonga, who reside in household compounds comprised of a central cooking hut, one or more sleeping structures, and occasionally a cattle kraal. In small households, a single sleeping structure may be occupied by a couple and their children, and large households may have several sleeping structures, each occupied by a wife and her young children, adolescent boys, the head of the household, or extended family members. Large households, especially those owned by men with several wives, may have as many as 24 residents in 9 sleeping houses and the head of the household may rotate between the sleeping houses of his wives. In this report, household refers to one of these compounds, and house refers to a structure.

Mosquito collection and handling. Mosquitoes were collected by using CDC light traps hung indoors next to persons sleeping under an ITN.^{17,18} The CDC light trap collections were conducted during the 2007–2008 and 2008–2009 rainy seasons at 9 households in Chidakwa, 12 households in Lupata, and 10 households in Namwalinda. Houses were selected on the basis of previous mosquito collection numbers, and collections occurred several times per month throughout the rainy season (December–April). During the 2009–2010 rainy season, collections were concentrated on five households each in Chidakwa and Lupata with high mosquito numbers, and occurred weekly. The location of each household was recorded by using a global positioning system. After collection, all specimens were killed by freezing, identified morphologically,^{19,20} packed individually in tubes containing silica gel desiccant and cotton, and stored at room temperature until processing.

Preparation of DNA and polymerase chain reaction. Mosquito heads and thoraces were separated from abdomens and DNA was extracted from each by using a modified salt procedure.²¹ Specimens morphologically identified as *An. gambiae* sensu lato were confirmed as *An. arabiensis* by a polymerase chain reaction (PCR).²² Specimens were screened for

P. falciparum infection by using a novel PCR protocol,²³ and blood meal species was identified by PCR.^{21,24}

Microsatellite analysis. Human blood meals were genotyped to determine the microsatellite alleles at 4 loci, and the sex of the host. Primers fluorescently tagged with HEX and FAM were used to amplify DNA at the CTT (CSF1PO and TPOX) and Silver STR (D7S and D13S) loci.^{4,25} Each 20-μL reaction contained 10 mM Tris, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 0.8 mM dNTPs, 3.2 units *Taq* polymerase, 25 pmol each forward and reverse primer, and 1 μL template DNA. The PCR conditions consisted of a 2-minute initial denaturation at 96°C; followed by 30 cycles of 1 minute at 94°C, 1 minute at 60°C, and 1.5 minutes at 70°C; and a 45-minute final extension at 70°C. Samples with > 2 alleles at any locus were scored as blood meals from multiple hosts.

For sex determination, the amelogenin²⁶ and SRY²⁷ loci were amplified by using HEX- and FAM-tagged primers. One microliter from each PCR product was multiplexed (CSF with TPOX, Amelogenin, and SRY; D7S with D13S) with 15 μL deionized formamide and 0.5 μL GeneScan-500 Rox size standard (Applied Biosystems Inc., Foster City, CA), and incubated at 95°C for 5 minutes before running on an ABI 3100 Avant Genetic Analyzer (Applied Biosystems Inc.). Data were analyzed with GeneScan Analysis 3.7 and Genotyper (Applied Biosystems Inc.). Archived, previously extracted DNA samples from 2005–2006 and 2006–2007 were also genotyped for pre-ITN blood meal sex determination.³

Household-scale heterogeneity analysis. Microsatellite genotype data from the blood meal analysis was used to determine the source of each blood meal. Although these were not matched to identified individuals, previous modeling shows that the microsatellite loci used are sufficient to distinguish between individuals in this population.³ Mosquito blood meal data was grouped into pre-ITN and post-ITN samples. Many households yielded a small number of blooded mosquitoes, making data analysis difficult and uninformative. Therefore, genotyping data from the eight households in each group with the highest number of identifiable blood meals were statistically analyzed. For consistency, and because the actual host genotypes were unknown, each unique genotype in a household was counted as one individual, even if it could be a mixed blood meal from 2 other individuals. Blood meals from multiple hosts, containing more than 2 alleles at any locus, were omitted because of difficulty in positively identifying each host. During the 2007–2008, 2008–2009, and 2009–2010 collection seasons, a census was taken at each household. This census asked the number of male and female inhabitants, and the number of persons who had not slept under a bed net that night. These data were used to ensure that the number of persons enumerated by genotype was a reasonable estimate. The Gini index and confidence interval (CI) for the distribution of blood meals between individuals was calculated by using the *inequal* and *somersd* functions in STATA version 10.^{28–30}

Village-scale heterogeneity analysis. To sample heterogeneity at the village level, additional CDC light trap collections were made at one house in each of 45 households in Chidakwa and 45 households in Lupata, for a total of 90 households. All households in each village were in a high-density area for *An. arabiensis* abundance, as predicted by the model of Clennon and others,³¹ and were in a contiguous area. Collections were made once per week for seven weeks in February and March, during the peak of mosquito abundance.

FIGURE 1. Proportion of blood meals from multiple hosts pre-ITN and post-ITN introduction, Zambia. ITN = insecticide-treated bed net. Error bars indicate 95% confidence intervals.

In some cases, collections were not always made because of equipment malfunction or household occupants being absent. To account for these factors, mosquito numbers were corrected by using the number of trap nights. The Gini index for the distribution of *An. arabiensis* was calculated by using the inequal function in STATA version 10.^{28,29} For spatial analysis, the top 25% of households for *An. arabiensis* density were classified as cases ($n = 23$), and the remaining low-risk households were classified as controls ($n = 67$). The bivariate K function (Kcross) in the spatstat library³² was implemented in R version 2.13³³ to estimate the spatial independence of cases and controls at distances of $r = 0$ meters to $r = 700$ meters. To estimate significance, the envelope function with 100 replications was used to generate theoretical minima and maxima for under complete spatial randomness. SaTScan version 9.0³⁴ was used to identify clusters of houses with high *An. arabiensis* density by using the Bernoulli model³⁵ with elliptical clusters³⁶ with a medium non-compactness penalty and 999 replications.

RESULTS

Post-ITN multiple feeding. During the 2007–2008, 2008–2009, and 2009–2010 rainy seasons, 1,659, 1,296, and 217 *An. arabiensis*, respectively, were collected by CDC light trap. Of these mosquitoes, 201, 196, and 78, respectively, had fed on a human host recently enough to be genotyped at the microsatellite loci used. The multiple feeding rate for each season was 7.0% (95% CI 3.5–10.5%) for 2007–2008, 12.2% (95% CI = 7.6–16.7%) for 2008–2009, 6.4% (95% CI = 0.97–11.8%) for 2009–2010. There was no significant difference between the three seasons ($P = 0.13$, by chi-square test) (Figure 1).

Overall, the multiple feeding rate for the three seasons after the introduction of ITNs was 9.1% (95% CI = 6.5–11.6%). When compared with the pre-ITN multiple feeding rate of 18.9%,³ there was a statistically significant decrease in multiple feeding after the introduction of ITNs ($P < 0.0001$, by chi-square test). This figure may be biased because of collection

methods. However, this bias was unavoidable because of limitations in collection methods that can be used with and without ITNs. In prior work we noted that *P. falciparum* infection had no significant effect on multiple feeding.³ Because after ITN introduction, *An. arabiensis* infection rates decreased to 0% for all of 2007–2008, 2008–2009, and 2009–2010, we were unable to make any conclusions about the effect of *P. falciparum* infection on multiple feeding behavior. A small proportion of mosquitoes collected had blood meals from multiple species (2005–2006: 1 human and dog; 2006–2007: 2 human and goat; 2008–2009: 2 human and cow, 1 human and dog, 2 human and goat, 1 goat and cow; and 2009–2010: 1 human and dog). These samples were not genotyped for multiple human blood meals.

Sex bias in host choice. Of 808 human blood meals from 2005–2006 through 2009–2010, 784 were successfully identified as male or female (Figure 2). Before ITN introduction, 57.2% were male and 42.8% female, which is a significant deviation from a 50:50 ratio ($P = 0.015$, by exact binomial

FIGURE 2. Sex bias in human blood meals by year, from 808 *Anopheles arabiensis* samples, Zambia. *Anopheles arabiensis* mosquitoes took significantly more blood meals from male hosts than females, both pre-ITN ($P = 0.015$) and post-ITN introduction ($P = 0.0015$). ITN = insecticide-treated bed net.

TABLE 1

Contribution of persons to total number of blood meals in the top eight households before and after introduction of ITNs, Zambia*

Household	Pre-/post-ITN	No. individuals from census	No. unique genotypes	No. blood meals	Gini index
WP 274	Pre	NA	4	7	0.250
WP 315	Pre	NA	6	8	0.167
WP 160	Pre	NA	3	6	0.333
WP 276	Pre	NA	4	5	0.150
Chilumbwe 7	Pre	NA	4	9	0.306
Chilumbwe 8	Pre	NA	6	6	0.000
Namwalinda 2	Pre	NA	5	7	0.171
Namwalinda 6	Pre	NA	4	8	0.313
Pre-ITN overall			36	56	0.269
Namwalinda 20	Post	8	20	78	0.596
Chidakwa 5	Post	10	12	37	0.426
Namwalinda 9	Post	13	17	30	0.341
Namwalinda 7	Post	8	8	25	0.365
Namwalinda 16	Post	7	11	23	0.285
Namwalinda 15	Post	10	9	20	0.344
Chidakwa 13	Post	7	5	16	0.375
Lupata 81	Post	1	1	19	0.000
Post-ITN overall		64	83	248	0.504

*Because the number of persons counted in the census varied by year, the maximum number of persons is given. ITN = insecticide-treated bed net; NA = not available.

test). Post-ITN, the proportions were essentially identical: 57.3% male and 42.7% female ($P = 0.0015$, by exact binomial test). For all years except 2009–2010, there were more male blood meals than female blood meals. However, census data shows that the Macha area is actually 46.3% male and 53.7% female (Fornadel C, unpublished data). In this case, the skew towards male blood meals is even more significant ($P = 0.0002$ pre-ITN and $P > 0.00001$ post-ITN).

Household-scale heterogeneity. Mosquitoes from the eight households with the highest number of blooded *An. arabiensis* were selected from the pre-ITN and post-ITN collections and analyzed to determine the blood meal contributions of persons (Table 1). Because of variation in sampling schemes throughout the years, there were no households that were

represented in the pre-ITN and post-ITN groups, and there were far fewer samples from households in the pre-ITN group. In the pre-ITN group, 56 blood meals were analyzed and 36 unique genotypes were identified. The Gini index, a measure of inequality, was calculated for each household and for the pre-ITN and post-ITN groups. For reference, a Gini index ranges from 0 to 1, with 0 indicating that each member of a household was bitten an equal number of times, and 1 indicating that biting was maximally unequal. The Gini index of individual households in the pre-ITN group ranged from 0 to 0.333, and the overall index was 0.269 (95% CI = 0.201–0.350), with 20% of individuals contributing 40% of blood meals. In the post-ITN group, 248 blood meals were analyzed and 83 unique genotypes were identified. The Gini index of individual households ranged from 0.385 to 0.596, and the overall Gini index was 0.504 (95% CI = 0.429–0.573), with 20% of individuals contributing 59% of blood meals. A special case in this group is Lupata household 81, which consisted of one person living alone, from whom all blood meals originated, giving a Gini index of 0.

In three of the eight households in the post-ITN group, the number of unique genotypes identified was greater than the number of individuals counted in the census. This finding could be caused by unamplified alleles in individual PCR samples, blood meals from multiple hosts that appeared to be from a single individual (for example, a blood meal from two hosts that were homozygous or shared alleles), or migration in and out of households over the three years of the study. In particular, Namwalinda household 9, an unusually large household, consisted of 9 houses and 24 persons. Only three of these houses were sampled, but persons in this area frequently moved between houses from night to night. Alternatively, because houses within a household are situated close together, mosquitoes could feed outdoors, or in another house, then enter the house where collections were ongoing.

Village-scale heterogeneity. A total of 430 *An. arabiensis* were collected from weekly trap nights at 90 households in

FIGURE 3. Cumulative *Anopheles arabiensis* and blooded *An. arabiensis* contribution by households household, Zambia. The identity line indicates a perfectly even distribution between households, and a steeper slope indicates greater inequality in distribution.

Chidakwa and Lupata, over the course of 7 weeks. There was significant variability in the number of *An. arabiensis* collected in each household: 80 *An. arabiensis* were collected from one household alone, and 20 households yielded no *An. arabiensis* during the course of the study. The overall Gini index for *An. arabiensis* was 0.721. When partitioned by village area, the Gini index was 0.728 for the Chidakwa village area and 0.570 for the Lupata village area. The top 25% of the households contributed 78.1% of the *An. arabiensis* mosquitoes (Figure 3).

Of the 430 *An. arabiensis*, 93 (21.6%) were blood fed. Previous data indicate that in this area, even with high ITN coverage, 94–97% of *An. arabiensis* blood meals from indoor CDC light trap collections are from human hosts (unpublished data). Inequality in the distribution of blooded *An. arabiensis* was even higher than that of total *An. arabiensis* (Figure 3). The overall Gini index was 0.856, and 25% of households accounted for 94.3% of blooded *An. arabiensis* collections.

K-function analysis showed that households with high *An. arabiensis* density (cases) and low *An. arabiensis* density (controls) were not independently distributed. Statistically significant clustering of high-*An. arabiensis* households occurred at spatial scales of 0–250 meters, and low-*An. arabiensis* households were clustered with each other at spatial scales of 0–700 meters (Figure 4).

Spatial scanning analysis with SaTScan detected a significant cluster of high-*An. arabiensis* households along the western edge of Chidakwa ($P = 0.036$) (Figure 5). This cluster of seven households (7.7% of the total) contributed 52.3% of the total *An. arabiensis* collection. The cluster was < 100 meters from a large, flat area that was intermittently flooded during the rainy season, which was noted to be the most highly productive

anopheline larval site found in the two villages (unpublished data). Three additional clusters were detected by SaTScan analysis: a cluster of 38 low-risk households that comprises most of Lupata, four high-risk households in Lupata, and a second high-risk cluster of three households in Chidakwa, but these were not statistically significant ($P = 0.079$, 0.737, and 0.993, respectively) (Figure 5).

DISCUSSION

After the introduction of ITNs to Macha, the multiple blood meal rate decreased from 18.9%³ to 9.1% (95% CI = 6.5–11.6%). It is possible that part of this decrease could be accounted for by a change in capture method. The pre-ITN multiple feeding rate was estimated from samples collected by pyrethrum spray catch, whereas the post-ITN estimate was from CDC light trap collections. Pyrethrum spray catches are only possible in households with no ITNs so that mosquitoes can freely feed, then rest indoors on the walls of the house. Therefore, CDC light trap collections were used to replace pyrethrum spray catches in Macha after the introduction of ITNs. Theoretically, CDC light traps are designed to collect mosquitoes that are searching for a blood meal. However, during the course of our entomologic studies, we have noted that CDC light traps may collect fully blooded mosquitoes that are not searching for a new blood meal. In Macha, 25–33% of *An. arabiensis* caught in CDC light traps are blood fed, and 4% are gravid (Fornadel and others⁶ and unpublished data), which was more than would be expected if the light traps were only collecting mosquitoes seeking a second blood meal. In human landing catches, the gold standard for collecting host-seeking mosquitoes, we consistently collected fewer blooded and gravid *An. arabiensis* than in CDC traps (unpublished data), which indicated that CDC traps may be collecting more than simply host-seeking mosquitoes. Finally, a portion of the collections in the 2006–2007 season were from CDC light traps, and there was no significant difference in blood meals from multiple hosts between collection methods.³

An alternate hypothesis is that sublethal exposure to ITNs may cause delayed mortality. If the blood meals are being taken from different hosts on consecutive nights, mortality caused by ITNs would cause a decrease in blood meals from multiple hosts. However, another possibility is that the presence of insecticide-treated nets protecting most of the human population has focused biting on the persons who do not own a net, whose nets are damaged or not used properly, or who are awake and out of bed during the hours when *An. arabiensis* are biting. In this case, the multiple biting rate may actually be the same, but that fact is obscured because multiple blood meals from the same individual are indistinguishable by our methods.

This last hypothesis is supported by household-scale heterogeneity data. Although these data are limited because of the smaller number of blood meals analyzed during the pre-ITN period, they suggest that inequality in mosquito biting risk among persons in a household increased after introduction of ITNs. The overall Gini index among the top eight households increased from 0.269 to 0.504. Combined with the apparent decrease in blood meals from multiple people, this finding suggests that a smaller number of persons are being bitten more frequently. We are currently planning follow-up studies in a new field site with lower ITN coverage. We expect that

FIGURE 4. Bivariate K-function plot for high-*Anopheles arabiensis* (case) versus low-*An. arabiensis* (control [ctrl]) households, Zambia. The red line indicates clustering of cases with each other relative to clustering to controls, the black line indicates clustering of controls with each other relative to cases, and the gray dashed line indicates the simulated minimum and maximum for significance testing. Deviations above the theoretical maximum indicate significant spatial clustering, and deviations below the minimum indicate negative autocorrelation, or a more regular distribution of cases.

FIGURE 5. Spatial distribution of *Anopheles arabiensis* collections in Chidakwa and Lupata villages, Zambia. Larger circles indicate greater densities of adult female *An. arabiensis*, and dark circles indicate high-risk clusters identified by SaTScan analysis. The seven high-risk households along the western edge of Chidakwa comprise the most significant cluster ($P = 0.036$), which provided 53% of the total *An. arabiensis* collected. Mosq = mosquito.

concurrent collections at houses with and without ITNs, combined with census data on individual ITN use and genotyping human inhabitants of the households, will help elucidate how ITNs affect host choice.

Heterogeneous biting in an infinite host population increases R_0 , the basic reproductive number of the vector-borne pathogen.⁸ However, in limited populations, such as that in Macha, heterogeneity in biting may lead to a decrease in R_0 because most bites would land on a subset of the population that is then super-infected.¹⁰ Multiple feeding, by increasing the human biting rate, would increase malaria transmission,⁴ but most likely within the subset of the population that is bitten more frequently.

However, LLINs are not effective indefinitely, and require replacement or re-treatment. In Macha, a number of LLINs distributed in 2007 have already begun to lose a significant amount of deltamethrin, and have an unacceptable number of holes.³⁷ As LLINs in use become less effective, differences in LLIN efficacy may become a key determinant in who is bitten by vectors, and, over the long term, mosquito host choice may revert to a more homogeneous pattern as more nets fail. Future studies will need to investigate how heterogeneity in

malaria risk changes as LLINs lose efficacy, either because of degradation or insecticide resistance.

For *An. arabiensis*, there was a significant bias towards biting male hosts, despite the presence of more women in the Macha area than men. This was apparent before the introduction of ITNs, when host choice would be expected to be based upon inherent attractiveness, and after ITNs, when available hosts were more restricted. The sole exception was during the 2009–2010 season, during which there were slightly more blood meals from female hosts. However, this may have been caused by smaller sample size from that year, and collections were reduced to a much smaller number of households, which may have biased results. Previous data from Macha has shown that women are 1.87 times more likely to use an ITN than men, and that children less than five years of age are more likely to use an ITN than other age groups.⁶ Although it is promising that these high-risk groups are being protected, it is important to note that older males are more likely to be bitten, and thus to be *Plasmodium* reservoirs.

Previous work has shown that, within the Macha region, most malaria cases occur within high-risk areas near where

An. arabiensis are predicted to breed, and that these 20% of households comprise 80% of the risk.¹⁵ The heterogeneity data presented here shows that within these high-risk areas, there is more stratification of risk. Even when collections were limited to high-risk areas, the Gini index for *An. arabiensis* distribution was 0.721. These data are similar to data for *An. gambiae* s.l. and *An. funestus* from Tanzania, in which the Gini index was 0.866 for heterogeneity within a village.^{9,13}

Statistical analysis showed that *An. arabiensis* density was not only heterogeneous, but spatially clustered. The distribution of *An. arabiensis* adult mosquitoes within households appears to be at least partially determined by distance to breeding sites because the most significant cluster of high-risk households was within 100 meters of the largest breeding site in the area. As has been shown with studies on large-scale heterogeneity in malaria risk, knowledge of the location of anopheline breeding sites can greatly aid in targeting individual households with the highest malaria risk. In an area with decreasing malaria transmission, such as Macha, targeting malaria control towards persons with the highest risk is the most efficient method to continue the decrease in transmission towards elimination.

Received September 22, 2011. Accepted for publication July 28, 2012.

Published online February 4, 2013.

Acknowledgments: We thank the entomology team at the Malaria Institute at Macha, Choma, Zambia, especially Shadreck Habbanti, Limonty Simubali, Harry Hamapumbu, and Petros Moono for invaluable assistance with collections; Gregory Glass and Julie Clennon for larval risk maps; Jason Persichetti for assistance with spatial statistics; and Timothy Shields for assistance with the geographic information system and mapping.

Financial support: This study was supported by a National Institutes of Health Training Grant (T32 AI 007417) to Laura C. Norris, research support to Douglas E. Norris from the National Institutes of Health (International Centers of Excellence for Malaria Research) (U19 AI089680) and the Johns Hopkins Malaria Research Institute, and additional training support from the Fogarty International Center (D43 TW001587).

Authors' addresses: Laura C. Norris and Douglas E. Norris, W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins School of Public Health, Baltimore, MD, E-mails: lcnorris@ucdavis.edu and dnorris@jhsph.edu.

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